

NUTRITIONAL EVALUATION OF
CLASSICAL MEALS PROCESSED BY
VARIOUS METHODS

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NUTRITIONAL EVALUATION OF BRASSICA MEALS
PROCESSED BY VARIOUS METHODS

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by

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BY VARIOUS METHODS

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ABSTRACT

Meals of the Brassica species generally have a low market value, per unit protein, compared with the more commonly used protein supplement, soybean meal. This inferiority can be attributed in part to the glucosinolate contents and the methods of processing. These studies were undertaken to gather more information on the harmful effects of glucosinolates, both with and without dietary myrosinase, on the metabolic rate, the growth response and the enlargement of the liver and kidneys of mice. Investigations were also carried out on methods of removing glucosinolate products from enzyme-inactivated rapeseed meal.

To assess the effects of meals of Brassica species on metabolic rate of mice, a simple automatic respirometer was designed and tested. The experimental results showed that the apparatus was capable of detecting abnormalities in thyroid activity with a high degree of reliability.

The effects of synthetic thyroid-active iodinated-casein, synthetic goitrogenic thiouracil and natural glucosinolates on the metabolic rate of mice were studied. Feeding meals of Brassica species containing thioglucosides and thiouracil resulted in a reduction in oxygen consumption, whereas iodinated-casein caused an increase in consumption.

Myrosinase supplementation of the meals of Brassica species consistently depressed oxygen uptake, but there was a wide range of response. This variation can be attributed to the differences in levels, types of glucosinolates and other unknown toxic factor(s) in the different meals.

It has been found that adding a certain amino acid(cystine) to rapeseed (Brassica napus) meal in acidic (pH5) media will neutralize glucosinolate products, hydroxynitrile, isothiocyanate and other known toxic factor(s). An improved Brassica napus meal has been developed by using L-cystine to give a significantly better nutritive value and acceptability. Growth response and organ weights (liver and kidney) of mice comparable to those obtained on Bronowski meal were possible when a certain quantity of this amino acid was added to the meal.

Glucosinolate contents, their hydrolysis products, isothiocyanates, goitrin, hydroxynitrile, and biological quality were investigated in rapeseed (Brassica napus) meals treated according to various techniques developed during these studies. Particular attention was given to the formation and toxicity of hydroxynitrile.

According to the present methods, a process is provided for the treatment of rapeseed meal containing a high level of glucosinolates to produce a rapeseed meal with a feeding value equal to casein. The feeding values of the rapeseed meals treated by these techniques were of the same magnitude as casein, while untreated enzyme-inactivated

meal was 40 percent below casein.

Aqueous extraction procedures for the removal of glucosinolates (as their non-enzymatic hydrolysis products) from rapeseed meal are described. Enzyme-inactivated meal is thoroughly mixed with certain levels of ferrous sulfate and L-cystine, then wetted with pH5 buffer solution to allow non-enzymatic hydrolysis of glucosinolates to occur. The meal slurry is then subjected to extraction. The residual meal contained small amounts of isothiocyanates, goitrin, hydroxynitrile and unknown toxic factor(s) of sufficiently low concentrations to be readily tolerated by laboratory animals.

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1. INTRODUCTION

Canada has become the world's largest exporter of rapeseed. The domestic market for rapeseed oil and meal has also developed rapidly. Acceptance of rapeseed meal as a protein supplement for animal feed has, however, been limited by its glucosinolate (thioglucoside) content, lower protein levels, higher levels of crude fiber and lower metabolizable energy contents compared with alternative feedstuffs.

The earlier work suggesting that foods contain goitrogenic material seems to have been that of Cheney and his co-workers in France, and of Marine's group in showing that Brassica species contain substances goitrogenic to animals.

The literature on toxic effects of glucosinolates and their hydrolytic products is extensive. Basic information in this field is, however, of recent origin and the major contributions having been made since about 1955. The recent knowledge includes fundamental studies on the biosynthesis of glucosinolates. Methods for determining glucosinolate products, extensive nutritional studies and changes in the methods of crushing seed are introduced.

Processing Brassica seed by methods that inactivate thioglucosidase, the enzyme responsible for liberation of isothiocyanates and oxazolidinethione (goitrin) from its substrates (glucosinolates), leaves the glucosinolates intact with a minimum of hydrolyzed products in the meal. When such a meal is included as a source of protein supplement in livestock and poultry rations the intact glucosinolate contents are decomposed

in the gastro-intestinal tract of animals by bacterial enzyme(s) and chemical hydrolysis.

The decomposition of glucosinolates in the meal of the Brassica family by catalytic (certain heavy metal ions) procedures has been proposed by Canadian investigators. Unfortunately, the chemically hydrolyzed products include organic nitriles. The meal containing the nitriles is more toxic than that containing mustard oils.

In spite of much research on growth response and thyroid enlargement, information is still limited on the overall effects of meals of Brassica species on metabolic rate, and hyperplasia and hypertrophy of different organs of animals. Moreover, the investigations made to obtain such information have yielded limited and sometimes confused results.

A new approach to evaluate the nutritive value of Brassica meal, particularly rapeseed meal, is, therefore, justified. It also appears that more attention should be given to the toxicity caused by products of glucosinolate hydrolysis other than enzymatic hydrolysis.

The investigations which are reported here deal with

- 1) the design of a simple respirometer for measuring oxygen uptake by mice,
- 2) the changes in the metabolic rate of experimental animals due to feeding meals of various Brassica species and varieties and a synthetic thyroactive compound, and
- 3) the methods of extracting and neutralizing glucosinolate products in rapeseed meal.

2. LITERATURE REVIEW

2.1 Biochemistry, Occurrence and Amount of Natural Glucosinolates in Plants

2.1.1 Biochemistry and occurrence of glucosinolates

The acrid nature of plants, such as horse radish, mustard green and mustard seed has been known to man from antiquity. It was reported (VanEtten et al., 1969; Bell, 1965) that a relationship between mustard oil (allyl isothiocyanate) which is the cause of the pungency and a precursor was first established by Bussy in 1840 following the first isolation of a thioglucoside named sinigrin from black mustard seed (Brassica nigra L.). Formation of the mustard oil appeared to be due to activity of enzyme systems associated with sinigrin in black mustard seed.

Although a tentative structure for glucosinolates containing a thioglucoside unit was proposed by Gadamer 1897, for nearly 60 years no progress took place in the understanding of the aglucone portion. The work of Schmeder in 1928 to 1931 supplied our fundamental knowledge of 1-thioglucopyranose (Ettlinger and Kjaer, 1968).

By 1914 only two additional thioglucosides were isolated, namely: sinalbin from white mustard seed (Sinapis alba L.) and glucocheirolin from wall flower seed (VanEtten, 1969). During the period 1954 to 1970, as a result of the introduction of chromatographic methods of analysis, the number of identified natural plant thioglucosides has increased to about 50 (Ettlinger and Kjaer, 1968) and (VanEtten, 1969).

The structure of all glucosinolates is that of the prototype shown either in Figure 1 or 2.

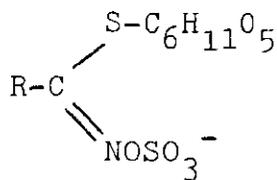


Fig. 1

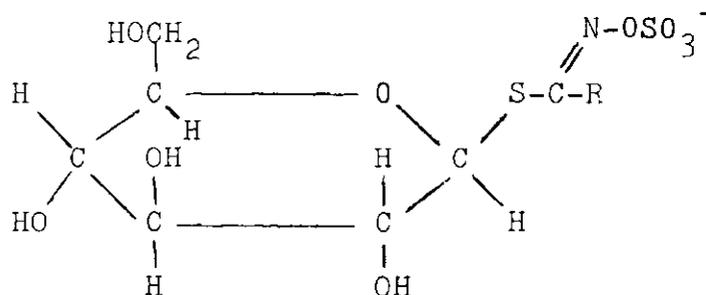


Fig. 2

Chemical Structure of Glucosinolate

This structure was established and synthesized by (Ettlinger and Lundeen, 1956 and 1957). This structure replaced that proposed by Gadamer (Fig. 3), in which the nitrogen was placed between the glucosinolate carbon and the R-group.

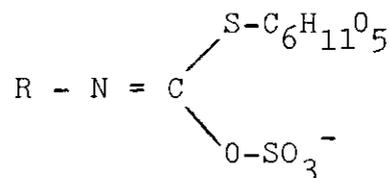


Fig. 3

Chemical Structure of Glucosinolate

The characterized glucosinolates differ only in the nature of their R-groups.

The natural thioglucosides found in plants are also called glucosinolates. Glucosinolates have been named by three different naming systems (VanEtten *et al.*, 1969):
 (1) by prefixing "gluco" to the botanical name of the plant from which it was first isolated, for example, gluconapin,

glucobrassicinapin, (2) by the trivial name such as, sinigrin, sinalbin, progoitrin and epi-progoitrin. (The first two compounds were so named before much was known about their chemistry and progoitrin was so named because it was the precursor of goitrin. Furthermore, progoitrin and epi-progoitrin differ from each other only in configuration at the asymmetric carbon in the thioglucoside's aglucone), and (3) by current nomenclature, the parent ion (R = H) receiving the trivial name "glucosinolate" to which is added a prefix that chemically describes the name of a side chain (R-group), for example, (R)-2-hydroxy-4-pentenylglucosinolate and 4-methylthio-3-butenylglucosinolate. Thus R-group is substituted and it distinguishes one glucosinolate ion from another. It should be mentioned that the current naming system was proposed by Ettliger and Dateo (1961).

Chemically, the simplest glucosinolate occurring in nature has a methyl side chain, that means $R = CH_3$ (Gmelin et al., 1970). Methylglucosinolates have been repeatedly encountered in species of the Capparidaceae. With one exception, it has not been reported in any of the more than 400 species of the Cruciferae examined. They consistently contain glucosinolates with side chains other than a methyl group (Ettliger and Kjaer, 1968). The one exception is, the occurrence of methylisothiocyanate in leaves of the crucifer Mattiola fruticulosa Maire reported by Matas (Gmelin et al., 1970). However, the occurrence of methylglucosinolate in Mattiola fruticulosa was not confirmed by Ettliger and Kjaer (1968). In addition,

Ettlinger and Kjaer (1968) tabulated 50 different natural glucosinolates, all possessing the same major functional group, and they classified these compounds into 3 groups, as follows:

1. Aliphatic glucosinolates containing a C-methyl-group or derived from diacids. This group includes (a) alkyl, alkenyl, hydroxyalkyl and acyloxyalkyl compounds, or (b) ketoalkyl compounds.
2. W-methylalkyl glucosinolates and derivatives.
3. Arylmethylglucosinolates.

Practically all plants of the Cruciferae that have been examined contained thioglucosinolate (Josefsson, 1967). In particular, the most valuable cultivated species of Cruciferae family belong to the genus Brassica. According to Kjaer (1966), 300 of 1500 species considered as members of the Cruciferae family examined for glucosinolates contained these compounds. Furthermore, all species from the related Cruciferae family, Capparidaceae, Moringaceae, and Resedaceae and some species from unrelated plant families possess glucosinolates. The general structure of glucosinolate already described and the chemical nature of R-groups in thioglucosides found in some of the Brassica plant are shown in table 1 (Joseffson, 1967) and (VanEtten et al., 1969).

Rapeseed contains three major thioglucosides: gluconapin, glucobrassicinapin and progoitrin (Youngs, 1968 and 1969). Furthermore, in 1968 and 1969 the presence of the following toxic compounds in rapeseed cakes have been reported (Kjaer et al., 1953).

Table 1. Brassica species and their glucosinolates

Glucosinolate			
Plant	Trivial name	Systematic name	R-group (side chain)
<u>Brassica nigra</u> , Black mustard		Allylgluco- sinolate ion	$\text{CH}_2=\text{CH}-\text{CH}_2-$
<u>Brassica juncea</u> , Indian (brown mustard)	Sinigrin		
<u>Brassica carinata</u> , Ethiopian rapeseed			
<u>Brassica oleracea</u> Cabbage, kale			
<u>Amoracia lapathifolia</u> , <u>A. rusticana</u>			
<u>Brassica oleracea</u> Broccoli, kohlrabi			
<u>Brassica campestris</u> , Rape, turnip rape, Polish rape, rubsen, naverte	Gluconapin	3-butenylgluco- sinolate ion	$\text{CH}_2=\text{CH}(\text{CH}_2)_2-$
<u>Brassica napus</u> , Rape, Argentine rape, winter rape			
<u>Crambe abyssinica</u> , Crambe, Abyssinian kale			
<u>Brassica campestris</u> , Rape, turnip rape, Polish rape, rubsen, naverte	Glucobrassica- napin	4-pentenylgluco- sinolate ion	$\text{CH}_2=\text{CH}(\text{CH}_2)_3-$
<u>Brassica napus</u> , Rape, Argentine rape, winter rape	Glucoiberin	3-methylsulphinyl- propylgluco- sinolate ion	$\text{CH}_3-\text{SO}(\text{CH}_2)_3-$

Table 1. continued

Glucosinolate			
Plant	Trivial name	Systematic name	R-group (side chain)
<u>Brassica campestris</u> , Rape, turnip rape, Polish rape, rubsen, naverte	Glucoraphanin	4-methylsulphinyl- butylglucosinolate ion	$\text{CH}_3\text{-SO}(\text{CH}_2)_4\text{-}$
<u>Brassica campestris</u> , Rape, turnip rape, Polish rape, rubsen, naverte	Glucoalyssin	5-methylsulphinyl pentylglucosinolate ion	$\text{CH}_3\text{-SO}(\text{CH}_2)_5\text{-}$
<u>Brassica campestris</u> , Turnip	Gluconasturtiin	2-phenylethyl- glucosinolate ion	$\text{C}_6\text{H}_5(\text{CH}_2)_2\text{-}$
<u>Amoracia lapathifolia</u> , Horse radish			
<u>Brassica napus</u> , Rape, Argentine rape, winter rape			
<u>Crambe abyssinica</u> , Crambe, Abyssinian kale			
<u>Brassica campestris</u> , Turnip, rape, turnip rape, Polish rape, rubsen, naverte	Progoitrin	2-hydroxy-3-butenyl- glucosinolate ion	$\text{CH}_2=\text{CH}-\underset{\text{OH}}{\text{CH}}-\text{CH}_2\text{-}$
<u>Brassica napus</u> , Rape, Argentine rape, winter rape			
<u>Sinapis alba</u> White mustard	Sinalbin	p-hydroxybenzyl- glucosinolate ion	p-OH-C ₆ H ₄ CH ₂ -
<u>Brassica napus</u> , Rape, Argentine rape, winter rape			

1. $\text{CH}_2 = \text{CH} - \text{CH}_2 - \text{CH}_2 - \text{N} = \text{C} = \text{S}$
Mustard oil
2. $\text{CH}_3 - \text{CH} = \text{CH} - \text{CH}_2 - \text{N} = \text{C} = \text{S}$
Isothiocyanate (trans-crotyl derivative)
3. $\text{CH}_2 = \text{CH} - \text{CH}_2 - \text{CH}_2 - \text{C} \equiv \text{N}$
Crotonyl cyanide

Daxenbichler et al. (1966) found that the major thioglucoside in the seed of Crambe abyssinica is epi-progoitrin (Fig. 4a) and in the rutabaga seed is progoitrin (Fig. 4b).

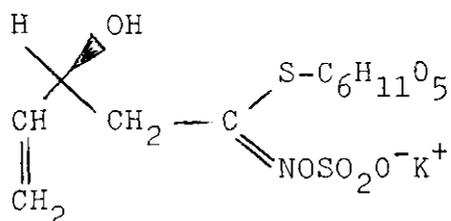


Fig. 4a
Chemical structure of epi-progoitrin

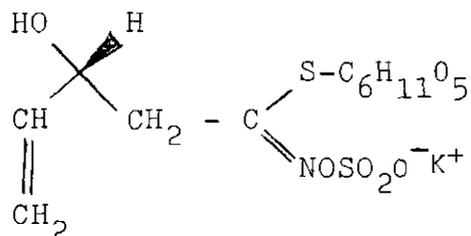


Fig. 4b
Chemical structure of progoitrin

Although over 40 thioglucosides have been reported (Kondra and Downey, 1969, 1970; Downey et al., 1969; and Youngs, 1969, 1967) in the plants only three major ones have been identified in two rapeseed species (Brassica napus L. and Brassica campestris L.): 3-butenylglucosinolate, 4-pentenylglucosinolate and 2-hydroxy-3-butenylglucosinolate. These compounds, when hydrolyzed with enzyme, myrosinase, give rise to 3-butenyl- and 4-pentenylisothiocyanates and (S)- or (R)-5-vinyl-2-oxazolidinethione, respectively. Moreover, the co-occurrence of sinigrin-glucoiberin, gluconapin-glucoraphanin and glucobrassicinapin-glucoalyssin have been cited in literature (Josefsson, 1967).

2.1.2 Amount of glucosinolates

Seed meals of winter and summer types of varieties of Brassica napus L. and Brassica campestris L. grown at different locations and years in Sweden were analyzed for glucosinolate contents by Josefsson and Appelqvist (1968). The reported values are given in table 2.

These authors also analyzed seed meals of winter rape (Var. Lembke) cultivated under conditions in Germany and the following data were reported (table 3).

In the fall of 1965, samples of seven varieties of rapeseed from seven stations across Western Canada were collected and analyzed by Wetter and Craig (1959) and the following mean values were reported (table 4).

Table 2. Content of glucosinolates (percent in dry matter) in seed meals of different varieties of rape, grown at different locations in Sweden.

Species and variety or strain	Glucosinolate content		
	Gluconapin ¹	Progoitrin ²	Gluconapin and progoitrin
<u>Brassica napus</u> , winter type			
Matador	1.71	4.69	6.40
Helmer	1.71	4.51	6.24
Vector	1.79	4.31	6.11
<u>Brassica napus</u> , summer type			
Regina II	1.27	3.04	4.31
Rigo	1.31	3.72	5.03
<u>Brassica campestris</u> , winter type			
Duro	3.10	0.22	3.32
Rapido II	2.95	0.50	3.45
<u>Brassica campestris</u> , summer type			
Bele	1.78	1.26	3.04
SV58/4	2.15	1.15	3.30

¹ -potassium 3-butenyl glucosinolate calculated as volatile isothiocyanates.

² -potassium 2-hydroxy-3-butenyl glucosinolate calculated as oxazolidinethiones.

Table 3. Glucosinolate contents (percent dry matter) in rapeseed (Var. Lembke) meals, grown in Germany.

Growing conditions	Glucosinolate content		
	Gluconapin	Progoitrin	Gluconapin and progoitrin
(a) Cultivated at the same location in different years (1962-1965)			
mean	1.74	4.06	5.80
range	1.49-2.04	3.81-4.5	5.30-6.39
(b) Cultivated at different locations (1965)			
mean	1.97	4.51	6.48
range	1.64-2.15	4.14-4.9	5.78-7.05

Table 4. The means values of isothiocyanate and oxazolidinethione contents of different varieties of rapeseed from Western Canada

Variety	Isothiocyanate ¹ (I)	Oxazolidinethione ² (II)	Ratio I/II
Argentine	4.90	5.90	0.87
Swedish	4.48	5.42	0.82
Golden	4.33	5.30	0.81
Regina II	4.64	5.42	0.85
Polish	5.35	1.55	3.45
Arlo	4.88	1.33	3.66
Gute	4.84	1.79	2.70

1,2, Isothiocyanate and oxazolidinethione contents expressed as mg/g of fat-free meal.

From table 4 one can see that these varieties fall into two general groups; Argentine-types (Argentine, Swedish, Golden and Regina II); and the Polish-types (Polish, Arlo, and Gute). The total glucosinolates (gluconapin, progoitrin and gluco-brassicinapin) in the seed of varieties of Argentine-type and Polish-type grown today in Canada vary from 1.5 to 3.5 percent of the rapeseed (Youngs 1968). Wetter (1965), citing values obtained by Clandinin, showed expeller meals from different varieties of rapeseed ranging from 2.10 to 3.08 and 1.04 to 3.35 mg/g for isothiocyanate and oxazolidinethione, respectively, and in prepress-solvent and solvent-processed meals ranges of 2.39 to 5.55 and 1.83 to 6.39 mg/g were reported. Furthermore, Youngs and Wetter (1969) stated that there is some variation in thioglucoside content of rapeseed with variety. Brassica napus L. varieties have a thioglucoside content equivalent to 5 mg/g to 10 mg/g oxazolidinethione on an oil-free basis, while Brassica campestris L. varieties contain only 2 mg/g to 3 mg/g. Thioglucosides equivalent to 5 mg/g isothiocyanate are present in both varieties. Fourteen domestic and foreign varieties of rapeseed meal were grown in Sweden under normal conditions. Twenty-eight rapeseed meal samples for above varieties were analyzed for isothiocyanates and oxazolidinethione by Appelqvist (1969) and the value obtained (on the basis of defatted meal) are shown in table 5.

Ballester et al. (1970) worked on the chemical composition and biological quality of the protein in rapeseed meal. At the same time, they chemically analyzed twelve samples of

Table 5. Isothiocyanate and oxazolidinethione contents in rapeseed meals grown in Sweden

Species	Isothiocyanate (I) mg/g	Oxazolidine- thione (II) mg/g	I and II	I/II
<u>Brassica napus</u> winter type	4.3	7.6	11.9	0.6
<u>Brassica napus</u> summer type	8.3	0.7	9.0	11.9
<u>Brassica campestris</u>	7.2	0.9	8.1	8.0

Table 6. Ranges of p-hydroxybenzyl, allyl-3-butenyl- and 4-pentenyl isothiocyanate and oxazolidinethione (ozt) contents in Brassica seeds (mg/g oil-free meal)

<u>Brassica</u> <u>Species</u>	<u>Crop Name</u>	<u>p-OH</u>	<u>allyl</u>	<u>butenyl</u>	<u>pentenyl</u>	<u>ozt</u>
<u>B. hirba</u>	Yellow mustard	12-20	0	0	0	0
<u>B. arthensis</u>	Wild mustard	12-20	0	0	0	0
<u>B. juncea</u>	Brown or leaf mustard	0	7-15	0	0	0
<u>B. nigra</u>	Black mustard	0	7-15	0	0	0
<u>B. carinata</u>	Abyssinian mustard	0	7-15	0	0	0
<u>B. oleracea</u>	Kale, cabbage, etc.	0	1-9	0-4	0	0-7
<u>B. napus</u>	Rape	0	0	1-4	0.5-1.5	4-12
<u>B. campestris</u>	Turnip rape	0	0	1-3	1-3	0.5-3
	Indian yellow sarson	0	0	8-16	0	0

solvent-extracted rapeseed presscake meals for glucosinolate contents. They reported the glucoside value, ranging from 2.65 to 3.3 g/100g 3-butenylisothiocyanate from 1.30 to 1.52mg/g and oxazolidinethione from 6.57 to 8.39mg/g with the mean values of 3.00, 1.40 and 7.61 in the same order.

Daxenbichler et al.(1964) obtained information about the parent thioglucosides by estimation of oxazolidinethione and steam-volatile isothiocyanate contents of enzymatic hydrolysates of the seed meals from sixty-five species of Cruciferae. Moreover, they reported the following values for a sample of Brassica napus: 5.9 to 6.0 and 4.3 to 6.2 mg/g for isothiocyanate and oxazolidinethione respectively. Kondra and Downey (1970) in a study of the effects of pod position and environment on the three major glucosinolates (gluconapin, glucobrassicinapin and progoitrin) contents of rapeseed meals from Nugget (Brassica napus L.) and Echo (B. campestris) found that butenylisothiocyanate ranged from 0.94 to 2.99, pentenyl isothiocyanate from 0.05 to 3.36 and oxazolidinethione from 2.13 to 8.01 mg/g on the basis of oil-free meals. Downey et al. (1969) in a study of breeding rapeseed for oil and meal quality by selection found that Brassica seeds varied markedly in the kind and amount of thioglucosides (table 6). Of particular interest is that, within the Brassica napus L. species, plants of variety Bronowski (Polish summer rape variety) were found to have extremely low levels of all three major compounds with respect to other varieties (Table 7).

Table 7. Hydrolysis products of thioglucoside contents of standard rapeseed varieties and selections (mg/g of oil-free meal)

<u>Species and variety</u>	<u>3-butenyl isothiocyanate</u>	<u>4-pentenyl isothiocyanate</u>	<u>oxazoli- dinethione (ozt)</u>
<u>Brassica napus</u>			
Tanka	2.5	0.6	11.5
Nugget	1.7	0.3	7.8
<u>Brassica campestris</u>			
Bronowski	0.3	T	0.3
<u>Brassica campestris</u>			
Echo and Arlo	2.0	1.5	1.7
sel.ozt	2.4	1.6	0.0
sel.pentenyl	1.1	0.0	1.0
sel.pentenyl+ozt	2.1	0.0	0.0

A large collection of Brassica napus L. samples compiled from various sources were analyzed in an effort to obtain low glucosinolate types. The lowest glucosinolate content in any rapeseed material investigated was found in the Polish summer rape variety Bronowski (Joseffson and Appelqvist, 1968). (Table 8).

Table 8. Content of glucosinolates (percent in dry matter) in seed meal samples of Bronowski summer rape and offspring from that variety

Sample	Source	Gluconapin	Progoitrin	Gluconapin and progoitrin
I.L.1997	Poland	0.15	0.79	0.94
I.L.2243	Czecho-slavakia	0.87	2.78	3.65
Bronowski IHAR	Poland	0.11	0.53	0.64
Sv64-1055	Sweden	1.29	2.35	3.64
Sv67-90181-11	Sweden	0.07-0.36	0.10-0.36	0.17-0.58
Bronowski IHAR	France	0.06	0.12	0.18
Bronowski 217865	France	0.05	0.15	0.20

A summary of data on the content of glucosinolates of some European rape and turnip rapeseed is given in table 9.

Table 9. Content of glucosinolates (percent in dry matter) in seed meals of some* European varieties of rape and turnip rape, grown at various localities

Species and variety	Locality	Year	Gluconapin	Progoitrin	Gluconapin and progoitrin
<u>Brassica napus</u>					
winter type					
Rapol	Germany	1965	1.66	4.22	6.58
Diamant	Germany	1965	1.45	4.98	6.43
Tonus	Nord.France	1965	1.73	4.16	5.89
Nain de Hamburg	Nord.France	1965	1.21	4.64	3.85
Sarepta	Nord.France	1965	1.10	2.81	3.91
Sarepta	Versailles,				
	France	unknown	2.30	3.28	5.58
Sarepta	Svalof, Sweden	1961	1.38	2.94	4.32
Valois	Versailles,				
	France	unknown	2.22	5.55	7.77
Valois	Svalof, Sweden	1961	1.43	5.45	6.88
Dublanski	Svalof, Sweden	1965	1.81	4.12	5.93
Niemierczanski	Svalof, Sweden	1965	2.00	4.73	6.73
Skrzeszowsicki	Svalof, Sweden	1965	1.77	4.04	5.81
Warszawski	Svalof, Sweden	1965	1.87	4.46	6.33
Poiwicki	Svalof, Sweden	1965	2.06	4.68	6.74
Gorczański	Svalof, Sweden	1965	2.03	4.30	6.33
<u>Brassica napus</u>					
summer type					
Zollerngold	France	1965	1.76	3.18	4.94
Cyzowski	Czechoslovakia	1965	1.50	4.05	5.55
Cyzowski	Svalof, Sweden	1965	1.27	3.78	5.05
Mtochowski	Czechoslovakia	1965	1.80	3.59	5.39
<u>Brassica campestris</u>					
winter type					
Gruber	Tikkurila, Finland	1965	3.84	0.46	4.30
Rapido I	Habbiala, Finland	1965	3.07	0.57	3.64

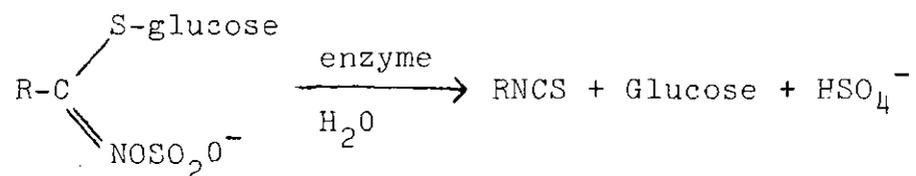
* (Joseffson and Appelqvist, 1968)

2.2 Myrosinase

Throughout the literature the name, myrosinase, has been used analogously with the names myrosin, sinigrinase, or mustard myrosinase. Furthermore, in 1962, the term glucosinolase was proposed (Ettlinger and Thompson, 1962).

Since then the International Union of Biochemistry (Webb, 1967) and (Stotz, 1964) has recommended the term thioglucosidase (an enzyme that hydrolyzes a thioglucosyl bond) as a trivial name, or thioglucosidase hydrolyzing enzyme as a systematic name with code number 3.2.3.1. The recommended names are commonly used by investigators in this field.

Thioglucosidase is the enzyme system responsible for the hydrolysis of the glucosinolates, natural substances, found in any species of the Cruciferae, Caparidaceae or Resedaceae (Calderon et al., 1966). The action of myrosinase on the thioglucosides may be simply represented as follows:



It has been reviewed (Gaines and Goering, 1960) and (Ettlinger and Lundeen, 1956) that early workers (Von Euler, 1926; Sandberg and Holly, 1932; Neuberg and Schoenebeck, 1933 and Ishimoto and Xamashino, 1940) believed that the enzyme, myrosinase, was composed of two entities: a glucosidase (thioglucosidase) capable of hydrolyzing S-glycosyl compounds, splitting off the glucose moiety, and a sulfatase capable of

removing sulfur. Gaines and Goering (1960) prepared the crude enzyme from defatted Oriental yellow mustard seed (Brassica juncea) and tested the activity of the prepared enzyme by using sinigrin ($R=CH_2=CH-CH_2-$) as substrate. These authors supported the concept of a two-enzyme system, namely a thioglucosidase and sulfatase, involved in the total hydrolysis of the mustard oil glucosides. Their experimental results indicated definitely that there is a sulfatase, or factor which possesses sulfatase activity, involved in the total hydrolysis of the mustard oil glucosides. After enzymatic removal of the glucose moiety by the thioglucosidase, the residual structure undergoes cleavage of the sulfate group and subsequent rearrangement, probably while bound to the sulfatase.

In 1959, Gmelin and Virtanen indicated that at least two factors are involved in hydrolysis and rearrangement of the thioglucoside molecule to its products. Gaines and Goering (1962) provided myrosinase from defatted Brassica juncea and salicin, amygdaline, phloridzin, sinigrin and p-nitrophenyl-B-D-glucoside used as substrates. Their results showed conclusive evidence for the dual nature of myrosinase. Schwimmer(1960) stated that there was an indication of separate functions for the sulfatase and thioglucosidase of myrosin.

Ettlinger and Lundeen (1956) established some doubt as to the necessity of the two-enzyme system for the hydrolysis of the mustard oil glucosides. Other investigator's work (Nagashima and Uchiyama, 1959 and Ettlinger and Lundeen, 1957)

favor the theory of a one-enzyme system for myrosinase. In 1966, Calderson et al. examined Black (Brassica nigra), Brown (Brassica juncea) and Oriental mustard seeds (Brassica juncea) for myrosinase activity by using methyl n-pentylketoxime-o-sulfate (potassium salt) and sinigrin as the enzyme substrates; they did not detect specific components for the hydrolysis of oxime sulfonates. These authors confirmed the theory of a one single enzyme system, which describes the action of thioglucosidase on the mustard oil compounds as the hydrolysis of glucose from the thioglucoside followed by a Lossen rearrangement of the aglycone to give isothiocyanate and sulfate. Nagashima and Uchiyama (1959) concluded from their studies that the myrosinase was not a mixture of the two enzymes, but it was a single B-thioglucosidase and liberation of sulfate would occur by non-enzymic reaction.

Tsuruo et al. (1967) studied the chromatographic behavior of myrosinase and stated that the myrosinase was originally a single B-thioglucosidase but might have two species. Tsuruo and Tadao (1967) investigated the mechanism of action of plant myrosinase by ascorbic acid; they did not support the concept of the effect of ascorbic acid activity on the myrosinase proposed by other investigators. Vaughan et al. (1968) developed a test for the identification of myrosinase after separation of seed protein in starch gel and immunoelectrophoresis of Brassica oleracea, Brassica campestris, Brassica nigra, Brassica napus, Brassica juncea, Brassica carinea, Sinapis arvensis and Sinapis alba. These authors

indicated that there might be some taxonomic distinction between Brassica and Sinapis based on the enzyme found in both genera. Vaughan and Gordon (1969) studied the relationship between myrosinase in the two species, Brassica juncea and Sinapis alba. Immunodiffusion results indicated that the enzyme is serologically similar in the two species but a considerable difference exists between them, that in Sinapis alba being greater than that in Brassica juncea. These investigators also stated that the enzyme may exist in two forms. It has been reviewed that (Vaughan and Gordon, 1969) total protein patterns following acrylamide gel electrophoresis showed that certain differences exist between Brassica juncea and Sinapis alba as well as considerable similarities.

From time to time, the presence of myrosinase without glucosinolate has been reported in common plants like beans, carrots, onions, wheat, or clover, but those claims do not appear substantial (Ettlinger and Kjaer, 1968). The evidence is that yellow mustard seed contains at least two enzymes that catalyze the same reaction, hydrolysis of mustard oil glucosides; one enzyme corresponding to the classical myrosin acts equally in presence or absence of ascorbate. The other enzyme needs vitamin C as cofactor. However the concept of the proper ascorbate-activate glucosinolase was not confirmed by Ettlinger and Thompson (1962).

From this limited review, one might conclude that the mechanism of myrosinase activity has been in question. Since

the discovery of the enzyme in the middle of the last century, a controversial question has been whether myrosinase is a one or two-enzyme system. However, almost all investigators now believe that only one enzyme is involved in the thioglucosinolate hydrolysis.

2.3 Hydrolysis of Glucosinolates

More than a hundred years ago, the sulfur-containing compounds responsible for the production of the bitter principle of black and white mustard were isolated in crystalline form. Several decades later they were recognized as glucosides, undergoing enzymic hydrolysis to mustard oil, glucose and sulfate. Thus, the pungent taste and odor of isothiocyanates can render the presence of glucosinolates in conspicuous plants. A variety of factors however, confuses the sensory detection. Isothiocyanates often are not discussed during botanical description or chemical analysis, partly because (1) non-volatile isothiocyanates do not stand out, and (2) the compounds are not formed in dry material or material in which the myrosinase is no longer active, once liberated or lost in storage.

In general, many investigators have reported that the thioglucoside content of Brassica seed meals may be altered. First, by enzymatic hydrolysis, the thioglucosides may be hydrolyzed by the action of an enzyme or enzyme complex known as myrosinase (thioglucosidase), which is present in all plants containing glucosides. Secondly, by chemical modification and processing methods the general structure of glucosides and their products by enzymatic cleavage can be represented as follows. (Fig. 5).

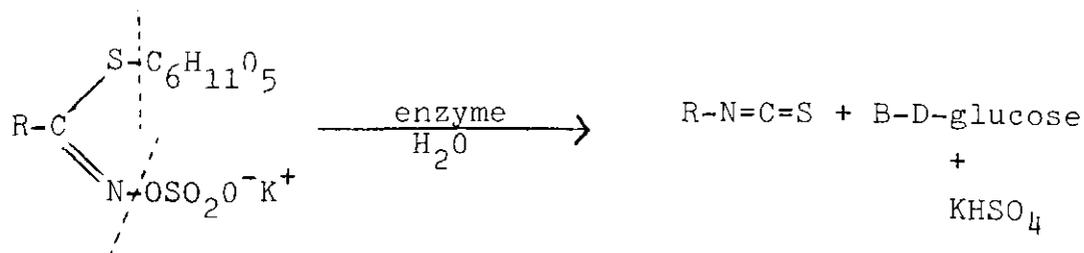


Fig. 5
Enzymatic cleavage of glucosinolate

The portion of the aglycon represented by (R) group varies considerably upon the rate of enzymatic hydrolysis (Gaines and Goering, 1960). Glucose and acid sulfate ion are released from thioglucosides, but these products are not often mentioned because they do not vary with the nature of the thioglucosides. Moreover, the broken lines illustrated above indicate the sites of hydrolysis by the thioglucosidase in the enzyme system.

2.3.1 Plant enzymatic hydrolysis

The distribution of glucosinolate can hardly be considered without thought of the enzymes that act on them. Two modes of enzymatic attack on glucosinolate are known: (1) removal of sulfate and (2) cleavage of the glucosylthioether link. Presumably, thioglucosidases do not show major specificity toward the glucosinolate side chain (Ettlinger and Kjaer, 1968). The typical action of the plant enzyme (myrosinase) on glucosinolates is shown in Fig. 5. Ettlinger and Kjaer (1968) observed that in 1839 and 1840 potassium myronate was isolated as the result of the first deliberate backward search from product to substrate of a known enzymatic reaction; and sinalbin (sinipin p-hydroxybenzylglucosinolate) was recognized to be an analogue.

As noted previously, Ettlinger et al. (1957) and Ettlinger and Lundeen (1956) assigned the structure that the liberation

of sulfate occurred by the Lossen rearrangement after the cleavage of the thioglucoside linkage. Nagashima *et al.* (1959) also supported the concept of Lossen rearrangement. Bell and Belzile (1965) demonstrated the oxazolidinethione arose from cyclization following the action of enzyme and other factors on thioglucosides as follows (Figs. 6 and 7).

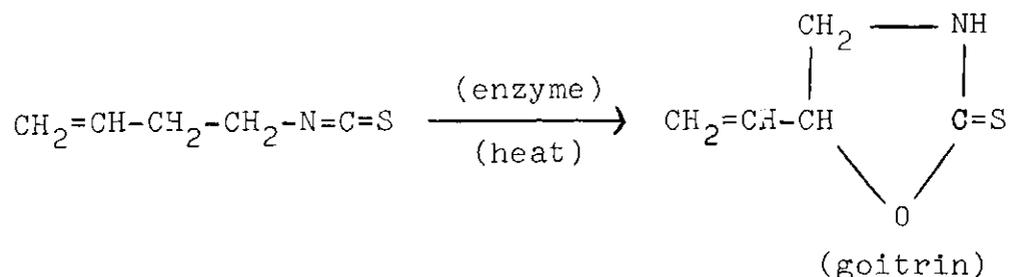


Fig. 6
Formation of goitrin

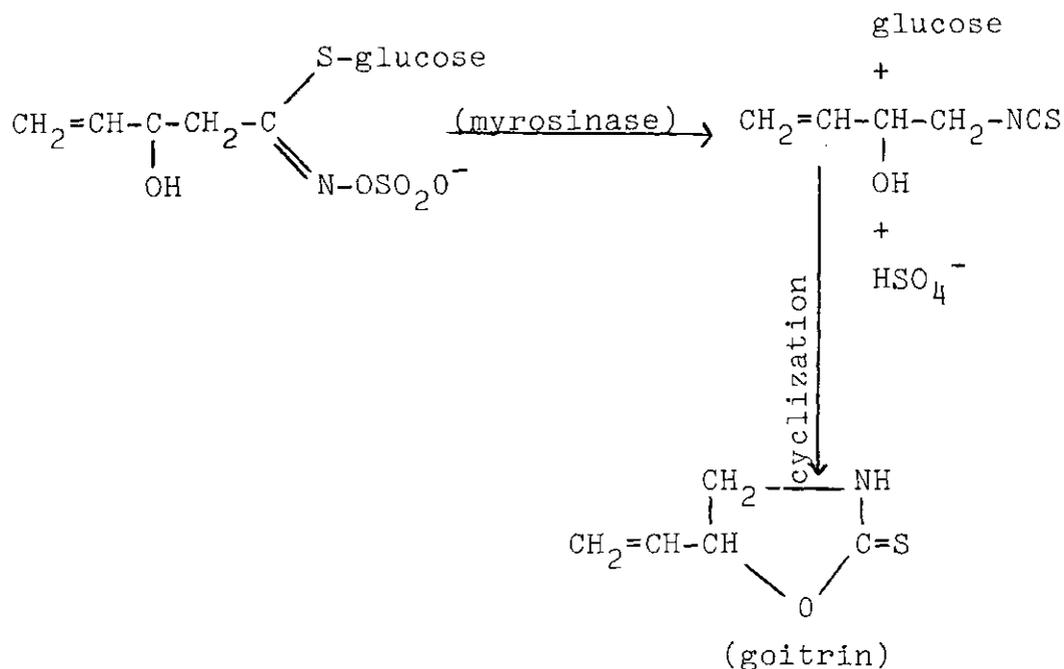


Fig. 7
Formation of goitrin

These authors also pointed out that the action of myrosinase on thioglucosides can give rise to isothiocyanates, thiocyanates or nitriles but the prominent reaction involves a Lossen transformation to yield isothiocyanates (in some cases thiocyanates).

Kirk et al. (1966) by using Wetter's method showed that epi-progoitrin thioglucoside was enzymatically converted to thioxazolidine as illustrated in Fig. 8. In this experiment the pH of the solution was adjusted to 5.9.

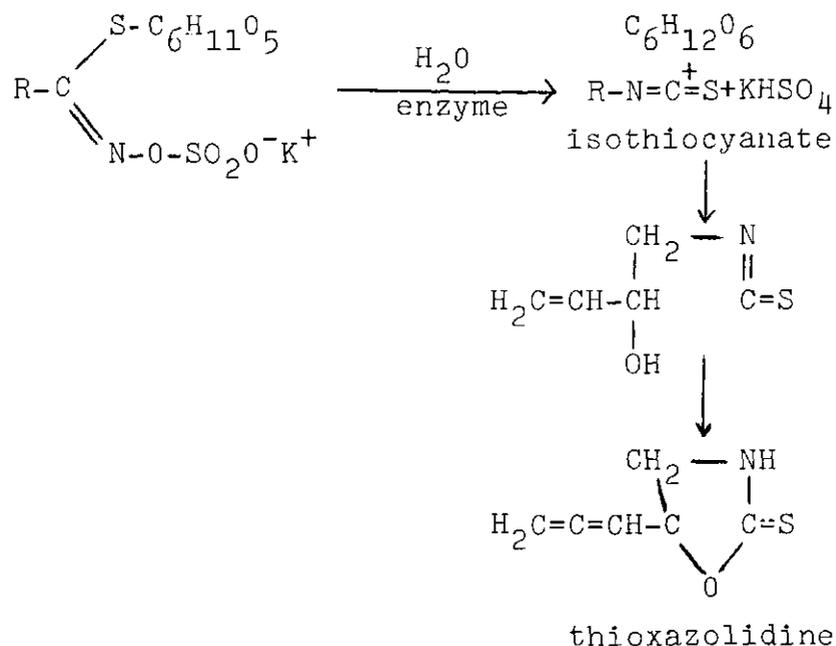


Fig. 8
Formation of Goitrin

Thus in the presence of a thioglucosidase from the enzymatic complex of myrosinase, progoitrin hydrolyzes, setting free 2-hydroxy-3-butenylisothiocyanate which owing to the instability of the hydroxylic group of the secondary alcohol undergoes a rearrangement of the Lossen-type and by ring formation forms goitrin.

The fresh rape (Brassica napus L. - Var. oleifera) leaf and stem tissue was steamed at atmospheric pressure for twenty

minutes to inactivate enzymes and precipitate proteins. The sap was adjusted to pH6.5 and incubated with cell-free myrosinase, then a new compound (Fig. 9) and isothiocyanate were extracted. The new compound was named (-)-5-vinylthiooxalidone (napaleiferin) and its presence also reported in turnip (Brassica campestris L.) (Tupper and MacGibbon, 1967).

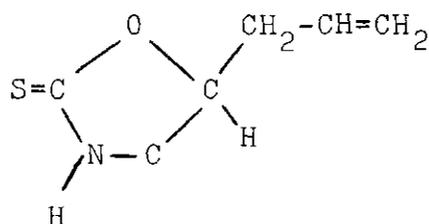


Fig. 9
Formation of Goitrin

The enzymatically liberated oxazolidinethiones and volatile isothiocyanates of the seed meals of thirty-two genera and species of Cruciferae were estimated by Daxenbichler et al. (1964). These authors concluded that: (1) the optimum oxazolidinethione yield was obtained near pH7, (2) thioglucosides that form oxazolidinethione are not as widespread as those that form volatile isothiocyanates, and (3) Brassica napus L. consistently formed more oxazolidinethione than Brassica campestris L.

VanEtten et al. (1966) reported that the thioglucosides of Cruciferae and related plant families also generally yield

isothiocyanates upon enzymatic hydrolysis. Oxazolidinethiones (goitrin) are formed if the thioglucoside contains a hydroxy group properly located in the aglycone to facilitate ring closure. Occasionally, nitrile(s) plus sulfur or thiocyanate are formed instead of the isothiocyanate. These authors (Van-Etten et al. (1966) also mentioned that the variation in the kind and amount of aglycone products (other than H_2SO_4) was derived from enzymatic hydrolysis of the thioglucosides when seed meals of Crambe and rapeseed (Brassica napus L.) are autolyzed under various conditions. For example, 1) both thioglucosides and myrosinase were easily destroyed by autoclaving in contact with steam; 2) (R) goitrin formation increased by raising the temperature to $68^{\circ}C$. with a concomitant decrease of the cyano-containing products, increase in pH from 6 to 11 caused an increase in (R) goitrin and a decrease in the formation of cyano-containing products.

The possible enzymatic formation of nitrile(s) is not to be confused with a two-enzyme system, reported for myrosinase in which thioglucosidase and sulfatase are separated. However, the pathway of enzymatic breakdown of epi-progoitrin or goitrin is not apparent.

Furthermore, the occurrence in nature of glucosides yielding R-hydroxy substituted isothiocyanate (Fig.10) (Greer, 1962) upon enzymic hydrolysis became evident when Greer in 1956 isolated the crystalline sodium salt of a glucoside, progoitrin, from rutabaga seeds and demonstrated it to be the progenitor of (-) -5-vinyl-2-oxazolidinethione (goitrin) (III) formed upon

spontaneous intramolecular cyclization of the initially liberated 2-hydroxy-3-butenylisothiocyanate (II).

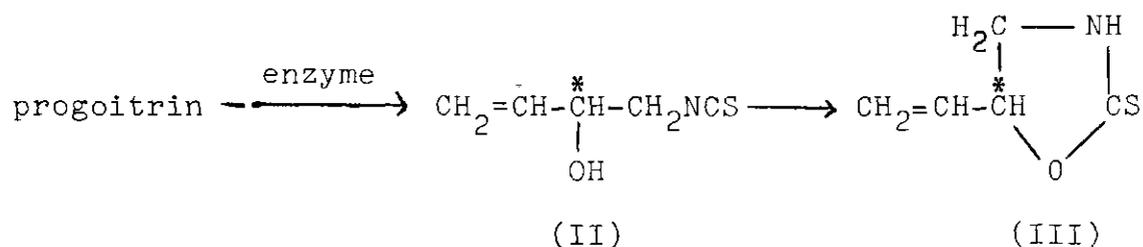
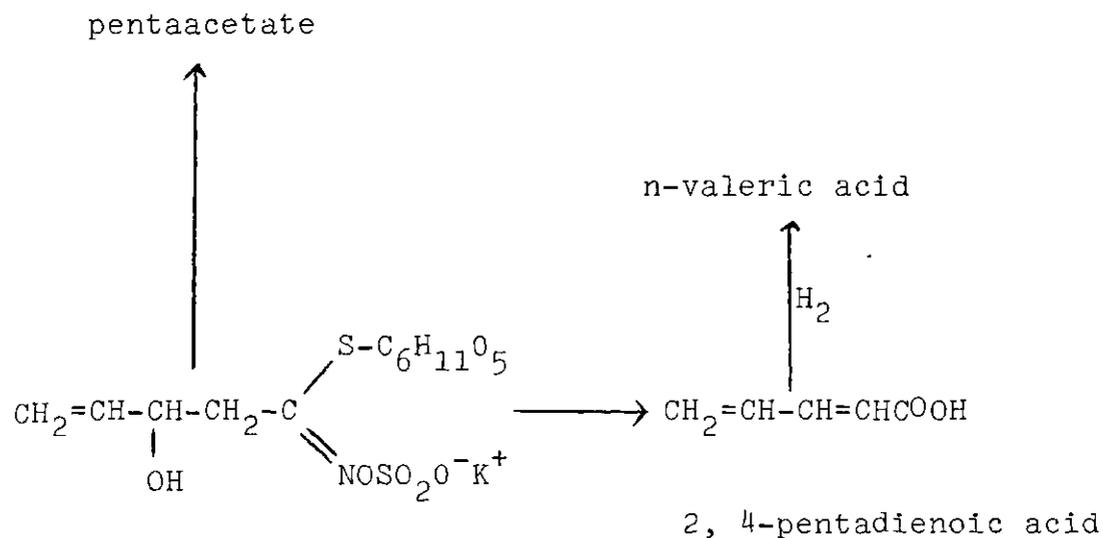


Fig. 10
Hydrolysis of glucosinolate

Ettlinger and Kjaer (1968) demonstrated that oxazolidine-2-thiones were formed by cyclization of β -hydroxyisothiocyanate.

Hydrolytic breakdown of thioglucosides takes place spontaneously by the action of native enzymes when the seed meal is merely brought into contact with water. The pathway is remarkably complex (Daxenbichler et al. 1966a;1966b; 1965 and VanEtten et al. 1966). Samples of Nugget (Brassica napus L.) and Echo (Brassica campestris L.) rapeseed meals, analyzed according to the method of Youngs and Wetter for three major glucosinolates: gluconapin, glucobrassicinapin, and progoitrin upon enzymic hydrolysis produced 3-butenylisothiocyanate, 4-pentenylisothiocyanate and (S)-5-vinyl-oxazolidine-2-thione respectively (Kondra and Downey, 1970

and Youngs and Wetter, 1967). Daxenbichler et al. (1965) isolated a thioglucoside from Crambe seed and identified it as (R)-2-hydroxy-3-butenylglucosinolate. This glucosinolate was hydrolyzed by myrosinase and mineral acid. The results were as follows:



(R)-2-hydroxy-3-butenylglucosinolate

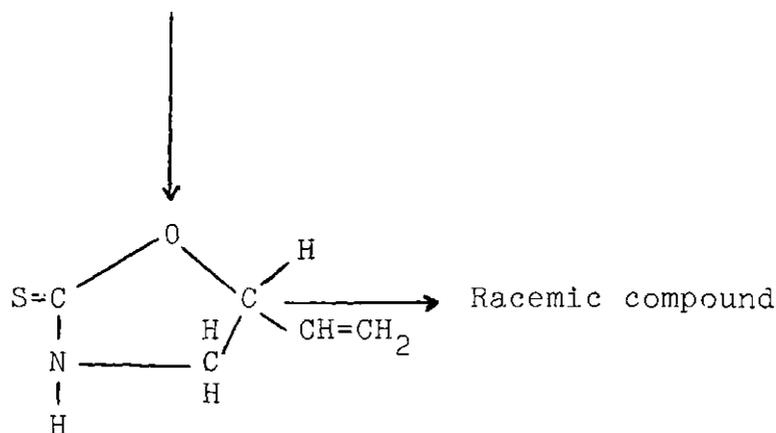


Fig. 11
Hydrolysis of epi-progoitrin

It has been reported (Ettlinger and Kjaer, 1968) that the aglycones produced by enzymic hydrolysis of glucosinolate in neutral solution decompose spontaneously to isothiocyanates and sulfate with half-lives of about a minute near room temperature; the reaction sequence can be diverted to other organic products (Fig. 12). In acidic medium, or very likely, often by the action of certain catalysts, the aglycones give nitriles which are not easily olfactorily detected (Ettlinger and Kjaer, 1968).

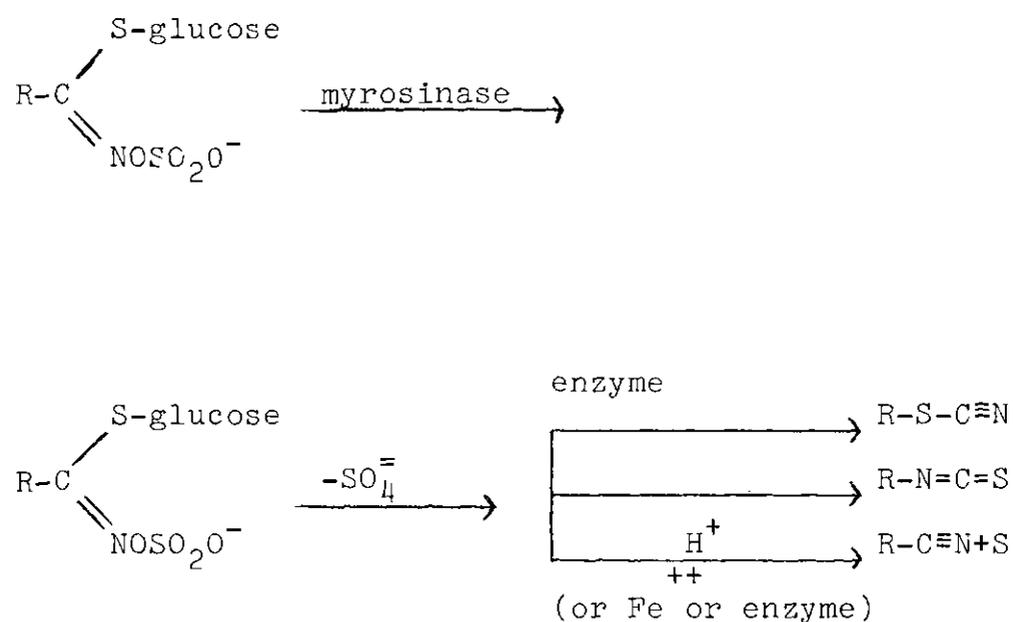


Fig. 12
Enzymatic and non-enzymatic hydrolysis of glucosinolate

VanEtten (1969) described the hydrolysis of progoitrin or epi-progoitrin as follows (Fig. 13).

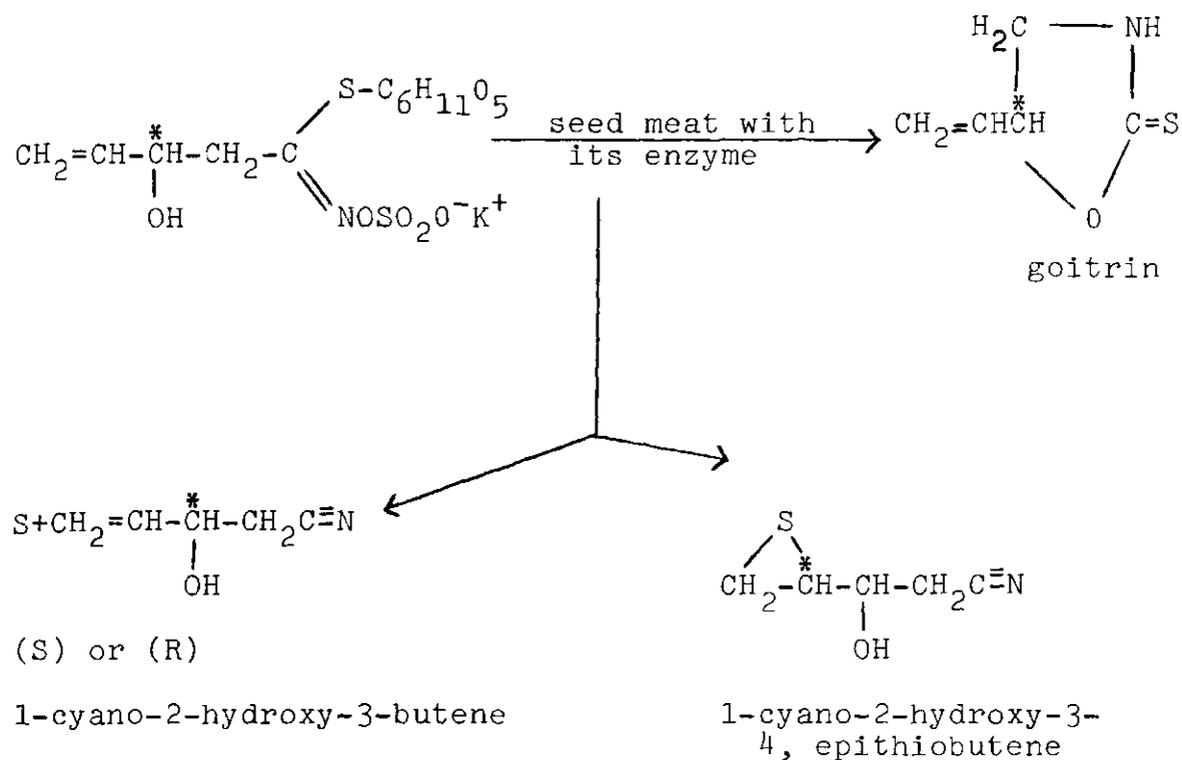


Fig. 13
Hydrolysis of glucosinolates

This author demonstrated that goitrin is formed through cyclization of the postulate of unstable isothiocyanate containing a hydroxyl group.

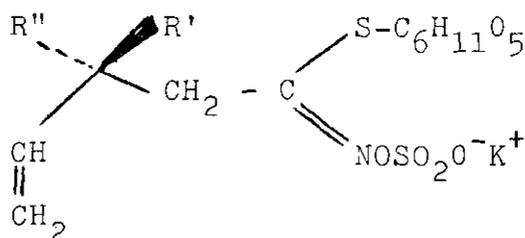
Schwimmer (1960) showed the formation of thiocyanate ion and organic thiocyanates from S-glycosyl-thiohydroxy-sulfate (isothiocyanate glucosides), and pointed out that the fate of thioglucosides can be enzymatically more diverse than that of a simple thioglucosidase action accompanied by Lossen rearrangement with concomitant sulfate displacement and formation of isothiocyanate. This author also showed that both pathway as well as the rate of degradation of thioglucosides are dependent upon the pH of the medium and upon the presence of foreign protein and ascorbic acid. This author indicated that other investigators have shown that nitrile was present in enzyme reaction mixtures containing ascorbic acid at pH3.0 but not at pH5.8, and that adding $FeCl_3$ increased the nitrile formation.

2.3.2 Micro-organism myrosinase

Although it has been assumed for many years that hydrolysis of thioglucosides similar to progoitrin could be accomplished only through the action of myrosinase of plant origin, later studies have indicated that this view is incorrect. Rease et al. (1958) reported that the fungus Aspergillus sydwi produced a B-thioglycosidase (termed "sinigrinase") active on the mustard oil thioglucosides; sinigrin, sinalbin and progoitrin. However, the enzyme could not be obtained from other fungal genera or from the bacteria, Bacillus subtilis or Pseudomonas aeruginosa, which also were found to consume sinigrin on prolonged (14 days) incubation with this substance (thioglucosides).

Thioglucosidase activity hydrolyzing several purinyl thioglucosides has been reported by Goodman et al. (1959) to be widespread in mammalian species, and occur in the micro-organisms Tetralymena pyriformis L. and Escherichia coli. Goitrin has been found in blood and urine after oral ingestion by man of pure crystalline progoitrin. These results suggested that the conversion of progoitrin to goitrin might have been carried out by thioglucosidase activity of bacteria in the gastro-intestinal tract. This hypothesis was supported by Greer, in 1962, who incubated the feces of man or rats with progoitrin and found the formation of goitrin. In 1965, Oginsky et al. (1965) after an extensive study, concluded that myrosinase activity was demonstrated in a variety of bacteria, particularly paracolobactrum, which commonly inhabit the intestinal tract of man. Nagashima and Uchiyama (1959) demonstrated that the only animal enzyme found to affect glucosinlates is one of the molluscap sulfatases. In the absence of thioglucoside-hydrolyzing enzymes, progoitrin can be hydrolyzed to give goitrin by micro-organisms commonly found in the non-ruminant intestinal tract (Logothetopoulos and Myant, 1965). Coop and Blakely (1949) noticed that the hydrolysis of free glucoside and glucoside in plant tissue in the rumen is rapid and can be complete in as a short time as 10-15 minutes. Hydrolysis in the rumen does not necessitate the presence of plant enzyme since the ruminal bacteria can hydrolyze cyanogenetic glucosides very rapidly.

in which the native enzyme had been inactivated were added. These authors concluded that chemical degradation is comparable to enzymic conversion under equally mild conditions. Youngs and Perlin (1967) reviewed that sinigrin is readily decomposed enzymatically and also chemically in a variety of ways. Enzymatic hydrolysis in nearly neutral solution gives allyliso-thiocyanate whereas in an acid medium allyl cyanide and sulfur are formed in place of the isothiocyanate. Moreover, these authors found that when certain metallic salts, notably ferrous sulfate, were added to Brassica campestris L. rapeseed meal, the thioglucosides present decomposed to yield nitrile (1-cyano-3-butene, 1-cyano-4-pentene, and 1-cyano-2-hydroxy-3-butene) and traces of the thioglucosides remained. Daxenbichler et al. (1967) autolyzed the defatted seed from Brassica napus L. (var. Regina II) and Crambe abyssinica seed meals and showed the degradation pathway of glucosinolates and the thioglucoside products as follows:



1a, progoitrin (R'=H, R''=OH)

(R) at asymmetric center

1b, epi-progoitrin (R'-OH, R''=H)

(S) at asymmetric center

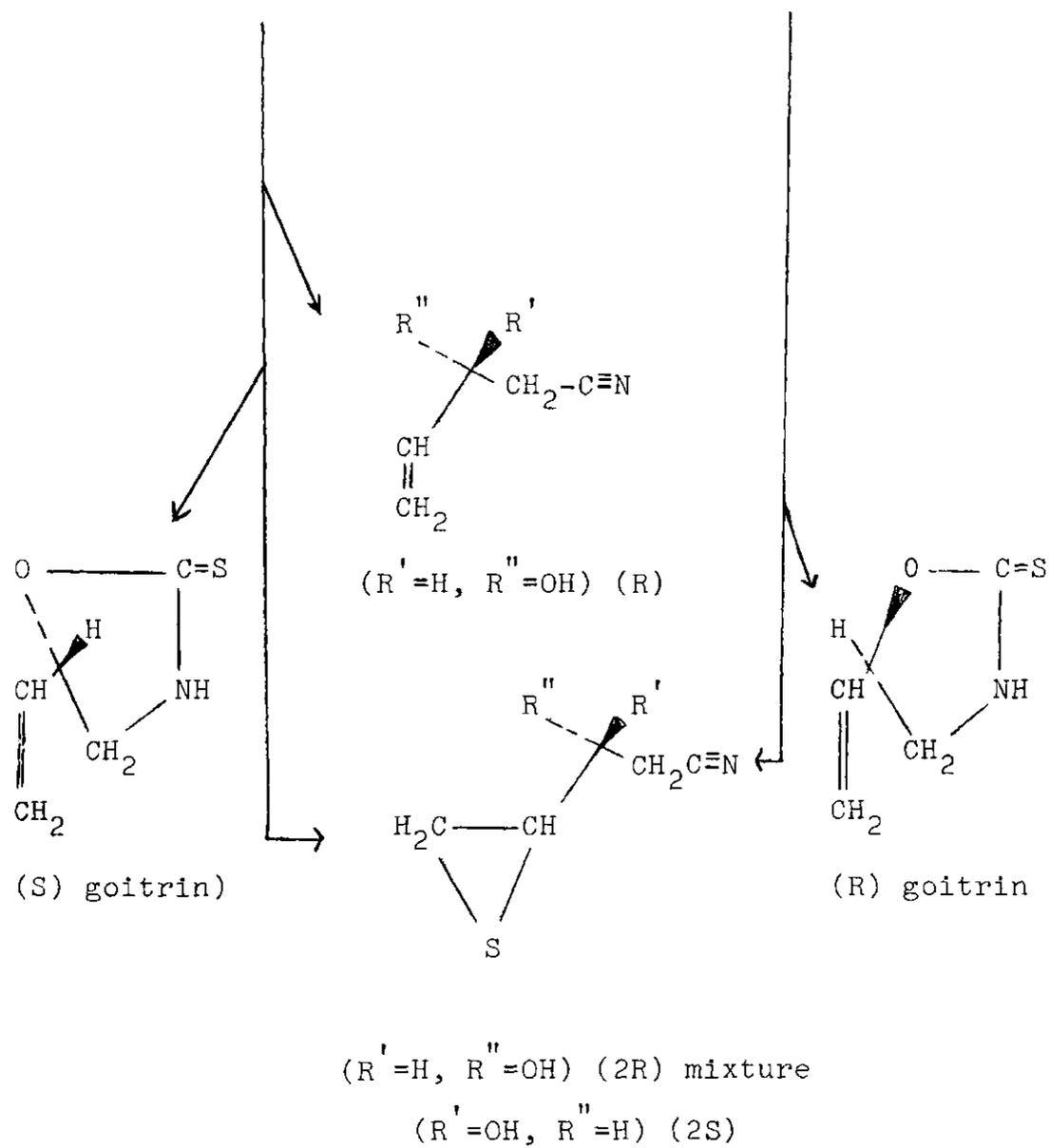
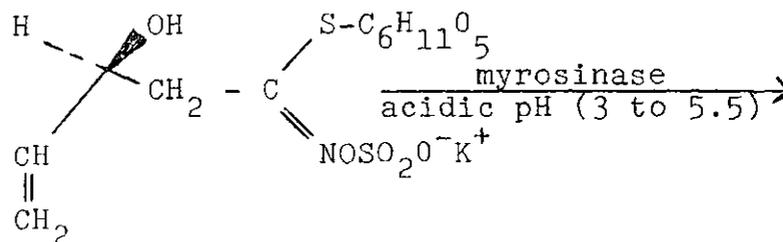
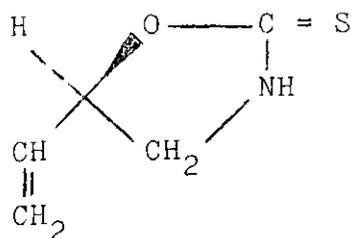


Fig. 15
Degradation pathway of glucosinolates

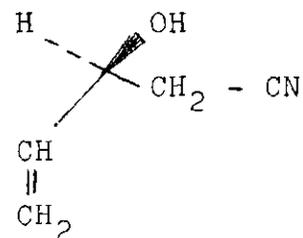
Youngs (1968) for detoxification of rapeseed meals, selected ferrous sulfate from among certain heavy metal ions which catalytically decomposed thioglucosides, because of its non-toxicity and low cost. In a typical run, when the author added hydrated ferrous sulfate to a commercial rapeseed meal and agitated the meal in the presence of moisture and heat, the thioglucoside content decomposed completely. However, the two thioglucosides, gluconapin and glucobrassicinapin, gave volatile nitriles while progoitrin content gave a hydroxynitrile and thionamide that were not volatile and remained in the meal. The alternatives to enzymic degradation of thioglucosides have also been studied by others (Austin and Gent, 1967).

Daxenbichler et al. (1966a) showed that the nitrile formed from epi-goitrin (in Crambe seed) and progoitrin (in rutabaga seed) by myrosinase hydrolysis at pH3 was (S)-1-cyano-2-hydroxy-3-butene(4a) and (R)-1-cyano-2-hydroxy-3-butene(4b) respectively. These authors concluded that enzymatic hydrolysis occurred over a wide range of pH under the conditions studied, favoring (R)-goitrin completely at pH7 and above and a mixture of (R)-goitrin and(S)-1-cyano-2-hydroxy-3-butene at lower pH values. They also pointed out that less than 5 percent mustard myrosinase hydrolysis of purified epi-progoitrin occurred at pH2-3 and pH12 on standing for 24 hours. The results of these are given as follows: (Figs. 16 and 17).

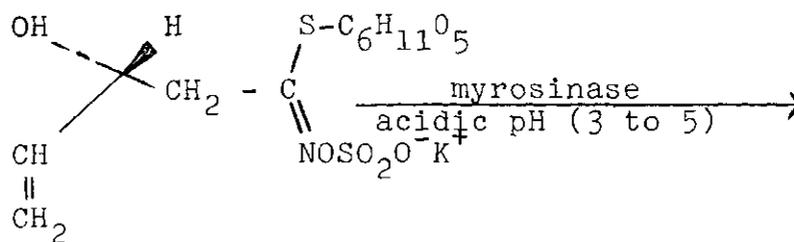
epi-progoitrin

(R) goitrin

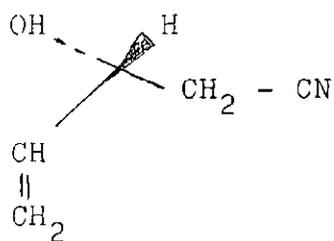
+



(S)-1-cyano-2-OH-3-butene (4a)

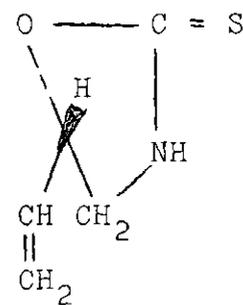


progoitrin



(R)-1-cyano-2-OH-3-butene (4b)

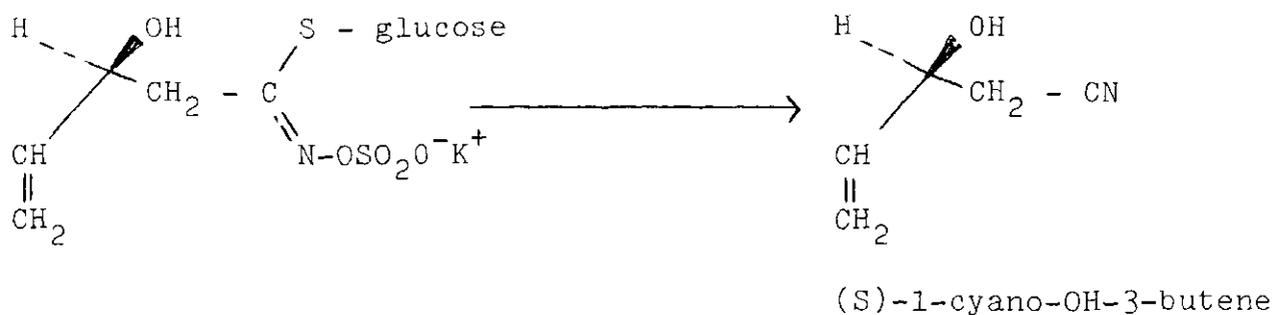
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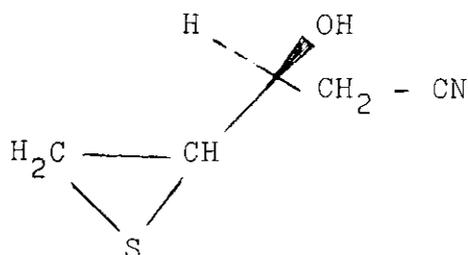
(S) goitrin

Fig. 16

Enzymatic hydrolysis of glucosinolates and formation of nitriles



+



Diastereomeric 1-cyano-2(S)-OH-3, 4-epithiobutene

Fig. 17

Enzymatic hydrolysis of glucosinolates and formation of nitriles

Gmelin and Virtanen (1961) isolated and characterized glucobrassicin (3-indoylmethylglucosinolate), from cabbage leaves. This indoyl-containing glucosinolate was hydrolyzed at acidic pH; nitrile was one of the hydrolyzed products.

Austin *et al.* (1968) performed further studies on the rate of formation of (S)-1-cyano-2-hydroxy-3-butene and (S)-3-hydroxy-pent-4-enethionamide by chemical catalyst (ferrous salt) under different conditions. The author summarized the following results:

- (a) The concentration of reactants influenced the course of reaction in concentrated solutions; the thioglucosides suffered complex degradation and

decomposition.

- (b) Thionamide production reached a maximum value at about 25 hours.
- (c) The reaction of ferrous salts with thioglucosides over a range pH from 1.4 to 5.4 was the same, except for a reduction in thionamide content in the most acidic solution (pH1-4).
- (d) The reaction of thioglucosides with ferrous salt was practically inhibited at 5°C. and at the extreme temperature the maximum amount of thionamide was formed.

These authors also studied the effect of ferrous ion with other glucosinolates. Their findings are reported in the following tables 10 and 11.

They concluded that thionamide production occurred only with those thioglucosides that possess a hydroxyl group in the 2-position of an alkylglucosinolate and this structural feature is the same which permits oxazolidinethione formation on enzymic conversion of thioglucosides. Youngs and Wetter (1967) showed that pH (over range 4.5 to 8.0) did not have any pronounced effect on released aglycones, 3-butenyl, 4-pentenylisothiocyanates and 5-vinyl-2-oxazolidinethione from a Brassica napus L. meal.

Daxenbichler et al. (1966a) isolated and identified the optical isomers of 1-cyano-2-hydroxy-3-butene formed from isolated progoitrin and epi-progoitrin after thioglucosidase

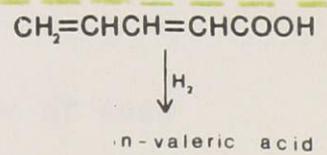
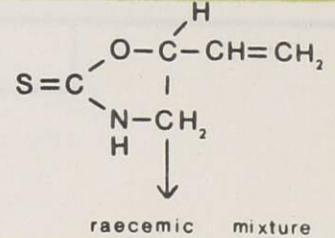
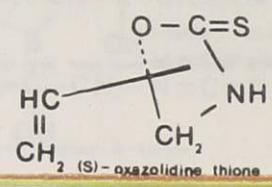
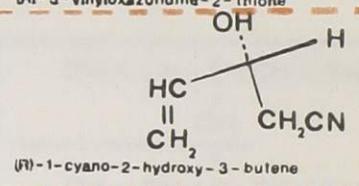
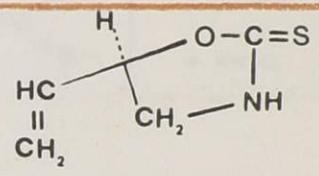
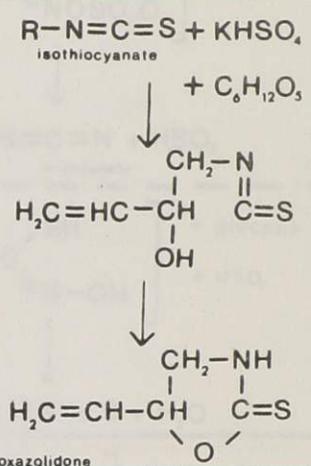
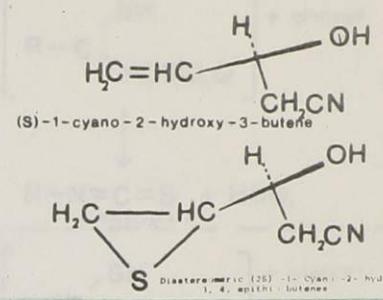
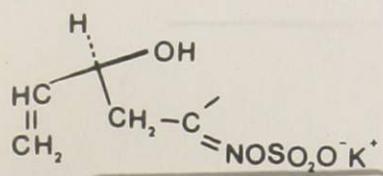
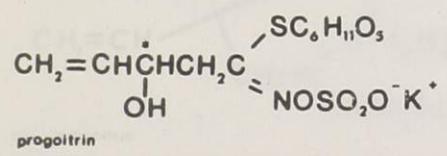
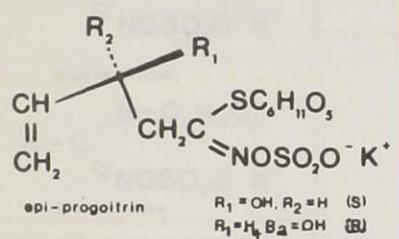
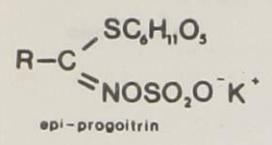
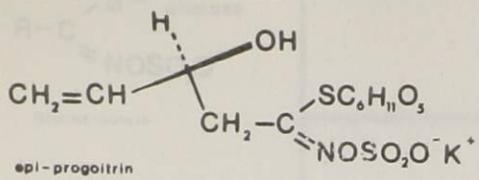
Table 10. Reaction of purified thioglucosides and ferrous ammonium sulfate

Thioglucoside	Products	
	Nitrile	Thionamide
Glucotropaeolin	+	+
Sinigrin	+	-
Epi-progoitrin	+	+

Table 11. Reaction of thioglucosides in seed meals with ferrous ammonium sulfate

Plant species	Major thioglucoside	Products	
		Nitrile	Thionamide
<u>Brassica nigra</u>	sinigrin	+	-
<u>Brassica napus</u>	progoitrin	+	+
<u>Crambe</u>	<u>epi</u> -progoitrin	+	+
<u>Barberae vulgaris</u>	glucobarbarin	+	+

Moreover, formation of isothiocyanate, thiocyanates, goitrin and nitriles depend upon conditions of the hydrolysis and other unidentified variables. (Fig. 19).



in crambe

in rape

neutral pH

acidic pH

neutral pH

enzymic hydrolysis

H⁺

H⁺ acidic pH

enzyme H₂O

acidic pH
45°C at pH 4.5-5.0

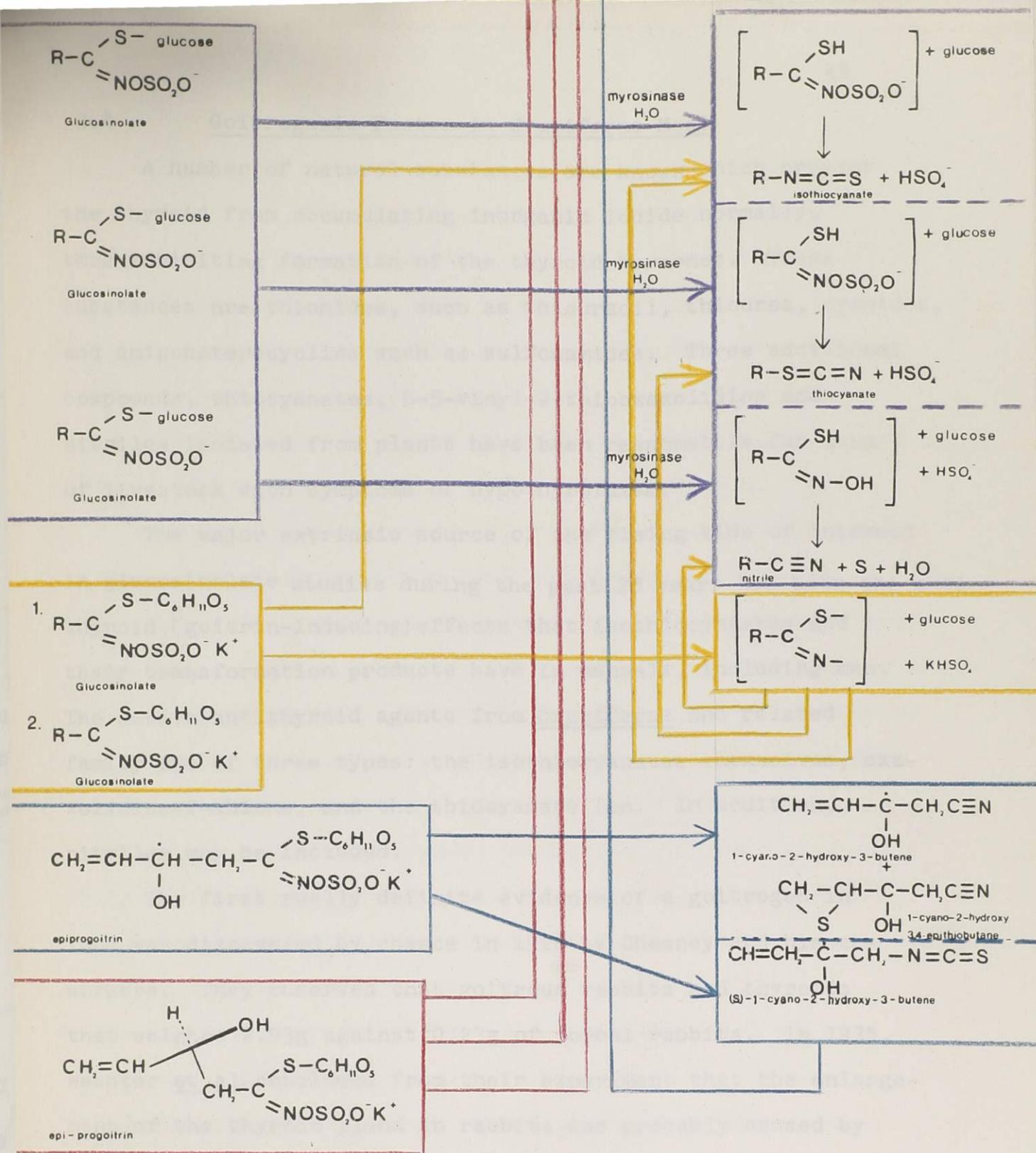


Fig. 19

Pathways of hydrolysis of glucosinolate contents of seed meals of Brassica species

2.4 Goitrogenic Factor in Cruciferae Meal

A number of natural substances are known which prevent the thyroid from accumulating inorganic iodide normally, thus inhibiting formation of the thyroid hormones. These substances are thiomides, such as thiouracil, thiourea, cyanides, and aminoheterocyclics such as sulfonamides. Three additional compounds, thiocyanates, L-5-vinyl-2-thiooxazolidine and nitriles isolated from plants have been responsible for loss of livestock with symptoms of hypothyroidism.

The major extrinsic source of the rising tide of interest in glucosinolate studies during the past 20 years has been the anti-thyroid (goitrin-inducing) effects that isothiocyanates and their transformation products have in mammals, including man. The active antithyroid agents from Cruciferae and related family are of three types: the isothiocyanates themselves, oxazolidine-2-thione, and the thiocyanate ion. In addition, nitriles may be included.

The first really definite evidence of a goitrogen in food was discovered by chance in 1928 by Chesney and his co-workers. They observed that goitrous rabbits had thyroids that weighed 2.93g against 0.23g of normal rabbits. In 1935, Webster et al. concluded from their experiment that the enlargement of the thyroid gland in rabbits was probably caused by feeding cabbage. The year after Chesney's report, Marine and his co-workers (1930) found that Brussel sprouts had the same effect as cabbage. Marine also discovered that the goitrogenic activity of the cabbage could be destroyed by drying it,

so he suggested that, the goitrogenic principle might be a glucoside. In 1936, Hercus and Purves fed cabbage seed, rapeseed, and steamed black or white mustard seed and found them to be goitrogenic. For this reason, the generic term, Brassica seed-goiter has become a common term. Greer (1950) quoted that other investigators (Spence, Wegelin, Herais, and Aitkend) were not able to detect a pronounced goitrogenic effect in rabbits fed Swiss or New Zealand cabbage.

Kennedy and Purves (1941) found that seeds of rutabaga (Swede), rape, soft turnip, hard turnip and chou moellier were goitrogenic when fed to young rats. These authors found that rats receiving a rapeseed diet for 19 days and injected with 1.3mg potassium iodide had thyroids averaging 22.5mg/100g body weight, as compared with 38.3mg/100g of body weight for the non-injected rats fed a rapeseed diet. The authors concluded that iodine has a moderate inhibitory effect on the size of the Brassica seed goiters. Greer and Astwood (1948) tested a total of sixty-one foods for antithyroid effect. They pointed out that the most of the positive goitrogenic effects were found in Cruciferae such as rutabaga, broccoli, cabbage, cauliflower, mustard, radish and turnip. These authors were unable to show marked goitrogenic effects when the Cruciferae was cooked.

Iino et al. (1961) administered graded doses of propylthiouracil (PTU) subcutaneously once daily to rats for three days. I¹³¹ was given immediately following the last dose of PTU and twenty-four hours later the thyroids were removed and studied.

The results indicated that relative amounts of DIT, and T_3+T_4 and thyroid I^{131} uptake decreased with increasing doses of PTU (Fig. 20).

Maloof and Soodak (1961) quoted that the antithyroid effect of 2-thiouracil may be due to its interference with nucleic acid metabolism.

Because of the role played by the lack of iodine in the appearance of endemic goiter, frequently encountered in mountainous regions poor in this element, the effect of iodine cannot be questioned. Several factors have, however, shown that iodine deficiency is not the only cause of this disorder. For example, regions are known in which, although the available amount of iodine is small, goiter does not occur, and goiter can be observed in areas where no deficiency of iodine exists. Moreover, research has shown that other generating factors exist which are capable of interfering with the utilization of iodine during the biosynthesis of thyroid hormone or which increased considerably the demands of the organism for this element.

Rapeseed meal, available during World War II as a feed concentrate however, produced goiter in animals if fed in more than small amounts. A goitrogenic substance, L-5-vinyl-2-thioxazolidine has been isolated from rapeseed (Astwood et al. 1949 and Carrol 1949). Rapeseed has also been found to contain volatile isothiocyanates which might prove troublesome if ingested in high concentration.

Bell (1955) reported that the use of rapeseed meal in the ration of swine resulted in decreased growth rates and enlarge-

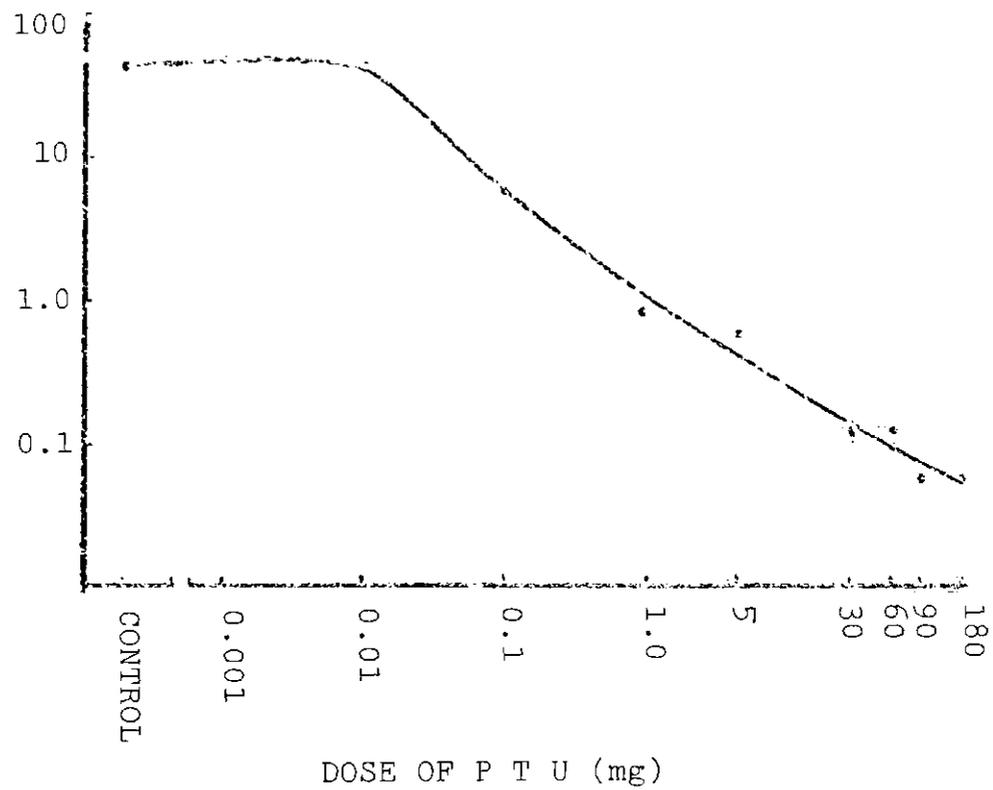


Fig. 20 Effect of graded doses of PTU on thyroidal I^{131} uptake. Log-log plot. Each point represents mean of 3-4 animals.

ment of the liver, kidney and thyroid.

It has been reported (Collins and March, 1967) that three commercial rapeseed meals were fed at the 15 percent level to chicks; all produced marked enlargement of the thyroid gland. Kirk et al. (1966) fed chicks prepressed-solvent extracted and untreated Crambe meal and found that chicks consuming untreated meal developed thyroids, grew poorly and suffered high mortality.

2.4.1 Oxazolidinethiones

Both (R)- and (S)-goitrin are potent antithyroid agents. The thyroid-inhibiting effect of goitrin was first determined by measuring the uptake of radio-active iodine, I^{131} , by the thyroid after feeding the compounds to animals (Astwood et al., 1949). Carrol (1949) fed goitrin to rats and found this compound caused hyperplasia of the thyroid slightly less than that due to thiourea (a thyroid-inhibiting drug). Ettlinger (1956) synthesized the optically active (R) and (S) forms and racemic goitrin, and Greer (1962) found both forms to be equally potent as antithyroid agents. Greer (1962) concluded that the compound possessed 2 percent and 133 percent of the activity of the antithyroid drug (propylthiuracil) when tested with rats and humans, respectively.

Clandinin et al. (1966) fed racemic goitrin to chicks as 0.15 percent of the ration and it caused depression of the growth rate, hyperplasia and hypertrophy of the thyroid glands. Radioiodine uptake of the thyroid and release of iodine by the gland into the blood stream were also depressed during feeding of goitrin, but after longer feeding of the compound the functions

of the gland were similar to those of the thyroid in the controls. The authors (Clandinin et al. 1966) concluded that chicks fed the goitrogen eventually reached physiological equilibrium with increased ratio of thyroid to body weight.

VanEtten (1969) reported that the 5,5-dimethyloxazolidine-2-thione isolated from hare's ear mustard tested by Astwood et al. in 1945 had about the same antithyroid activity as goitrin. Szewczuk et al. (1968) stated that 5-vinyloxazolidine-2-thione is goitrogenic. Faiman et al. (1967) assessed the antithyroid activity of rats with orally and parenterally administered propylthiouracil and goitrin (D,L-5-vinyl-2-oxazolidinethione). They found (1) a dose-response relationship for both propylthiouracil and goitrin, with increasing doses resulting in a progressive increase in thyroid weight and a diminution of radioactive iodine-uptake, with a long-linear response for propylthiouracil (0.0003 to 0.0009 percent of diet) and for goitrin (0.06 to 0.18 percent of diet). (2) propyl thiouracil was 150 times as potent as goitrin after parenteral administration. These authors concluded that the discrepancy in potency between oral and parenteral administration of these compounds may be related to differences in gastrointestinal absorption or hepatic metabolism.

When Royan et al. (1968) fed rats 0.12 percent goitrin (dl-5-vinyloxazolidinethione) and 0.0006 percent propylthiouracil, the results indicated that (1) goitrin feeding produced significant enlargement of the thyroid, suppression of I^{131} intake by the thyroid and depression of serum PBI and (2) propylthiouracil feeding gave results similar as goitrin.

The goitrogenic effects of goitrin, methiomazol and methiocil on growing chicks were investigated by Matsumoto and Akiba (1969) by using radioiodine. They fed 0.05 percent (-)-5-vinyl-2-oxazolidinethione, 0.05 percent 1-methyl-2-mercaptoimidazole (methiomazol) and 0.05 6-methylthio-uracil (methiocil) to one-day old chicks and 10 day old chicks and found that (a) goitrin caused thyroid enlargement high thyroidal uptake of I^{131} and a slow release of I^{131} . (b) methimazol exhibited severe thyroid enlargement, high I^{131} uptake and rapid release of I^{131} . They concluded that goitrin displayed a different goitrogenic effect on iodine metabolism of growing chicks than either methiomazol or methiocil.

Willis (1966) reported that the epithelial hyperplasia within the thyroid gland that underlies the development of goiter has been found to be dependent on an increased secretion of thyrotropin by the pituitary gland, because the thyroid atrophied invariably following hypophysectomy even though the goitrogenic influence was not removed. Thus, one mode of action of L-5-vinyl-2-thioxazolidine might be that goitrin stimulates secretion of thyrotrophin by the pituitary.

Gontzea and Sutzescu (1969) reported that progoitrin was quickly hydrolyzed when it was added to feces of rats and humans. This compound was not decomposed when added to boiled feces and incubated. Furthermore, it has been reported that (Gmelin and Virtanin, 1961 and Greer, 1956) progoitrin has been very successfully used for the treatment of hyperthyroidism because of its slow absorption and prolonged

thyroidostatic effect.

VanEtter et al. (1969) performed a feeding experiment with rats and from their results showed that 0.23 percent of (R)-goitrin in diets decreased growth to 85 percent of that of a control group. A mild hyperplastic goiter and a mild degenerative non specific alteration of liver cells were the only microscopic changes detected. In addition, similar growth restriction resulted when comparable levels of epi-progoitrin (0.5 and 1.5 percent) were included in the diet either as isolated or present in the inactivated meal. Peltola (1965) demonstrated that the weight of the thyroids of rats increased significantly with increased doses of goitrin fed as 0.0,0.1,0.5,1.0 and 2.0mg per day over a long period. In New Zealand, turnips found to contain goitrogenic substance, L-5-vinyl-2-thioxazolidine (Astwood et al. 1949) and demonstrated that this compound was the cause of congenital goiter in lambs (Hercur and Purves, 1936).

Willis (1966) added that the results of feeding rats a diet contained Brassica seed suggested that L-5-vinyl-2-thioxazolidine does prevent the binding of iodine by the thyroid gland. Kingsbury (1964) reported that L-5-vinyl-2-thioxazolidine is a goitrogenic factor. Gontzea and Sutzescu (1969) carrying out an investigation with twenty-three human male volunteers, and with rats, established that when radiiodine uptake by the thyroid is prolonged to 24 hours, the antithyroid effect of heat-treated goitrogenic materials and crystalline progoitrin (free of the enzyme) is as potent as that of goitrin.

They explained that thioglucoside (progoitrin) may be hydrolyzed inside the body, a fact which has been confirmed by detecting goitrin both in blood and urine. These authors gave 10 millimol.(1.29g) of goitrin by mouth and found it reached its maximum concentration in the blood within about 30 minutes, whereas after the equivalent quantity of 10 millimol. (4g) of progoitrin, the highest level in blood was reached only after 35 hours. They concluded that this delay is due to the fact that the thioglucoside is decomposed by enzymes cell free by the bacteria in the colon.

2.4.2 Thiocyanates

As thiocyanate has a similar ionic radius to iodine it can substitute or displace this element, preventing its accumulation in the thyroid as well as its uptake in an acute experiment. (Miller, 1966).

Barker in 1936, noticed that some of the patients fed inorganic thiocyanate to reduce hypertension, developed enlarged thyroids, so he concluded that thiocyanate acts as a goitrogen. In 1947, Vanderlaan and Vanderlaan demonstrated that thiocyanate ion inhibited uptake of iodine by the thyroid. Maloof and Soodak (1959) by intraperitoneally administering thiocyanate and thiocarbamide-type antithyroid compound, found that (1) there is no concentration gradient for thiocyanate ion between the thyroid and serum, (2) thiocyanate ion was metabolized by the thyroid and the major product was sulfur. In addition, thiocyanate metabolism was completely inhibited by thiourea, sodium iodide, and sulfadiazine, but

was only slightly inhibited by sodium perchlorate. Those authors mentioned that it has been demonstrated that the inhibitory action of iodine on I^{131} binding can be reversed by simultaneous administration of thiocyanate.

When adult hamsters were given S^{35} -thiocyanate by intramuscular injection and their thyroid glands were studied by autoradiography (Logothetopoulos and Myant, 1965), the results showed thiocyanate concentration in the follicles of the thyroid as unbound, some of them undergoing chemical changes in the thyroid, probably by oxidation to unbound sulfate. These authors also pointed out that the pattern of blocking S^{35} -thiocyanate was similar to that found in propylthiouracil. They also suggested the inhibitory action of the thiocyanate ion on the concentration of iodine by the thyroid as being competition between the ions for the same transport mechanism.

Oxazolidinethione and thiocyanate caused hyperplastic enlargement of the thyroid and symptoms of hypothyroidism. Most livestock lost had been lambs from ewes which were fed a plant or plant products containing a goitrogenic factor. The ewes themselves showed few signs of hypothyroidism, although greater or lesser enlargement of the thyroid was observed on post-mortem examination. A large fraction of the lambs were born dead, others were listless, seemingly disinterested in nursing and poorly developed, with enlarged palpable thyroid glands, distinctly obvious upon post-mortem examination. Other investigators (Wiels, 1966)

reported that thiocyanate in cabbage may be an important factor in determining the goitrogenicity of cabbage.

2.4.3 Isothiocyanates

Bell (1955) reported that, in 1920, allylisothiocyanate in rapeseed and crotonyl in mustard seed were detected. Although the relative toxicities of these substances with rabbits were also established, they were not associated with thyroid dysfunction.

The mustard oils (isothiocyanates) are secondary products in the sense that they are not appreciably present in intact plant material but are formed after released either by enzymatic or chemical hydrolysis of glucosidic precursors, as reviewed previously. Three of the mustard oils (allyl-, 3-butenyl- and 4-pentenylisothiocyanates) may be important to animal health, including that of the human, because they yield antithyroid properties.

It has been reported (Ettlinger and Thompson, 1962) that in Brassica campestris, allylisothiocyanate is found only in trace amounts while compounds with a larger carbon chain, 3-butenylisothiocyanate and goitrin are more dominant in the seed. Brassica napus as rapeseed, furnishes, like Brassica campestris, 3-butenylisothiocyanate and goitrin but is accompanied as well by a higher concentration of 4-pentenylisothiocyanate. In general, Brassica napus resembles Brassica campestris.

Liener (1970) stated that certain cruciferous oil seeds, such as rapeseed and mustard seed, are known to contain thio-glycosides that, upon enzymatic hydrolysis, release goitrogenic

substances, such as allylisothiocyanate and L-5-vinyl-2-oxazolidinethione. Lo (1969) fed one steamed yellow sarson meal in 4 different feeding levels which contained 1.32, 2.64, 5.28 and 7.92mg/g diet of 3-butenyl-glucosinolate to mice. The results indicated that the mice receiving 1.32 or 2.64mg/g of 3-butenyl-glucosinolate did not show any adverse effects. However, the mice fed 7.92mg/g of this isothiocyanate gained only 35 percent of the control. This author also demonstrated that these yellow sarson meals did not produce enlargement of the thyroid gland. Langer and Stolc (1965) administered 2 or 4mg doses of allylisothiocyanate to rats by stomach tube, and injected 0.5mg of I^{131} one hour after administering allylisothiocyanate and found that 2 or 4mg of allylisothiocyanate significantly depressed the radioiodine uptake by the thyroid gland of the rat. These authors also fed this compound to rats and obtained the similar results. Furthermore, they concluded that cabbage contains other antithyroid agents such as thiocyanide and goitrin in addition to mustard oils.

2.4.4 Nitrile (cyanogenetic glucosides)

In early experiments with processed rapeseed meals fed to either livestock or laboratory animals as reviewed by Bell (1955), few attempts were made to relate biological response to meal constituents derived from glucosinolate.

Since 1955, many investigators (VanEtten et al. 1969 and 1965, Youngs 1968, Korsrud and Bell 1967, Bell 1965, 1957 and 1955b, Bell and Baker, 1957, Bell and Williams, 1953, Tookey et al. 1965, Nakya 1964, Bowland et al. 1963, Nakya and Nakamura 1963) reported evidence for the presence of unidentified toxic

substances other than known goitrogens in seed from Cruciferae.

It was reported by Graicosa, in 1883, that acetonitrile ($\text{CH}_3\text{-CN}$) or methylcyanide was converted into acetic acid and ammonia in the dog. However, Lang in 1894, concluded that acetonitrile was converted into formate and cyanide. Pozzani et al (1959), found that a median lethal dose (LD50) of acetonitrile was (1) 5ml/kg when injected subcutaneously into rats, (2) 3.8g/kg if given by gastric intubation to male rats, or (3) 5ml/kg by cutaneous application to rabbits. Pozzani et al used various assays to study males and females over a five-year interval and found that LD50 of acetonitrile ranged from 1.7 to 8.5ml or g/kg by gastric intubation. These authors also mentioned that the LD50 undiluted acetonitrile was 0.71ml/kg when injected into the portal vein and 1.68ml/kg when injected via the tail vein. Amdur (1959) after a post-mortem examination of the cerebral, thyroid, liver and renal system of a man exposed accidentally to acetonitrile concluded that organic cyanide might be the responsible agent. This author also made the following comments:

1. The usual detoxication (nitrile) process becomes more orderly if cyanide is made available at a slower rate.
2. This detoxication is enzyme-dependent, employs rhodanase (thiosulfate sulfurtransferase (2.8.1.1) or cyanide sulfurtransferase, which is in mitochondria and microsomes, acts by means of a disulphide group in its molecule, which probably acts as carrier of (S) from combination with sulfate to cyanide (Dixon and Webb, 1967),

which is present in all tissues except in blood and muscle, to convert the cyanide into a relatively "harmless" thiocyanate ion.

3. Thiosulfate and colloidal sulfur function as well as substrates for this enzyme, but other substances such as the sulfur-bearing amino acids as cystine, cysteine and glutathione, may also serve but less effectively.

Magos (1962) studied the toxicity effect of acrylonitrile ($\text{CH}_2=\text{CHCN}$), and found that the lethal dose (LD50) of this compound was 1.3 millimole/kg or 95.8mg/kg. He emphasized that the toxicity of acrylonitrile cannot be due solely to cyanide liberation.

Greer (1950) reported that prior investigation showed that nitriles caused thyroid enlargement in rabbits given daily doses of the compound and added that nitriles formed from the thioglucosides may act as goitrogens. However, he also noted that latter workers were not always able to confirm the reported goitrogenic properties of organic nitriles.

VanEtten (1969) reported that Jisourek and his co-workers isolated an organic polysulfide from cabbage and other Brassica, which when fed to rats caused histological changes in the thyroid like those caused by feeding cabbages. Nowry, 1948 mentioned that nitriles are toxic because of the toxic nature of related unsaturated nitriles and cyanohydrin.

Tookey et al. (1965) presented a method which led to the hydrolysis of Crambe seed meal containing (+)-1-cyano-2-hydroxy-

3-butene (and other C≡N) compounds. These workers fed rats the treated Crambe seed meal at a level of 28 percent (corresponding with a 1.4 percent nitrile in diet) and found the rats which received this diet did not survive for two weeks. Furthermore, these results were in complete agreement with the results obtained when rats were fed the ration containing 28 percent of untreated Crambe seed meal. These authors concluded that meal containing hydrolysis products derived from thioglucosides are at least as toxic as untreated meals. VanEtten *et al.* (1959) fed rats an isolated nitrile mixture as 0.1 percent and 0.2 percent of ration, approximating the amount in a ration that contained 10 percent of autolyzed meal and 40 percent of the rats receiving 0.1 percent nitrile died after 84 days, but those fed 0.2 percent died after 14 days. These authors pointed out that the nitrile mixture, either isolated or unisolated, was more harmful than (R)-goitrin or epi-goitrin in isolated or unisolated form. When Lo (1969) fed four synthetic levels (0.8, 1.6, 3.2 and 6.4mg/g of diet) of 2-hydroxy-3-butenyl-nitrile to mice, the animals fed 0.8mg/g diet had a 25 percent reduction in weight gain relative to control mice while the level of 6.4mg/g diet caused the death of animals. The relative actual toxicities of nitriles and (R)-goitrin with mice viewed (VanEtten *et al.* 1969) and reported (data are shown in table 12); these data indicate that the nitriles either separately or as a mixture are about eight times as toxic as (R)-goitrin.

From these reviews might be concluded that the action of glucosinolate products are distinguishable. Thiooxazolidine act similarly to thiourea and thiouracil.

Table 12. Toxicity of Nitrile and (R)-goitrin

Compound	Estimated LD50*
(S)-1-cyano-2-hydroxy-3-butene	170
(2S)-1-cyano-2-hydroxy-3, 4- <u>epi</u> -thiobutene A	178
(2S)-1-cyano-2-hydroxy-3, 4- <u>epi</u> -thiobutene B	240
Mixture of nitrile containing compound from Crambe meal	150
(<u>R</u>)-5-vinyloxazolidine-2-thione	1260-1415

* mg/kg of body weight

2.5 Biochemical Action of Thyroid Hormones

Thyroid function has been estimated by numerous methods such as basal metabolic rate, thyroid weight, histological appearance of the tissue for which biochemical correlations are often lacking, protein-bound iodine (PBI) levels, thyroidectomy followed by thyroxine or triiodothyronine replacement, blocking or preventing synthesis of thyroid hormone by propylthioracil (PTU) or goitrogenic substances.

It has been known for some time that some hormones may cause the multiplication of mitochondria, which play an important role in catalysis of aerobic oxidation in the Krebs' tricarboxylic acid cycle, as well as in the energy transfer system.

Any hormonal action resulting in mitochondriogenesis is of importance to the cell's ability to fulfil its intended function. This is so because thyroxine is generally mitochondriogenic (Nalbandov, 1964). Furthermore, like most hormones, thyroid hormones elicit multiple biological actions (Tata, 1965). The best known of these are:

1. Calorigenic action or regulation of basal metabolic rate (BMR)
2. Regulation of total body growth essential for development of the brain and bone and some other organs (table 13)
3. Anabolic effect with respect to protein, lipid and carbohydrate metabolism
4. Effect on water and ion distribution
5. Reproduction-maintenance of fertility in mammals.

Table 13. Comparison of Control and Hypothyroid tissues of rat *

Tissue	Control	Hypothyroid	% Control
Brain weight	1450	1240	85
Lung weight	445	275	62
Kidney weight	712	353	50
Heart weight	275	120	44
Liver weight	2940	1150	39
Muscle weight	318	124	39
Spleen weight	252	68	27

* This table reproduced from Brasel and Wink (1970)

2.5.1 Thyroid hormones and energy

In hypothyroidism, a generalized hypotonic and sluggishness is a characteristic finding. Both hypothyroidism and hyperthyroidism are characterized by disturbances in muscle tissue itself (table 13). Since the muscle mass constitutes about 45-58 percent of body weight, and since muscle has the widest range of oxygen requirement of any tissue, muscular activity must contribute very substantially to the elevated or lowered basal metabolic rate (Peltola, 1965).

The thyroid has a specific effect on maintenance of energy production through secretion of its hormone(s). Surgical removal of the thyroid causes a marked lowering of the basal metabolic rate. Subjects with hypothyroidism exhibit a subnormal basal metabolism and the converse is found in cases of hyperthyroidism (McHenry, 1963), Tata (1965) summarized the long-term effects of thyroidectomy and injection of near-physiological and toxic amounts of L-thyronine (T₃) on BMR, growth rate and mitochondrial oxidative phosphorylation in the rat (table 14).

Belzile (1963) reported that feeding myrosinase-containing rapeseed oil meal at a potentially toxic isothiocyanate and oxazolidinethione level, reduced the rate of basal metabolism of the mouse.

Ganong (1969) explained that the excess of thyroxine and its analogues decreases the efficiency of energy transduction by uncoupling of phosphorylation from oxidation in the mitochondria. Therefore, less of the produced energy by catabolism

Table 14. Effects of thyroxine on BMR and growth of rat

Status of rat	Dose of T3 Mg/100g.	(% of normal)	Change in BMR Growth (% change for 5 weeks)	P/O
Normal	-	-	+49	2.76
Thyroidectomized	-	-22	+36	2.79
Hyperthyroid	20	+41	+71	2.69
Thyrotoxic	1,700	+79	-24	0.73

stores in energy-rich phosphate compounds and more appears as heat. This author added that apparently this action is accompanied by swelling of the mitochondria as a result of the mitochondria imbibing water when treated with thyroxine, Ca^{++} or hypotonic media or simply as a result of the mitochondria aging. The overall result is lowering of the P:O ratio. More recently, Tata (1965) has also suggested, as a result of studies on the swelling of mitochondria, that modification of the mitochondrial membrane permeability by thyroid hormones may explain their effect on oxidative phosphorylation. Therefore, thyroxine alters the permeability of mitochondrial membrane and mitochondrial function is regulated by the altered rate of entry and exit of substances involved in respiratory activity and energy transfer. The calorogenic effect of thyroxine has been fully reviewed by Werner and Nauman (1968); they stated that until recently the oxidative-process concept was thought to represent the primary locus of action of thyroid hormones. Oxidative stimulation of so many substances however, suggested involvement of the electron transport as a common denominator. In addition, intercellular cytochrome and cytochrome oxidase concentrations respond to changes in level of thyroid hormone. These authors reported that when other investigators injected thyroxine into thyroidectomized rats whose liver mitochondria had lost nearly half of their normal electron transport capacity, and although normal capacity was restored within three hours, recovery was not associated with synthesis of new respiratory assemblies.

2.5.2 Thyroid hormone and protein metabolism

Thyroxine also acts as a "growth hormone", for failure to grow is an outstanding feature of thyroxine deficiency in young animals (Peltola, 1965). So thyroxine is one of the factors essential for normal growth and skeletal maturation. In the absence of thyroxine, pituitary growth hormone content and secretion are depressed, thus thyroxine also potentiates the effects of growth hormone on tissue (Ganong, 1969).

Tata (1965) pointed out that the capacity of both mitochondria and microsomes to incorporate amino acids into protein was severely depressed in thyroidectomized rat. DNA-dependent RNA polymerase stimulates increased incorporation of amino acids into protein by mitochondria and microsomes, and it also increases mitochondrial respiration and phosphorylation. This author tested the possibility that thyroxine may activate genes by promoting the separation of complementary strands of specific segments of the DNA double helix prior to transcription. The suggestion there was made that only mitochondrial RNA responds to thyroid hormones. Werner and Nauman (1968) stated that acceleration by thyroid hormones of transfer of acyl sRNA to ribosomal protein, increase in RNA polymerase activity, and enhancement of mRNA synthesis may reflect only some of many actions of these hormones at the cellular level. As a rule, however, in small doses thyroid hormones are anabolic agents and promote growth but at high doses they act catabolically, leading to a negative nitrogen balance and enhancement of lipid breakdown.

To summarize, it seems that the mitochondrion is the primary locus of thyroid hormonal effect, although no single mechanism of action has been identified.

2.5.3 Thyroid hormones and nutrient absorption

The regulation of food intake and food intake behavior are not regarded primarily as local gastro-intestinal tract functions (Peltola, 1965). A prominent gastro-intestinal tract function that is often markedly influenced by thyroid disfunction is motility. The cretin or adult hypothyroid often suffers from constipation; moreover, hypothyroidism is associated with slow absorption. The rate of intestinal absorption of glucose parallels the basal metabolic rate and the general level of thyroid activity (Gang, 1968 and Peltola, 1965). Samuels (1948) reported that the rate of absorption of those substances which pass through the intestinal wall by other means than simple diffusion is dependent upon the presence of the thyroid hormone. This author also cited from other workers that the absorption of sugars and fatty acids is delayed in myxedematous patient and in thyroidectomized rats. If thyroid hormone is administered in excess, the rate of absorption of these substances will be increased above normal (table 15).

Samuels (1948) believed that the absorption of amino acids does not seem to be disturbed by changes in thyroid function. It was reported (Selenkow and Schatz, 1968) that when N-15 glycine was used as protein label the rate of incorporation of this amino acid into body nitrogen is reduced in the absence

Table 15. Intestinal absorption of carbohydrates in normal, hyperthyroid and thyroidectomized rats

Experimental condition	Substance administered	Amount (mg) absorbed/ hour/100g weight
Normal	Dextrose	171 ± 14
Hyperthyroid	Dextrose	284 ± 30
Thyroidectomized	Dextrose	91 ± 5
Normal	Glucose	187 ± 27
Hyperthyroid	Glucose	273 ± 18
Normal	Starch	126 ± 24
Hyperthyroid	Starch	196 ± 14

of thyroid hormones. This decreased incorporation, however, does not appear to be related directly to the availability of adenosine-5-triphosphate (ATP).

In summary, thyroid hormones participate in the regulation of most biosynthetic processes, presumably by affecting the energy sources upon which oxidative mechanism is dependent. Thus oxygen uptake and oxygen utilization is influenced by lack or surplus of thyroid hormones. Furthermore, at a cellular level the ubiquitous actions of thyroid hormones produce measurable changes in protein, carbohydrates, fat and mineral homeostasis. However, Selenkow and Schatz (1968) pointed out that these events remain to be defined.

2.6 Feeding Value of Rapeseed Meal

The seeds of the genus Brassica, especially rapeseed, mustard seed and seeds of related species, are regularly processed to obtain a vegetable oil which is used in Canada and in some Oriental countries as a substitute for soybean oil. Such processes furnish a by-product, an oil meal cake. At the present time, this proteinaceous oil meal has limited use in feeding of livestock and poultry, partly because of the unpalatable taste and odor of the meal, partly because the meal contains substances which are converted into toxic substances in the digestive tract of animals and partly because the by-product contains an unknown toxic factor(s). The current status of feeding studies of rapeseed meal to various classes of livestock and poultry are summarized in the following section:

2.6.1 Swine

The problems involved in the feeding of rapeseed meal to swine have been comprehensively reviewed by Bell (1955). In this review, the author suggested that it would seem permissible to use rapeseed oil meal up to one-third of the protein supplement in rations of growing and finishing market hogs.

In 1954, Nordfeldt et al. reported that feeding 10 and 20 percent rapeseed meal to growing pigs caused growth depression and enlargement of thyroid glands, liver, and kidney. When these authors fed extracted rapeseed meal treated with hot water, they found that the growth rate has improved and that the body organs were essentially normal. Bell (1963) from his experimental results suggested that growing pigs were not affected by either 5 or 10 percent rapeseed oil meal in the rations. However, when 2 percent of ground rapeseed screenings were added to rations containing either 5 or 10 percent of rapeseed meal, there was a 25 percent reduction in daily gain, and feed efficiency was also adversely affected. Bell (1965) fed three levels of Polish-type (Brassica campestris L.) solvent-extracted rapeseed meals (0, 5 and 10 percent), free of active myrosinase and containing all of its original complement of thioglucosides, two levels of iodinated casein (0 and 0.22 percent) and two levels of ground rapeseed screenings (0 and 2 percent) as a source of thioglucosidase to growing pigs. The author concluded that (1) the use of either 5 or 10 percent rapeseed meal in grower rations significantly depressed rate of weight gain and daily feed intake without affecting efficiency

of feed utilization, (2) iodinated-casein reduced daily gain and increased feed consumption regardless of the presence or absence of rapeseed meal in the ration and (3) the inclusion of 2 percent of rapeseed screenings to grower rations containing processed rapeseed meal resulted in reduction of weight gain and efficiency of feed intake without affecting level of feed consumption. Investigators (Nordfeldt et al. 1954) also reported that supplementation of the untreated rapeseed meal with iodinated-casein in a feeding trial with growing pigs, failed to counteract the adverse effects of rapeseed meal in terms of the growth rate of the pigs.

There is insufficient data, however, to exclude the formulation of recommendations for other classes of swine. Bell et al. (1961) stated that no adverse effects were observed when solvent-extracted rapeseed meal was included at a 4 percent level for young pigs (4.5 to 90kg live weight) in the rations. In another report, (Bell, 1965), no more than 4 percent rapeseed meal in creep or starter rations and 10 percent in finisher rations for pigs was recommended. In 1961, Bell suggested that the use of rapeseed meal in rations of baby pigs and pregnant sows is prohibited, but is allowed up to a certain level in grower-finisher rations for market hogs.

Mans and Bowland (1963) have indicated deleterious effects of rapeseed meal on swine and rats, when rapeseed meal replaced half or all of soybean meal of the diets. Bowland (1965) in a review, made the following suggestions for using rapeseed in swine rations: at 4 percent level for young pigs, during the

starting period (25kg live weight), 10 percent for market pigs (25 to 90kg live weight) and 3 percent for females during gestation and lactation. In 1969, Bell recommended that the maximum level of rapeseed meal for breeding gilts is 3 percent of total ration, for weanling pigs 4 percent and for growing pigs less than 15 percent. He also mentioned that rapeseed meal at 15 percent when fed to growing pigs, resulted in a feed efficiency and metabolizable energy less than soybean meal by 5 percent and 12 percent respectively. Schuld and Bowland (1968) fed zero and 8 percent solvent-extracted rapeseed meal, substituted on an isonitrogenous basis for soybean meal and wheat to twenty-four sows from 29 to 106kg live weight. Their results indicated that 8 percent rapeseed meal should not be fed to growing gilts that are to be saved for reproduction, but the meal is less likely to present problems when used with mature sows. The gross composition of colostrum and milk and fatty acid composition of colostrum and milk fat from sows which received rapeseed meal 0 or 8 percent soybean meal or wheat during growth, reproduction or both were studied by Schuld and Bowland (1968). They indicated that dietary rapeseed meal for growth was associated with significantly higher fat levels in colostrum and an apparent increase in fat levels in milk. Total solids were also higher in the colostrum of sows receiving rapeseed meal during growth or reproduction. Moreover, they did not find other differences in composition associated with dietary treatment.

In another report, it was demonstrated that 8 percent

solvent-extracted rapeseed meal in the dams' diet did not influence weight gain, efficiency of feed utilization, digestible (DE) and metabolizable energy (ME) or digestible nitrogen (DN) and nitrogen retention in the first and second progeny. In addition, this report also mentioned that in pigs from first litters that received 8 percent rapeseed meal compared with 0.0 percent rapeseed meal, feed intake and rate of gain in the finishing period (55 to 90kg live weight) was depressed, and a reduction in weight gain and poorer efficiency of feed utilization for the overall experiment for pigs with a 6kg initial weight was observed. Carcasses from gilts and barrows which received rapeseed meal however, were leaner. Furthermore, significant differences were found in the digestible energy (DE), metabolizable energy (ME) or digestible nutrient (DN) and nitrogen retention between pigs fed 0.0 or 8 percent rapeseed meal in either the starting diets at 6 weeks of age or the growing diets at 40kg live weight (Bowland and Schuld, 1968).

As regard to the palatability of rapeseed meal different flavors were added to the rapeseed meal containing-rations fed growing pigs and the results revealed that (1) flavor did not affect either grain or feed efficiency, (2) the protein digestibility and amino acid composition of rapeseed meal which has been properly processed was similar to that of soybean meal and (3) the lower energy digestibility, as possibly related to higher fiber content, and in some cases the palatability of the rapeseed meal may be somewhat of a problem (Bell, 1966).

Furthermore, Bowland (1965) stated that lack of palatability does not seem to be a major factor affecting the use of rapeseed meal in the starter rations.

2.6.2 Poultry

It was mentioned that, as far as poultry was concerned, levels above 10 percent of the rapeseed meal in the ration have invariably produced a reduction of live weight gain and efficiency of food conversion, with thyroid hyperplasia being normally associated with growth depression (Renner et al. 1955 and Blakley and Anderson, 1948). Holmes and Roberts (1963) from their experimental results found gross growth depression and thyroid hyperplasia when they fed solvent-extracted rapeseed meal to chicks at the level of 30 percent. Hesketh et al. (1963) compared Crambe abyssinica meal with soybean meal as a protein source in broiler chicks, and they found rations containing 5, 10, 20 and 42 percent of Crambe meal produced a growth depression which was indirectly proportional to the amount of meal consumed. They also found that efficiency of feed utilization was decreased. Lodhi et al. (1969) found that the average metabolizable energy content of nine samples of prepress-solvent and solvent processed rapeseed meal was 1,203, 1,313 and 1,782Kcal/kilogram, for four week old chicks, 6 week-old chicks and laying hens fed 30 percent rapeseed meal at least for 21 days, respectively. These authors suggested that possibly the adverse effects, resulting from influence of hypothyroidism on absorbability, held within the first five days of the experiment, probably no longer exist over a longer period

of time (26 days) because compensatory changes have taken place in the thyroid of chicken. Growth depression and enlarged thyroids of chickens caused by feeding of rapeseed were reported in 1949 by Petitt et al. and in 1946 by Turner. These reports were confirmed by other workers (Blakely and Anderson 1948). In 1955 Renner et al. indicated that the Argentine type rapeseed meal is more goitrogenic to poultry than the Polish type. They also found either type gave less growth when feeding levels of meals increased. Bell(1955) suggested that the use of more than 10 percent rapeseed meal in poultry feeds would seem inadvisable at the present time. Clandinin (1969 and 1965) reviewed the work of many other investigators and stated that 10 to 15 percent of low-temperature expeller, prepress-solvent or solvent-processed rapeseed meal can be used in chick starter rations. Insofar as laying and breeding chickens and turkeys are concerned, he added that less than 10 percent prepress-solvent or solvent-processed rapeseed meal have been shown to yield production, feed conversion, fertility, and hatchability as satisfactory as a corresponding amount of protein from soybean meal. The above statements were supported by other investigator (Bell 1967). It has been reported that commercial prepress-solvent and solvent-processed rapeseed meals were included at 15 percent level in the ration of growing chicks. The results obtained indicated that growth of chicks and feed conversion were as good for rats fed rations containing rapeseed meals as those fed rations containing soybean meal, and feeding solvent-extracted rapeseed to starting

and growing turkeys also showed satisfactory results (Bell et al. 1967). Collins and March (1967) found that rapeseed meal can be included in chick starting rations at levels up to at least 15 percent without showing adverse effect on growth rate or feed efficiency. Salmon (1970) fed diets containing 10 and 20 percent of either prepress-solvent commercial rapeseed meal or thioglucoside-free Bronowski rapeseed meal to large white turkeys from 6 days of age to market age (144 days) and he concluded that, 20 percent commercial rapeseed meal depressed gains in comparison with a similar amount of soybean meal. But the effect of diets containing either 10 percent commercial rapeseed meal or 10 and 20 percent Bronowski meal was essentially equal to that of the control diet fed to birds. The diets were utilized more efficiently from 55 to 111 days than from 6 to 55 days of age when compared with control diet. This author also discussed that the growth depression caused by rapeseed meal was not overcome by the addition of fat to the diet to compensate for the low metabolizable energy value of rapeseed meal.

2.6.3 Ruminant

According to Bell's review on the nutritive value of rapeseed meal (1955), the feeding value of rapeseed oil meal was the subject of a German report in 1872. Much of the previous work also dealt with the composition of rapeseed meal and its digestibility by ruminants. However, little detailed information is available on the value of rapeseed for ruminants in comparison with that for other livestock. Bell in 1952,

observed that ewes consumed rapeseed oil meal and mustard oil meal less readily than linseed meal. But he did find thyroid enlargements by gross examination. Bell (1955), suggested that ruminants are less susceptible than other classes of livestock to the effects of possible toxic compound(s) produced by rapeseed meal. It has been reported that calves, sheep and cows have shown that rapeseed oil meal at high level was somewhat unpalatable but otherwise ruminants seem to be unaffected by toxic factors (Bell, 1961). Bell (1965) concluded that evidence indicates that ruminants (cattle, sheep) are not affected by the toxic compounds in rape, and the palatability of modern meal is not likely to be a problem at the usual level required for balancing ration for dairy or beef cattle. Also, from Whiting's review (1965), it might be concluded that rapeseed meal is not as palatable initially as linseed meal to young cattle and sheep (from birth to six months); but this review recommends up to (1) 10 percent rapeseed meal be allowed for growing and fattening ruminants with a precaution, (2) 20 percent for breeding and production and (3) 5 pounds per day per head for dairy cows. In another report (Bell, 1967), it was demonstrated that dairy cattle, beef cattle, and sheep do not appear to be affected by thioglucosides or mustard oils, except as they may relate to palatability and abrupt feed changes. Solvent-extracted rapeseed meal and linseed meal gave similar results when fed to sheep at 20 percent of the total ration in terms of (1) body weight gain of ewes during pregnancy (2) quantity and quality of wool produced by

ewes and (3) the weight of lambs at birth and by 6 weeks of age. However, when extracted rapeseed meal was fed at a 30 percent level to the sheep, the adverse effects were observed for all mentioned criteria (Bell et al. 1967). In a short experiment in which extracted solvent or prepressed solvent-processed rapeseed meal was fed to cattle at 10 percent level of total dry-matter intake, feeding values were equivalent to those found for linseed meal. Results obtained with dairy cattle and practical experiences in feeding beef cattle indicated rapeseed meal is a satisfactory protein supplement for beef cattle. Bell (1969) supported the recommended 10 percent level of rapeseed meal in rations for dairy cattle. Bell and Linton (1961) studied digestibility and effects of rapeseed screenings on feeder lambs and they concluded that rapeseed screenings may be used successfully in ruminant rations.

It was of particular interest that treatment of rapeseed meal with iron salts did not improve or alter the feed value of rapeseed meal for breeder calves (Bell, 1969).

2.6.4 Laboratory animals

Goering (1961) conducted a series of feeding experiments with rats with rapeseed meal. He found that the feed efficiency of treated rapeseed meal was equal to that of soybean meal, in ration containing up to nearly 50 percent rapeseed meal. However, growth was somewhat less. This author pointed out that this occurrence along with the reduced efficiency on 100 percent rapeseed meal seems to be due to a deficiency in certain amino acids such as valine and leucine which may be inadequately

supplied in rapeseed meal but which are adequate in the soybean. This finding was true for mustard seed meal as well. Bell (1957b) fed Argentine and Polish-types of rapeseed meal at the 25 and 50 percent levels (containing the equivalent of 0.4 and 0.3 percent allylisothiocyanates, respectively) to mice and found no marked differences between types of rapeseed meal. However rapeseed meal at levels of 17 to 50 percent have been fed to mice and evidence of a toxic factor(s) in rapeseed meal was shown (Whiting, 1965). It was reported (Bell, 1965) that amino acid contents and biological values of protein with laboratory animals indicated that modern rapeseed meal protein is a very good quality protein for extensive use in conjunction with cereals for animals.

However, Wetter (1965) citing from several workers, reported that rapeseed meal may be an inferior meal because of its low lysine, leucine and isoleucine content in relation to soybean meal.

Drouliscos and Bowland (1969) evaluated the biological value of solvent and prepress-solvent processed rapeseed meals with weanling and mature rats and found that (1) N intake was minimal with weanling rats receiving the prepress-solvent rapeseed meal diet followed by those having the solvent-rapeseed meal diet, the casein and the soybean meal (in the case of mature rats feed intake increased, but with soybean meal and casein diets it remained above rapeseed diets), (2) body weight gain and gross energy intake per rat per day were in descending order for treatments soybean meal, casein, solvent and pre-

press-solvent rapeseed meals and (3) the fresh weights of the thyroid averaged 12.0 - 12.5mg for rats fed soybean meal and casein diets and 17.4 - 23mg for the solvent and prepress-solvent rapeseed meal diets. The larger thyroid gland observed in the prepress-solvent rapeseed meal treatment are in accordance with the high oxazolidinethione and isothiocyanate content of the prepress-solvent rapeseed meal.

2.7 Detoxification of Brassica Seed Meals

In attempting to alleviate the effects of the harmful factors in rapeseed meal for feeding animals, two main approaches have been used: counteraction by dietary additives or extraction by various solvent compounds and different methods.

Rapeseed oil meal fed to chicks resulted in goitrogenic effects which were not counteracted by cold water extraction or dry heating of the meal (Allen and Dow, 1952). Potassium iodide was found completely ineffective in reducing the toxicity of a diet containing 25 percent rapeseed oil meal. Hot water extraction improved the meal only slightly. This report (Allen and Dow, 1969) also indicated that female chicks were more sensitive to rapeseed meal toxicity than were male chicks. Kratzer et al. (1954) showed water extraction and pyridoxine supplementation were ineffective in counteracting the toxicity of rapeseed oil meal. Potassium iodide in a ration containing 35 percent rapeseed meal did little to counteract thyroid enlargement. Iodinated-casein (protamone) fed with rapeseed oil meal diets to turkey poults resulted in normal thyroid size

but did not restore growth to normal. Neither iodinated-casein nor potassium iodide improved the weight gain of chicks when included in the rapeseed meal diet (Klain et al. 1956).

Frölich (1953) stated that a considerable amount of the toxic material in rapeseed meal could be extracted with (1) cold water or (2) 70 percent aqueous alcohol and (3) by heating at 150°C. The extraction of rapeseed meal with water at 100°C was reported by Nordfeldt et al. (1954) to have largely removed or inactivated the toxic factors. Some workers (Klain et al. (1956) and Nordfeldt et al. (1954) indicated that the addition of iodinated casein into untreated rapeseed meal did not improve the quality of rapeseed meal in terms of toxicity.

Further studies with mice fed rapeseed meal plus different counteracting compounds have been reported (Bell and Baker, 1957) that (1) Protamone (iodinated-casein) significantly depressed growth even though the intake of feed was increased, (2) stabilized potassium iodide (KI-calcium stearate) improved gains on rapeseed oil meal diets, and (3) Aurofac (aureomycin) had no counteracting effects on toxic factors. Bell (1957a) showed that acid hydrolysis produced little reduction in the rapeseed meal toxicity. On the whole, hot water extraction produced a greater improvement of rapeseed oil meal than cold treatment. Erfle (1959) in a study of removal of toxic factors from rapeseed oil meal by enzyme digestion and solvent extraction demonstrated that most extractions in absence of enzyme treatment were beneficial, HCl and ethanol separately or in combination being the most

favorable, but there was no advantage in combining enzyme (Rhozymes) digestion and extractions. Belzile (1963) showed that treatment of rapeseed meal with hot water or steam eliminates most of the growth depression associated with feeding this to weanling mice. Bell et al. (1967) claimed the treatment of rapeseed meal with a compound of iron or copper at a temperature between approximately 200° to 275°F. and with a moisture content of at least 15 percent. When these authors fed iron-treated rapeseed meal to mice, the mice fed the treated meal gained 10.2g per 14 days, while those fed the untreated meal (control) gained 8.8g in 14 days. Lo (1969) stated that neither buffer solution plus steaming treatment nor thyroxine addition to rapeseed meal resulted in the improvement of growth rates and feed intakes of mice. When this author also treated rapeseed meal with ferrous sulfate and fed it to mice, the average gain was 9.56g per 14 days, whereas, the average gain for control in the experiment was 6.55g. A number of methods have been proposed to remove the toxicity from rapeseed meal, yellow sarson meal and Crambe seed meal by other workers (Mustakes et al., 1965 and 1963). Kirk et al. (1966) have developed an improved Crambe meal by using ammonia heat treatment. The treated meal by this method gave significant results when fed to animals. Other investigators mixed rapeseed meals with water (1:4 ratio) in a planetary mixer for 30 minutes. The extract was then separated by filtration and the residue re-extracted three times in the same manner. The data obtained showed that 93.6 percent of the total thiogluco-

side in the extract was removed (Eapen et al.,1969).

Ballester et al. (1970) attempted to remove thioglucosides in rapeseed meal by steaming and water extraction. They found that double water extraction gave the best results. This technique resulted in a reduction of 84 percent in oxazolidinethione and 77 percent in isothiocyanates. Net protein utilization of treated meal by rats increased from 40 to 69 percent relative to the control. The improvement in digestibility was reflected in the growth of rats.

A general summary of the literature reviewed in regard to counteracting or methods for removing either the glucosinolates or their products in rapeseed meal is as follows:

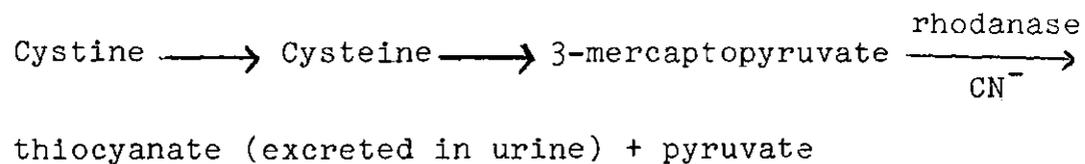
1. Iodinated-casein in most cases was effective in preventing thyroid enlargement of animals but the growth depressing effect of rapeseed meal was not counteracted by this compound, except in the case of turkey poults.
2. Antibiotics had no beneficial effects on neutralizing the toxic factor (S).
3. Vitamin supplementation was commonly ineffective.
4. Iodide did not improve the quality of meal for mice, swine, or turkeys, but the evidence for this was contradictory.
5. Autoclaving, steam stripping, chemical modification and aqueous extraction for detoxifying rapeseed have been tried. Variable results have been reported. Thioglucosides were catalytically decomposed by heating

with salts of iron, copper or nickel and resulted in production of nitrile and thiomide which are highly toxic. Removal of thioglucosides from rapeseed meal by water extraction resulted in an end product which was more toxic than the original material.

6. Since other organic substance may be formed instead of goitrin, further study in this field seems imperative.

2.8 Cystine

Literature on the detoxification of glucosinolates by cystine is limited. Blakely and Coop (1942) reported that cystine, cysteine, glutathione and thioglycollic acid injected intravenously into rats all prevented death from cyanide which had been injected subcutaneously one minute previously. They also showed that the body does not utilize its own intact molecules of cystine or cysteine (either as free or as peptide form). They stated that their findings were in agreement with other reports in this field. Muldoon et al. (1924) and Montgomery (1969) were agreed that under cyanide or bromo-benzene poisoning the animal's demand for cystine or cysteine is very acute. These workers were believed that the metabolic disposal of inorganic cyanide is as below:



2.9 Apparatus for Measuring Respiratory Exchange

An animal calorimeter is an apparatus for measuring the heat produced or given off by an animal.

The two biocalorimetric categories are direct and indirect. The direct animal calorimeter is an apparatus for measuring directly the amount of heat given off by an animal in a given time.

In the direct method the subject is placed in a well insulated closed box just large enough to hold the animal that is being subjected to the test with recording devices that measure the escaping body heat (Crampton and Lloyd, 1959 and Dukes, 1955).

Indirect calorimetry's (oxycalorimeter) fundamental principle is the determination of the value of oxygen required to burn a weighed sample of food or feed and calculation of the energy value from the amount of oxygen used. By indirect calorimetry one arrives at the amount of heat given off by an animal or feed sample without actually measuring the actual heat. The indirect method is thus based on the fact that the source of body heat is the oxidation of metabolism, normally oxygen consumption and CO₂ production. Consequently, oxygen consumption is almost perfectly correlated with body heat production and can be used as an index of measurement (Crampton and Lloyd, 1959).

It was reported (Kleiber, 1961) that in 1778, Crawford constructed the first combustion calorimeter, and he is said to have been the first to measure animal heat. At almost the

same time Lavoisier was the first to recognize that animal heat is produced by oxidation in the body. Lavoisier and Laplace devised an animal calorimeter to measure this heat, using a guinea pig as the subject (Maynard and Loosli, 1969). In 1892 the German physiologist Rubner (cited by McHenry, 1963) developed a highly accurate calorimeter in which simultaneous measurements were made in heat production, oxygen consumption and carbon dioxide output. Brody (1964) said that indirect calorimetry is based on the fact that normally, oxygen consumption and carbon dioxide production are closely correlated with heat production. In 1932, Koehler described an apparatus, which designed by him, could measure the oxygen consumption of dogs. Koehler demonstrated that if soda-lime has the proper moisture content, the CO_2 is practically all absorbed and no correction has to be made for residual CO_2 . His figures (table 16) show the residual CO_2 in the chamber was never nil nor did it accumulate with time.

This author showed that by the choice of some ordinary salts, practically any desired humidity can be obtained. This data (compiled by Koehler) show the range of humidity that can be obtained with some ordinary salts (table 17).

Morrison (1947) used soda-lime for absorption of CO_2 and wet calcium chloride to keep the humidity within a reasonable limit. The oxygen consumption by experimental animal was recorded mechanically and electrically with devices used by Hart (1950). He used magnesium perchlorate and ascarite for absorbing water vapor and carbon-dioxide in the chamber respectively.

Table 16. Rate of carbon dioxide absorption by soda-lime

Observation No.	Time-soda-lime used in hour	Run before analysis in hour	CO ₂ percent
1	8	5	0.030
2	17	1	0.045
3	24	3	0.040
4	7	6	0.036
5	25	2	0.022
6	39	1	0.040
7	48	6	0.048
8	9	4	0.028
9	17	5	0.032

Table 17. The effect of some normal salts on relative humidity in closed system

Solid phase	Temperature C°	Humidity percent	Aqueous tension
$H_3PO_4 \cdot \frac{1}{2}H_2O$	24.5	9	2.07
$LiCl \cdot H_2O$	20.0	15	2.63
$CaCl_2 \cdot 6H_2O$	24.5	31	7.15
$Ca(NO_3)_2 \cdot 4H_2O$	24.5	51	11.75
$NH_4 NO_3$	28.0	61	17.30
$K_2C_4H_4O_6 \cdot \frac{1}{2}H_2O$	28.0	73	20.70
$NaCl$	28.0	75	21.28
KCl	28.0	84	23.83
K_2SO_4	28.0	97	27.50

In 1933, Davis and VanDyke (1932) modified Koehler's apparatus to adapt it for use with small animals, such as mice, rats, and guinea pigs.

Werthessen (1937), in order to study the metabolic rate of rats, constructed a special device and measured the rate of oxygen consumption electrically. A closed circuit system for the automatic measurement of the rate of oxygen consumption by white mice was described by Morrison (1947). Smith (1955) developed a new closed circuit apparatus for continuous recording of oxygen consumption over periods of several days with automatic correction for changes in barometric pressure. Furthermore, the capacity for carbon dioxide absorption in the animal chamber was determined. He concluded that his results confirmed the findings of another investigator (Hitchcock) for the rate of absorption is proportional to the carbon dioxide gradient and inversely proportional to the liquid viscosity.

Teitelbaum and Harne (1941) developed different types of respiratory apparatus. In 1948, Farmer and Crampton (1948) modified one of the Teitelbaum and Harne's apparatus for measuring oxygen consumption of guinea pigs under the conditions of their procedure, and demonstrated that each pound of soda-lime was able to absorb carbon dioxide equivalent to 10 liters of water.

In addition, the findings of Farmer and Crampton agreed with those of Herrington (1940) in that the critical temperatures for the usual laboratory strains of rats, mice and guinea pigs lie within the range from 27°C. to 31°C. and

common practice has favored the use of a 28°C. and 30°C. range. Herrington also concluded that the region of thermal neutrality for mice is between 30° and 33°C. and 31.5°C. is acceptable as a condition of thermal neutrality for mice when normal activity is allowed.

More recently, Hayward et al. (1963) have described an electrolytic respirometer for small animals which relates oxygen production to current flow and embodies a recording ammeter. Bailey et al. (1957) have represented a volumetric respirometer so that the basal metabolic rate of Swiss albino mice could be determined. These authors also indicated that the temperature of thermal neutrality for mice was about 30°C. Bailey et al. (1957) faced considerable difficulty in obtaining a state of complete rest in mice with a body weight below 10 grams when food was withheld for a period longer than 12 hours.

2.10 Oxygen Consumption by Mouse

Metabolic heat production results from the oxidation of foodstuffs by atmospheric or supplied oxygen with the production of carbon dioxide. The magnitude and nature of this gaseous exchange varies with the type of foodstuff, or mixture of foodstuffs. Theoretical relationship between oxygen consumption and carbon dioxide production can be calculated from the stoichiometry of equations for the oxidation of carbohydrates, lipids and proteins, respectively (White et al., 1964).

Among the most fundamental of dynamic chemical events related to life are the oxidations which yield energy to the cell. The simplest and perhaps the most accurate method for

measuring energy metabolism is then, by the rate of oxygen consumption as fed from a calibrated oxygen source and computing the heat production by the caloric value of oxygen (Brody, 1964).

The first measurements of heat that can be produced by oxidation of foodstuffs outside the body were conducted by Rubner who compared the values obtained in a bomb calorimeter with those given by direct measurement of heat production by a dog placed in a calorimeter and then fed a known quantity of carbohydrate, protein or lipids. Rubner said that the energy liberated as heat for activity equals the potential energy value of utilized food.

It has been demonstrated (McHenry, 1963) that the amount of heat calculated from carbon dioxide measurement was found to be 90 percent of the total amount of heat actually given off by the animal. The heat production of the body during a given period can be estimated using the thermal equivalents if the amount of oxygen consumed or amount of carbon dioxide produced during that time is known. The estimate of heat production is more reliable when based upon oxygen consumption. The heat or caloric values (thermal equivalent) of a liter of oxygen at different respiratory quotient is 4.686 to 5.047kcal/liter O_2 (Best and Taylor, 1966).

As mentioned by Lusk in 1924, in 1901 Zuntz and Schumburg published a standard table of caloric values when a liter of oxygen used to oxidize mixtures of carbohydrate and fat. About two decades later Zunta and Schumburg's figures were elaborated

by Lusk (1924) who also introduced columns showing the relative quantity in calories of carbohydrate and fat consumed (table 18). Lusk (1924) by using respiratory quotient (R.Q.) and calorific value of a liter of oxygen for lipid, carbohydrate and protein also estimated the total metabolism and percentage of calories furnished by carbohydrate, fat and protein.

In 1933, Davis and VanDyke performed an experiment on fasting white mice, whose oxygen consumption for the quiet-relaxed-awake state was found to average 37.5 liters of oxygen per kilo of body weight of mouse per 24 hours. They mentioned that this figure was lower than most of the data that has been reported. When these authors carried out two other metabolic trials, in Chicago and Boston, the following values, 38.3 and 40 liters of oxygen per kilo of body weight of mouse per 24 hours were obtained, respectively. In all experiments, the mice were kept at an environmental temperature of about 28°C. before as well as during the metabolism determination. In all cases, the length of the experimental period was 5 to 25 minutes. Davis and VanDyke (1932) determined the volume of oxygen consumption by fasting mice at 28°C. The average oxygen uptake for the quiet-sleeping state was 26.8 (STP) and 37 ± 0.72 liter per kilo per body weight of mouse per 24 hours for quiet-relaxed state.

Bois (1924) demonstrated a graphic representation of respiratory quotient and the percentage of calories from proteins, fats and carbohydrates. By using these graphs as a map,

Table 18. Analysis of the oxidation of mixture of carbohydrate and fat

R.Q.	Percentage of total oxygen consumed by:		Percentage of total heat produced by:		Number (5)	Calories per liter O ₂	Logarithm (6)
	Carbohydrate (1)	Fat (2)	Carbohydrate (3)	Fat (4)			
0.70	0	100.0	0	100.0	4.686	0.67080	
0.71	1.02	99.0	1.10	98.9	4.690	0.67114	
0.72	4.44	95.6	4.76	95.2	4.702	0.67228	
0.73	7.85	92.2	8.40	91.6	4.714	0.67342	
0.74	11.3	88.7	12.0	88.0	4.727	0.67456	
0.75	14.7	85.3	15.6	84.4	4.739	0.67569	
0.76	18.1	81.9	19.2	80.8	4.751	0.67682	
0.77	21.5	78.5	22.8	77.2	4.764	0.67794	
0.78	24.9	75.1	26.3	73.7	4.776	0.67906	
0.79	28.3	71.7	29.9	70.1	4.788	0.68018	
0.80	31.7	68.3	33.4	66.6	4.801	0.68129	
0.81	35.2	64.8	36.9	63.1	4.813	0.68241	
0.82	38.6	61.4	40.3	59.7	4.825	0.68352	
0.83	42.0	58.0	43.8	56.2	4.838	0.68463	
0.84	45.4	54.6	47.2	52.8	4.850	0.68573	
0.85	48.8	51.2	50.7	49.3	4.862	0.68683	
0.86	52.2	47.8	54.1	45.9	4.875	0.68793	
0.87	55.6	44.4	57.5	42.5	4.887	0.68903	
0.88	59.0	41.0	60.8	39.2	4.899	0.69012	
0.89	62.5	37.5	64.2	35.8	4.911	0.69121	
0.90	65.9	34.1	67.5	32.5	4.924	0.69230	
0.91	69.3	30.7	70.8	29.2	4.936	0.69339	
0.92	72.7	27.3	74.1	25.9	4.948	0.69447	
0.93	76.1	23.9	77.4	22.6	4.961	0.69555	
0.94	79.5	20.5	80.7	19.3	4.973	0.69663	
0.95	82.9	17.1	84.0	16.0	4.985	0.69770	
0.96	86.3	13.7	87.2	12.8	4.998	0.69877	
0.97	89.8	10.2	90.4	9.58	5.010	0.69984	
0.98	93.2	6.83	93.6	6.37	5.022	0.70091	
0.99	96.6	3.41	96.8	3.18	5.035	0.70197	
1.00	100.0	0	100.0	0	5.047	0.70303	

Table 18 - cont'd.

		(R.Q. = R)
Formula for Column	%	
(1)	%	$= 100 \frac{R - 0.707}{0.293}$
(2)	%	$= 100 \frac{1.00 - R}{0.293}$
(3)	%	$= \frac{504.7 (R - 0.707)}{5.047 (R - 0.707) + 4.686 (1.00 - R)}$
(4)	%	$= \frac{468.6 (1.00 - R)}{5.047 (R - 0.707) + 4.686 (1.00 - R)}$
(5)	Calories	$= 4.686 + \frac{R - 0.707}{0.293} \times 0.361$
(6)	Logarithm	= Log of Column 5

This Table produced from Lusk (1924). The J. Biol. Chem. - Volume 59; pp.41-42.

the changes in metabolism caused by disease or by the administration of diets may be obtained. Michealis (1924) contracted the charts which enable one to determine the percentage of the total calories derived from proteins, carbohydrates or fats, and the amount of oxygen consumed for oxidizing proteins, carbohydrates or lipids, by providing urinary nitrogen, total oxygen consumed and the total respiratory quotient.

Lee (1928) measured oxygen consumption and carbon dioxide production of the rat by the gravimetric method and heat production per day by the rat was calculated by this author and he calculated respiratory quotient for 14 rats (starved 18 hours); the mean value obtained was 0.746 with a coefficient of \pm 2.5 percent. Lee commented that this figure was in agreement with the data published by Mitchel and Corman.

Morrison (1947) described an apparatus for a study of oxygen consumption in study of metabolic rate in small animals. By means of a respirometer, the average basal metabolism of the white mouse (Swiss strain, average weight 21g acclimated to 22-24°C. and measured at 28.5°C.) was determined to be 7.5 calories per gram per hour. He stated that this value compared favourably with values of 8.3, 7.5, 7.4, 7.1, and 6.8 calories per gram per hour reported by other investigators. The daily metabolic cycle and the oxygen consumption of fully fed, adult white mice (average weight 26.72g) was measured during rest and work at seven temperature

levels from -9.6° to 37°C by Hart (1950). The average respiratory quotients at 26.4°C , was 0.9 and the average oxygen consumption versus temperature levels and state of the mouse are illustrated in figures 4 and 5 respectively.

In 1957, Bailey et al. measured the oxygen consumption of mice and calculated their respiratory quotient. The respiratory quotients were 0.78, 0.79, 0.73, and 0.81 for 7.4, 15.1, 21.3, and 27.4g body weight, respectively.

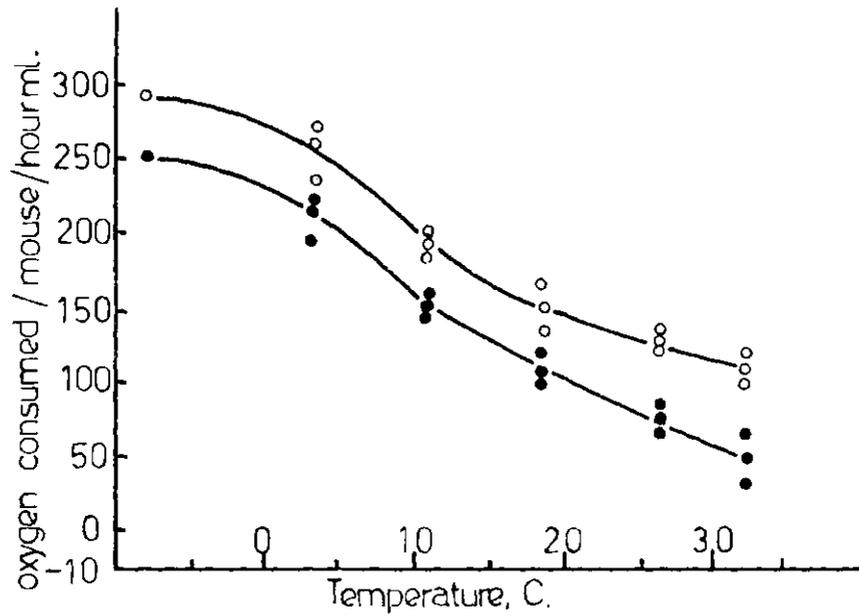


Fig. 21

Oxygen consumption in daily metabolic cycle in relation to temperature for each of four mice

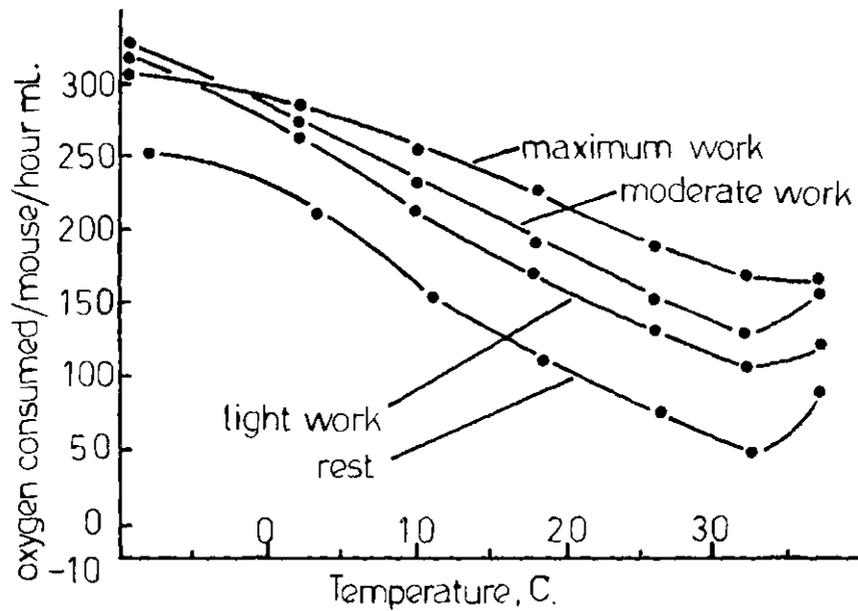


Fig. 22

Effect of temperature upon oxygen consumption of mice during rest, light work, moderate work and maximum work

3. EXPERIMENTAL

3.1 Experiment 1

A simple electrolytic respirometer for small animals.

3.1.1 Introduction

The feeding value of meals of Brassica species produced and processed in Canada is of economic importance. Many feeding experiments with livestock and chemical assays have been conducted by Canadian investigators to evaluate the feeding value of these meals. There is, however, no information about these meals with regard to their effects on metabolic rate of either small or large animals.¹ Thus, to assess the effects of Brassica meal on the metabolic rate of small animals, it was necessary to develop a suitable technique.

3.1.2 Objective

The objective of this study was to design an apparatus capable of rapid accurate measurement of oxygen consumption by mice and suitable for assessing the goitrogenic effects of Brassica meals included in the diet.

3.1.3 Materials and methods

The main parts of the instrument (Fig.23) are briefly described as follows:

3.1.3.1 The Oxygen Generator consists of a 300ml glass jar (5cm. diameter and 16.25cm. high). This part functions as the electrolyte vessel. A glass sleeve (2cm. diameter and 14cm. high) with a platinum or nickel wire coil inside was used as the cathode and outer side with another wire coil acted as the anode. An air tight rubber stopper

¹An exception is in the case of fasting animals.

(#10) was fitted to hold the coiled sleeve, electrodes and oxygen and hydrogen outlet tubes.

3.1.3.2 The Mercury Switch consists of two separate components: a float switch and a mercury container. The float switch is made up of a wedge-shaped copper foil chamber (3x4x3cm.) with an open bottom and this floats in a water tank (7x9x4cm.) and covering top of the open tube of the tank. The central tube of the tank is opened to the oxygen connecting tube which carried oxygen from the generator to the respiratory chamber. The float switch is pivoted at one end of the water tank and it has an adjustable balance weight and a provision for an electrical wire. The movement of this element is directly proportional to the volume or pressure of the air in the system.

The mercury containers are made up of two rubber stoppers in each of which are inserted 1.5 ml. glass tubing receptacles with electrode wiring inserted through the sealed bottoms. These two receptacles are connected to one another at the top through the float switch electrical wire. Details of the microswitch and electrical circuit of the instrument are shown in Fig. 23.

3.1.3.3 The Respiratory Chamber. This part serves as the oxygen reservoir with a capacity of 750 ml. This glass chamber is closed with a dome-shaped top. The glass top has two inlets for entrance of oxygen and a thermometer into the chamber. The bottom of the respiratory chamber is divided into three sections by an aluminum paper for keeping different

absorbants separated.

3.1.3.4 The Recorder. This component records the amount of oxygen liberated by the generator. In addition the recorder registers the time of electrolysis of the electrolyte (oxygen liberation) and also the time of oxygen consumption by the subject.

3.1.4 Operation of the respirometer

The outer jar of the oxygen generator is partially filled with 30 percent NaOH (electrolyte) (Hayward et al. 1963) and closed air tight by assembling the part which contains the coiled sleeve and electrodes as well as the oxygen and hydrogen outlet tubes. The unit is then sealed with wax. The water tank is filled with water. The mercury containers are filled with mercury. An absorbant container is filled with soda-lime and calcium sulfite and calcium chloride and placed at the bottom of the animal chamber to absorb CO_2 and H_2O . The wire-screen cage with the animal inside is inserted into the respiratory chamber; this is set about 2 inches above the absorbants by protuberances on the inside wall of the chamber. Then the chamber is closed, sealed with wax, submerged in the water bath and the electrodes are immediately connected to the power supply. Thus the animal breathes oxygen and exhales carbon dioxide and water vapor. Carbon dioxide and water are absorbed by the indicator soda-lime and calcium sulfite, respectively. In addition, the absorbant action calcium chloride controls the relative humidity within reasonable limits 31 percent, (Koehler, 1932). If the water vapor is not condensed

the moisture will wet the animal and make it uncomfortable. A certain amount of ammonia is expected to form from animal excretions and this can reduce absorbability of soda-lime. This ammonia problem is counteracted by moistening the calcium chloride with dilute acid (HCl). Thus the air in the respirometer is used repeatedly while carbon dioxide and water vapor produced are removed. As the oxygen is used, gas volume is reduced, causing the float switch to descend slowly into receptacles and completing the current circuit. The current hydrolyzes the electrolyte in the generator and releases oxygen and hydrogen. The liberated oxygen enters the system by a fine plastic connecting tube. When the rate of oxygen supply exceeds the rate of consumption, the air volume in the apparatus increases and breaks the current circuit by micro-switch and prevents hydrolysis.

It is known that the rate of oxygen uptake and carbon dioxide production could be altered by changes in environmental temperature and the activity of the animal. To overcome this factor, before recording the oxygen consumption, a period of 30-40 minutes was allowed to establish an equilibrium between the water in the water bath and the respiratory chamber and to acclimatize the animal (Fig. 24 and 25). After the adaptation period, the actual oxygen uptake by the subject is recorded. The amperage and voltage should be carefully controlled during the operation to ensure that oxygen liberation is not limited. In this experiment this demand was met by using a variable power transformer.*

*Hewlett-Packard (Canada) Ltd.

3.1.5 Calibration

It is necessary to maintain constant current flow through the system. The achievement of this condition depends upon two or more factors; (1) the constancy of voltage and amperage output from the power source used, (2) uniformity and maintenance of constant current conductivity through the electrolyte solution. In order to minimize conductivity changes due to gradual increase in concentration of electrolyte solution due to prolonged use, the electrolyte was discarded after every 8-10 hours of use and the generator tank was refilled with fresh solution. The increase of electrolyte is negligible for short periods. (Hayward et al. 1963). To ensure that these conditions are met three different tests were carried out:

(a) The amperage and voltage were measured at several points along the circuit (to and from oxygen generator, to and from microswitch, and to and from recorder).

These measurements were found to be the same and were equal to the reading shown by voltmeter and amper-
-meter of transformer. Furthermore, the baseline on the chart paper was constant for certain amperage and voltage which was set during corresponding time.

(b) Oxygen and hydrogen collection. Oxygen and hydrogen liberated by the generator were collected into two similar graduated cylinders and measured several times. The volume of released oxygen was found to be equal to half of the volume of liberated hydrogen.

(c) Indirect comparison. The evolved volume of hydrogen was collected as described already for a certain period of time and at the same time, the volume of released oxygen was calculated by recording on the chart paper of recorder (see calculation in the following section). The data obtained from these determinations were found to agree completely with results obtained by other methods.

Since maintenance of a constant temperature in the animal chamber (respiratory chamber) is a first prerequisite, repeated measurements were made to compare the ambient temperature, surrounding subject with that of water in a water bath with its temperature thermostatically regulated. Chamber temperature was measured with a telethermometer and water temperature was measured with ordinary centigrade thermometer. After temperature of respiratory chamber and water bath reached equilibrium, (during 15-20 minutes) the fluctuation of thermometers never exceeded 0.3°C . This deviation was within the ranges reported by others.

The pressure within the closed system maintained itself relatively constant due partly to the shortness of the recording period and partly to the constant ambient temperature of the laboratory and water bath. Barometric pressure of the laboratory was recorded daily for 120 days (from June to October). From day to day the laboratory barometric pressure, however, varied from 755 to 762mm. Hg. during this period.

3.1.6 Calculation

The electrolytic current strength (I) is accurately determined. The oxygen released from the generator can be easily calculated by using the electrochemical equivalent equation, $m = IeT/96,500$, then corrected to ml. per minute. Where m is grams of oxygen liberated by electrolysis, e is the electrochemical equivalent weight of oxygen, T is the total time of hydrolysis (oxygen production time) in seconds and 96,500 is the volume of 1 Faraday of current which is expressed in coulomb units. In addition, the time of oxygen consumption by the animal in the respiratory chamber is recorded on the chart paper. Thus with these values the amount of oxygen produced by system and the amount of oxygen consumption by the subject can be calculated.

3.1.7 Testing apparatus for leaks

The closed system should be checked for leaks as oxygen may leave the unit and give higher values. This factor has been practically eliminated. In addition, the assembled apparatus was tested by running the instrument as blank for several hours (8-10 hours weekly). During running of the blank the base line on the chart paper of recorder remained in the same position (horizontal) for corresponding current and voltage. This means the float switch of the microswitch does not contact the mercury in the receptacles, due to unchanged air volume in the system.

3.1.8 Results and discussion

Several respirometers for indirect animal calorimetry

are routinely used. A description of a simpler electrolytic and convenient method is given. The apparatus reported is designed, assembled and used for measuring oxygen consumption of individual mice on particular and normal diets.

The reliability of our respirometer was confirmed by measuring oxygen consumption by non-fasting white mice at different ages with different body sizes and fed different diets. The response of the animals to different periods of season was not significant (table 19). That means, (1) the changes of ambient temperature and the pressure within the unit were small and (2) the amount of oxygen uptake by inbred mice fed the similar diet at different periods of season was measured accurately.

Moreover, several hundred (700-800) oxygen consumption estimates were made on mice on 40-50 different rations and relevant data is reported in experiments 2, 3 and 4. Fig. 24 and 25 show that the age and body weight of the test animal has an effect on oxygen consumption. This might be due to the activity and behaviour of the animals at different stages. In addition, our experiment showed that a mouse weighing 24g or more was quieter than a young mouse. Fig. 25 shows the fluctuation of oxygen consumption of mice before and after adjusted period.

During the last two decades, many respirometers were built for the study and energy exchange of small animals. Our method is considerably cheaper and simpler. The advantages of the instrument may be summarized as:

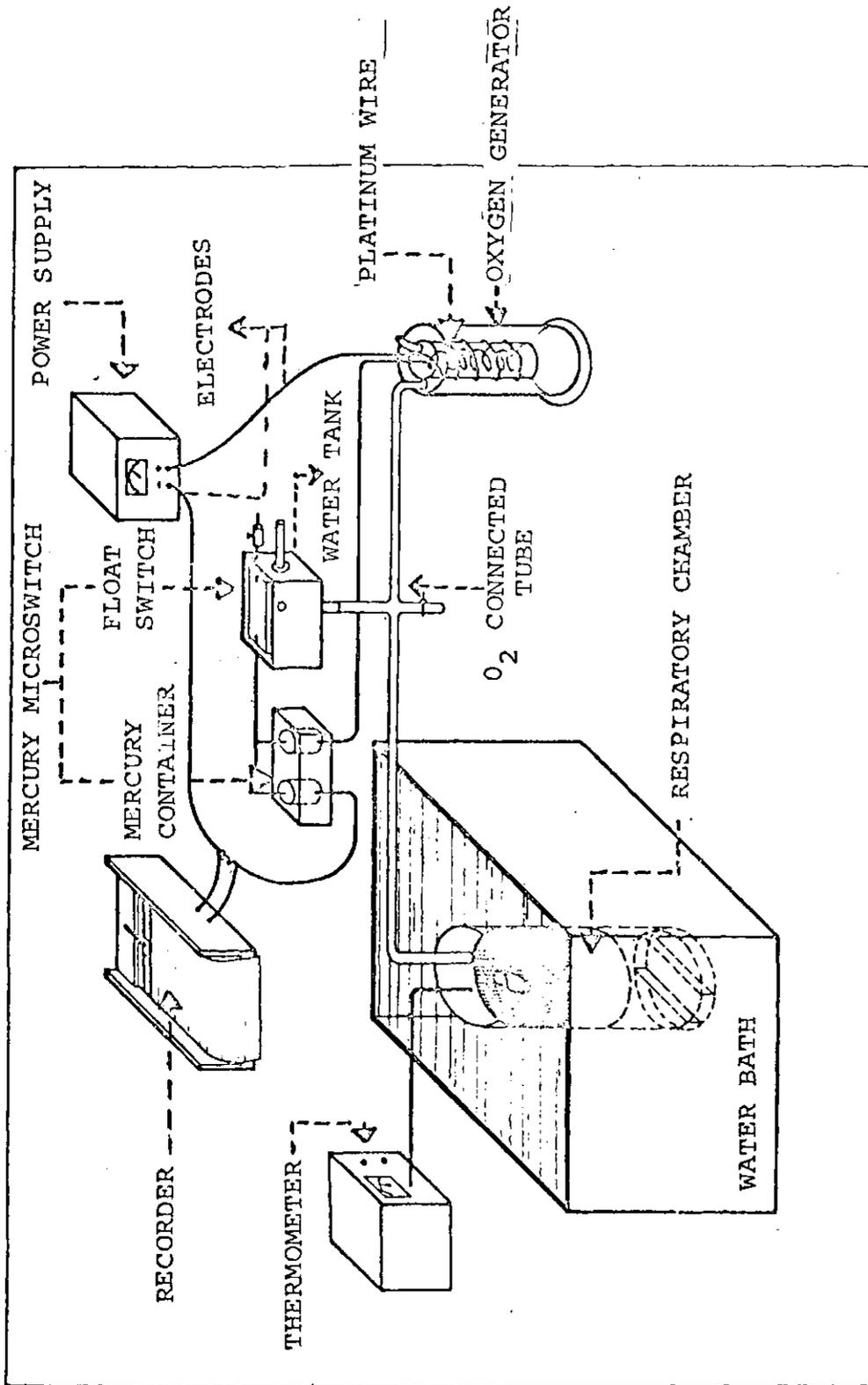


Fig. 23
 A diagrammatic representation of a simple electrolytic
 respirometer

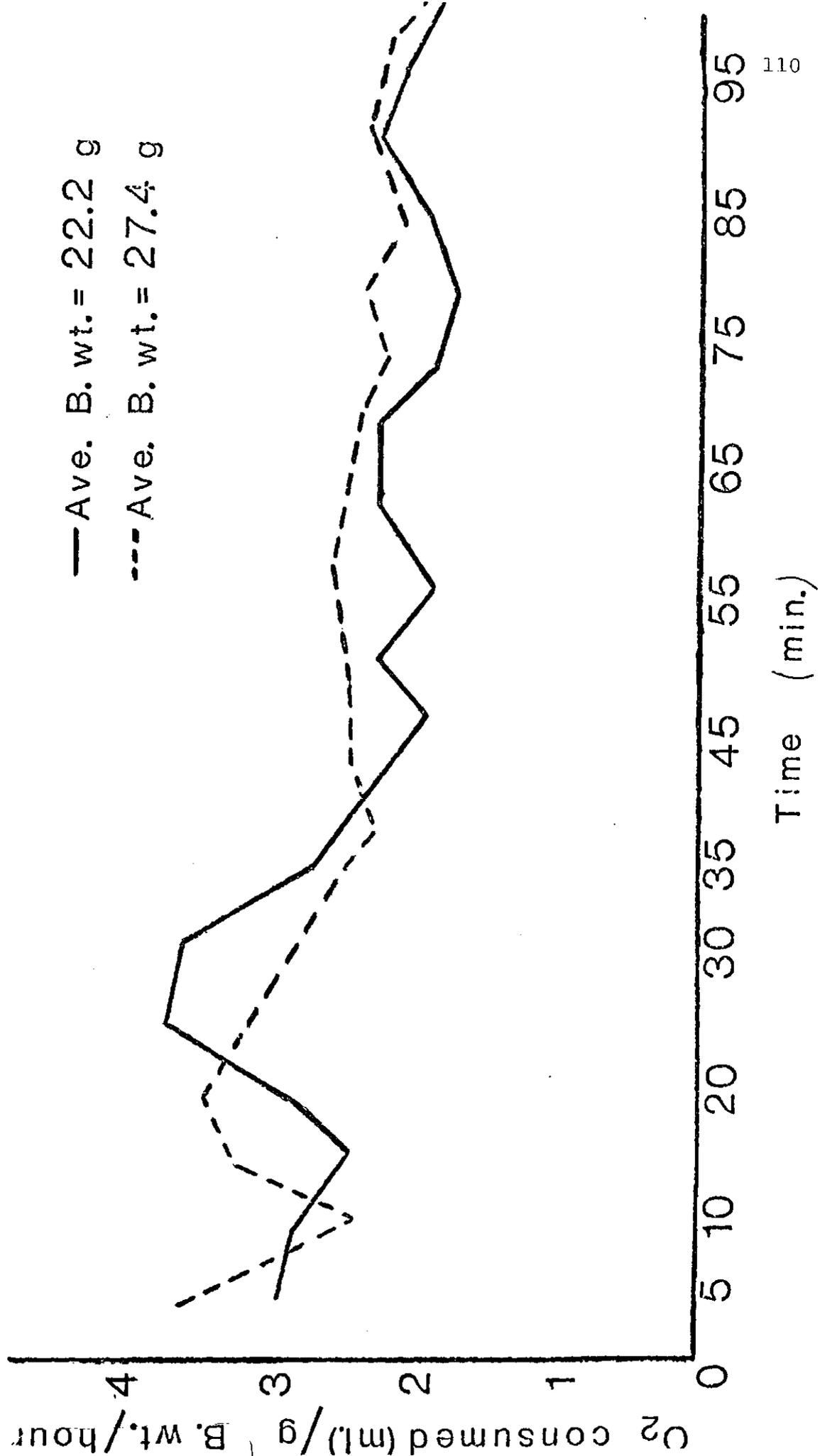


Fig. 24
 A graphic example of the results obtained with respirometer showing relationship between time and rate of oxygen consumption by mice with different weight

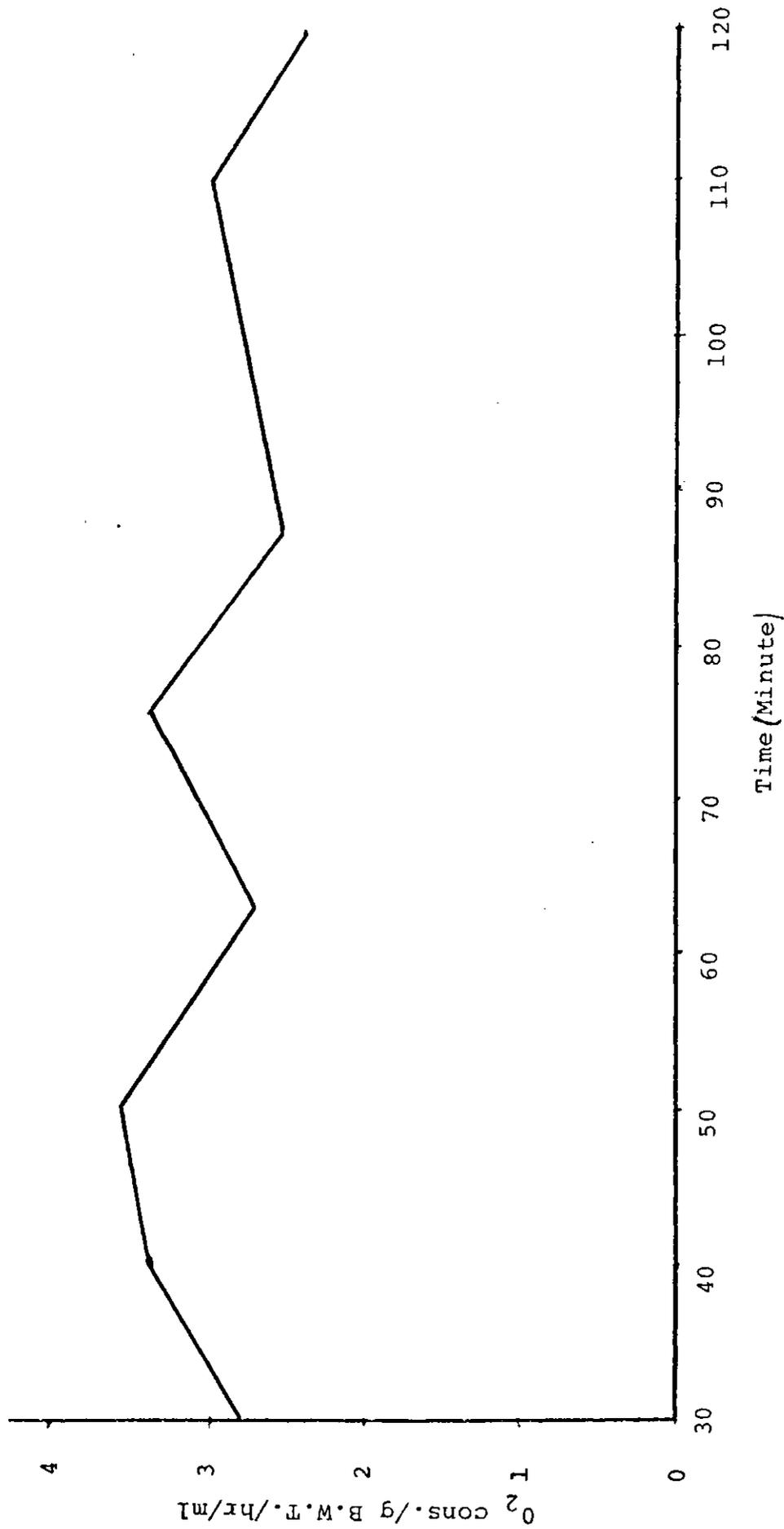


Fig. 25
Oxygen consumption in relation to time for each group of five mature (25g) mice

Table 19. Oxygen consumption of non-fasting white mice fed same ration in different time intervals

No. of mouse	April	July	August
1	3.36 ^{ab}	3.15 ^{ab}	3.22 ^{ab}
2	3.60	3.20	3.28
3	2.70	3.00	3.44
4	3.44	3.10	3.06
5	2.60	2.90	3.13
6	2.82	2.95	3.30
7	3.05	3.19	3.00
Average	3.08	3.07	3.20
SD	± 0.16	± 0.36	± 0.45
SE	± 0.04	± 0.01	± 0.14

a, ml. oxygen uptake/gram body weight/hour

b, Values are means of two measurements on one mouse

SD, Standard deviations

SE, Standard error

- (1) It is almost automatic only requiring attention for regulating current and voltage.
- (2) Oxygen production and consumption are easily measured during a succession of periods of time including periods as short as one minute to periods as long as desired.
- (3) It is convenient to maintain normal atmospheric air composition in the chamber.
- (4) No correction factors are needed for any of the measurements.
- (5) The operation, calibration, and computation of results are easy and reliable.
- (6) The animal does not necessarily breath pure oxygen.
- (7) The apparatus is portable and easy to set up in laboratories.
- (8) This instrument can be used to study metabolic rate of chicks at pre- and post-hatching periods with equal efficiency.

3.2 Experiment 2

Effects of certain goitrogenic and anti-goitrogenic compounds on oxygen uptake by mice.

3.2.1 Objective

The physiological and pharmacological properties of goitrogenic or anti-thyroid drugs such as thiourea, thionamide, aniline, thiocyanate, etc., and of antigoitrogenic drugs like thyroxine, thyronine, iodinated-casein, dinitrophenol, etc., have been studied repeatedly (Rivers, 1960; Bell and Williams,

1953; Purves, 1943 et al.). Response criteria included thyroid weight, histological changes in the tissues (liver, kidney, thyroid, spleen), PBI (protein-bound iodine) levels, T/S (radio iodide concentration in the thyroid to that in serum), basal metabolic rate of animal tissue (in vitro) and growth response.

The main purpose of this investigation was to study the effect of propylthiouracil (6-n-propyl-2-thiouracil) and iodinated-casein on metabolic rate by feeding to mice and measuring their oxygen consumption. Another objective of this experiment was to establish a primary reference for our future studies of nutritive value of meals of Brassica species, with respect to metabolic rate.

3.2.2 Materials and methods

3.2.2.1 Experimental design. The experimental design was a completely random design involving three treatments (control, thiouracil and iodinated-casein). Five mice were allotted to each treatment.

3.2.2.2 Diet formulation. The diets were employed of a semi-purified basal protein containing barley, sucrose, lard, cellulose, starch, vitamins, minerals, and soy-bean meal to which the required levels of iodinated-casein and thiouracil were added (Appendices A and B).

3.2.2.3 Animal management. Adult white male mice¹ weighing 23-25g were used in the 8-day feeding trial. Animals were individually housed in wire-bottomed metal cages equipped with collecting pans. The temperature of the laboratory

¹ Carworth farms No. 1 strain

was thermostatically controlled at $25^{\circ}\text{C} \pm 0.5$ (The temperatures in different parts of the laboratory as well as in different points of the battery were measured three times per day for one month and no significant differences were found). Feed and water were provided ad libitum; these were replenished every second day and the feed jars were cleaned of urine and feces daily.

3.2.2.4 Measurement of oxygen consumption.

The small animal respirometer used in this experiment was described in the previous chapter. On the seventh day of the trial the mice were weighed individually and subjected to respirometer measurements. After inserting the test animal into the respiratory chamber 30 to 40 minutes were allowed for acclimatization of the animal to the chamber and for temperature equilibrium to be reached in the system. Next the oxygen uptake of the mouse was recorded over a 90 minute period with the mouse at rest or asleep in chamber temperatures. Oxygen consumption for each mouse was determined on two consecutive days (7th and 8th days). Weight gain and total feed intake (for 8 days) were recorded at the end of the trial.

3.2.3 Results

Oxygen consumptions by individual mice fed thiouracil (0.075g/100g diet), iodinated-casein (0.12g/100g diet) and neither of these drugs were measured (table 20). Thiouracil feeding resulted in a reduction of oxygen consumption ($P < 0.01$) compared with the control diet whereas iodinated-casein caused an increase (table 20 and Appendix C). Furthermore, the oxygen consumption pattern for mice on the three treat-

Table 20. Estimation of O₂ uptake by mice fed iodinated-casein, control and thiouracil*

No. of mice	No. of observation	I-casein (0.12 percent)	Control	Thiouracil (0.075 percent)
1	1	4.269	3.589	2.998
	2	4.588	3.136	2.878
2	1	3.358	3.322	2.833
	2	3.237	3.240	2.664
3	1	3.691	3.401	2.212
	2	3.498	3.055	2.608
4	1	3.636	3.064	3.401
	2	3.248	3.273	3.369
5	1	3.775	3.064	2.560
	2	4.119	3.199	2.708
	mean	3.737 ^A	3.274 ^B	2.801 ^C

* Oxygen uptake expressed as ml. per gram of body weight per hour

A, B, C means are significantly different ($P > 0.01$)

ments, as illustrated in Fig. 26 indicated that there is a positive correlation between time and oxygen uptake although regression coefficients among treatments differed substantially. The differences among means for feed intake were, however, not significant (Appendix C), even though relatively large differences appeared (table 21). It, therefore, appears that animal response to these drugs was individually highly variable with respect to feed intake. Depressed gain of weanling mice fed thiouracil and iodinated casein has been reported (Bell and Williams, 1953).

In addition, data obtained on the first versus second day for oxygen consumption were compared statistically with the *t*-test (Steel and Torrie, 1960). This test indicated no significant difference between first and second observations.

In conclusion, the results of this experiment reveals that the electrolytic respirometer, operated as described, is capable of differentiating departures from normal thyroid activity (metabolic rate) of the order of 15 percent of normal, with a high degree of reliability. The apparatus, therefore, holds promise of being effective in studying the goitrogenic properties of Brassica meal.

3.3 Experiment 3

Effects of glucosinolates from several Brassica species, fed with and without myrosinase, on the metabolic rate of mice.

3.3.1 Description of anti-thyroid compounds

The term anti-thyroid agents is generally employed to designate substances inhibiting normal thyroid function; these agents include the following:

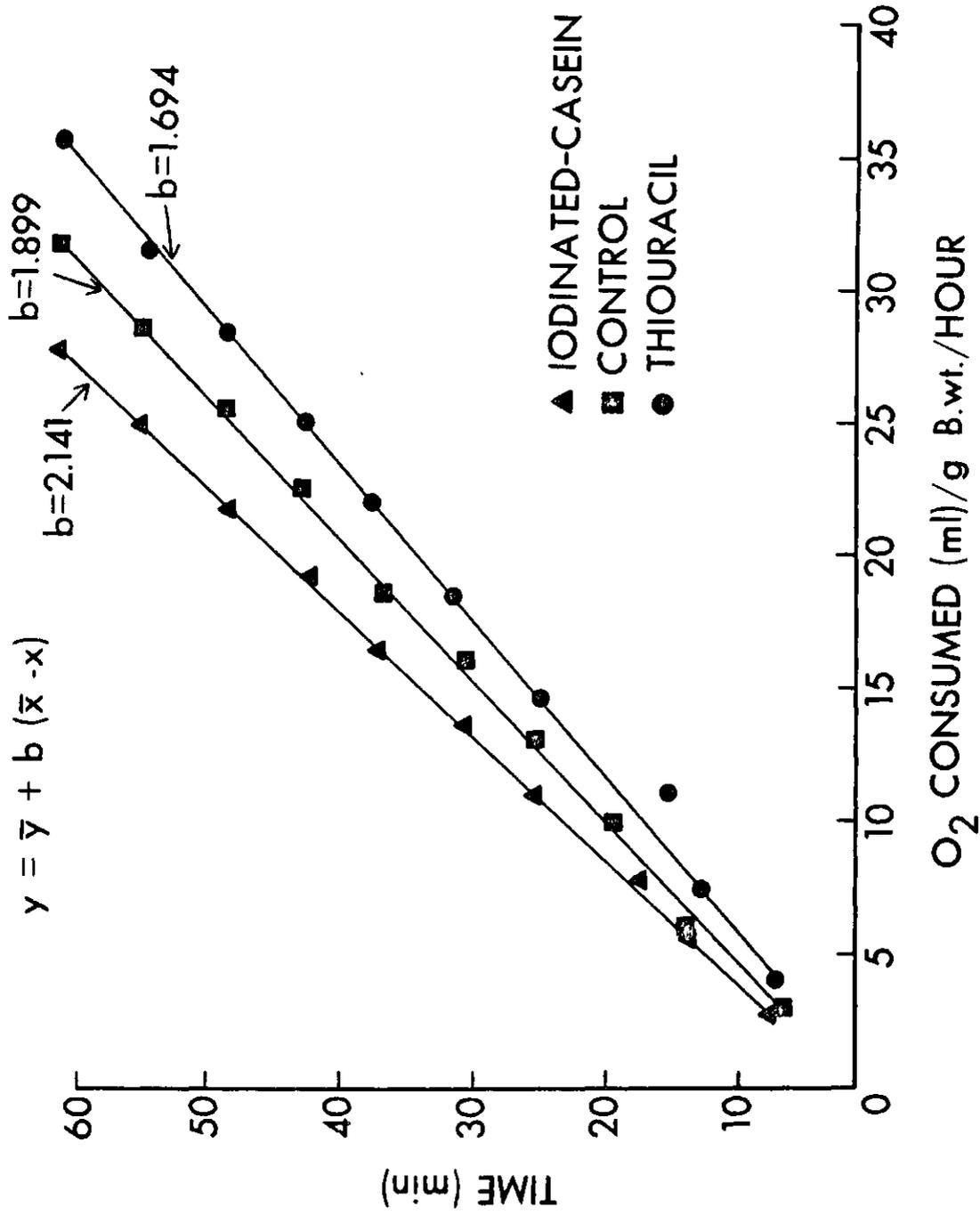


Fig. 26
 Oxygen consumption of mice fed thiouracil, iodinated-casein and control in relation to time

Table 21. Feed intake/g body weight/8 days

No. of mice	(g) I-casein (0.12 percent)	(g) Control	(g) Thiouracil (0.075 percent)
1	31.22	33.18	29.85
2	19.59	30.66	27.22
3	25.91	30.29	25.62
4	19.03	30.14	25.90
5	31.93	28.52	33.57
mean	25.67	30.55	28.43

- (1) Those which prevent the release of hormone(s) from the thyroid by a feed-back mechanism (eg. thyroxine and its related group).
- (2) Agents which retard synthesis of thyroid hormone(s) (eg. glucosinolate products, which either inhibit iodide uptake by thyroid through competition or by blocking the synthesis of thyroid hormone by affecting a specific enzyme. This group of anti-thyroid compound is of interest in this experiment).
- (3) Substances that inhibit utilization of thyroid hormones (viz. structural analogues).

Thyroid hormones participate in regulating rates of most biosynthetic processes, presumably by affecting the energy sources upon which the oxidative mechanism is dependent. Furthermore, it is well known that the measurement of oxygen uptake by animals provides a good index of thyroid function as it relates to the ingestion and anti-thyroid agents.

3.3.2 Objective

The objectives of this experiment were to study the effects of various kinds and levels of glucosinolates (table 22) as found naturally in certain Brassica species, on the metabolic rates of mice and, further, to study the effects of myrosinase on the anti-thyroid activity of the various glucosinolates.

3.3.3 Materials and methods

3.3.3.1 Experimental design. The experiment was one of 8 x 2 factorial design consisting of eight meals and 2 levels of myrosinase enzyme. Five weanling mice were allotted to each ration.

Table 22. Content of thioglucosides and residual oil in seed meals of Brassica species

Meal	Residual Oil Percent	Thioglucosides*				Total
		Allyl ITC	Butenyl ITC	Pentenyl ITC	OZT	
Echo	2.9	0	3.1	2.1	3.0	8.2
Nugget	2.3	0	2.2	0.4	8.9	11.5
Target	1.6	0	2.4	0.7	10.4	13.5
Bronowski	1.9	0	0.14	0.03	0.20	0.37
Yellow sarson	2.2	0	15.4	0	0	15.4
Oriental mustard	2.2	11.8	0	0	0	11.8
Oriental mustard + FeSO ₄	2.2	0	0	0	0	0

* Expressed as mg of the enzymatically released aglycones per g of meal

ITC = Isothiocyanate

OZT = (S)-5-vinyloxazolidine-2-thione (goitrin)

3.3.3.2 Diet formulation. The test diet was composed of the basal (oat meal, lard, casein, soyprotein, cellulose, amino acids, vitamins, minerals) and the source of protein (Appendix D). Each of the meals (as source of protein) was incorporated at a level of 20 percent of total diet. Each test diet was adjusted to contain 16 percent crude protein. Crude myrosinase was isolated from yellow sarson seed and added at 0.3 percent to half the diets. The diets were formulated to meet or exceed requirements of mice as defined by Bell.* Sixteen diets containing eight different meals as protein source with and without myrosinase were prepared (table 23).

All diets except for the daily allowance were kept in refrigeration during the course of the test in order to preserve myrosinase activity and to minimize a possibility of hydrolysis of thioglucosides prior to ingestion of the diets by animals.

The management of the animals was similar to that of Experiment 2.

3.3.3.3 Oxygen consumption measurement. The method and apparatus for measuring oxygen uptake were also as described in Experiment 2.

3.3.4 Results

Average of oxygen consumption data and feed intakes by mice are given in tables 24 and 25 and the obtained data were

* Unpublished data.

Table 23. Protein source and dietary myrosinase contents of the experimental diets

Protein Source	Myrosinase(percent in meal)
Soybean meal	0.0 0.3
Bronowski	0.0 0.3
Target	0.0 0.3
Oriental mustard	0.0 0.3
Oriental mustard + FeSO ₄	0.0 0.3
Yellow sarson	0.0 0.3
Echo	0.0 0.3
Nugget	0.0 0.3

subjected to analysis of variance as well as to co-variance (Appendix E). There was significant ($P < 0.01$) variation in oxygen uptake by mice due to being fed different meals. This significant difference is supported by the fact that the content of thioglucosides of meals are different in terms of quantity and quality (table 24). The greatest oxygen uptake depression occurred in mice fed Target and Nugget meals (Brassica napus L. varieties). This adverse effect occurred with and without added myrosinase. It should be pointed out that the responses of animals to Echo meal (Brassica campestris L.) and oriental mustard meal plus myrosinase (Brassica juncea L.) were quite similar with respect to oxygen consumption. Even though the allyl isothiocyanate mustard oil (formed from allylglucosinolate (sinigrin)) content of oriental mustard is about 30 percent higher than the content of the total glucosinolate products (3-butenyl, 4-pentenylisothiocyanate and (S)-5-vinyloxazolidine-2-thione) in Echo meal. Furthermore, allyl isothiocyanate (in oriental mustard meal) and 3-butenylisothiocyanate (in yellow sarson meal) produced similar effects on oxygen consumption. This was evident both in the absence and presence of myrosinase. By consulting tables 22 and 24, it seems that (S)-5-vinyloxazolidine-2-thione (goitrin) or its corresponding thioglucoside (progoitrin) had a more deleterious effect than either allyl or butenyl isothiocyanate. The animals fed the diets included Target and Nugget meals, (containing 0.208 and 0.178g goitrin in 100g diet, respectively) consumed less

oxygen than those fed soybean meal) or other meals under conditions of this experiment.

Bronowski meal showed a slightly harmful effect ($P > 0.05$) on oxygen consumption by mice relative to the soybean meal control. This effect became more severe in the presence of myrosinase. This finding indicates that the Bronowski seed contained enough identified glucosinolates to affect oxygen consumption rate or that the meal contained some unidentified anti-thyroid materials.

Ferrous treated oriental mustard showed greater oxygen consumption in mice than untreated Oriental mustard. This improvement could be attributed to the effect of the ferrous ion. However, the mice fed ferrous treated Oriental mustard meal still consumed less oxygen than those which received soybean or Bronowski meals. Those differences bring up the possibility that, detoxification of this meal with ferrous sulfate might result in the production of another toxic factor(s) (organic nitriles, thionamide, etc.), not indicated in table 22.

There were no statistical significant differences for meals in regard to a daily feed intake (Fig. 25). Bell also evaluated the effects of the meals studied in this experiment by feeding them to weanling mice in growth trials and found significant differences among the meals in terms of gain and feed intake. Thus the results of this experiment were not in agreement with Bell's findings with respect to feed intake.¹

The results indicate also that the inclusion of thioglucosidase in diets containing glucosinolates depressed oxygen uptake ($P < 0.01$). The differences among means of oxygen

¹Unpublished data

consumption due to the inclusion of myrosinase in meals were compared.¹ This comparison revealed that supplementary myrosinase had no effect on soybean and Ferrous-treated oriental mustard meals and only slightly affected Bronowski toxicity. The presence of myrosinase, however, enhanced the harmful effects of other meals. By referring to the amount and type of thioglucosides on the basis of their enzymic released aglycones (table 22), it might be postulated that the supplementary thioglucosidase activity was dependent upon the type and amount of substrates (thioglucosides).

The addition of myrosinase generally resulted in reduced feed intake ($P < 0.01$), although at the interaction level significant reduction occurred only in the case of soybean meal. The author is unable to explain why thioglucosidase reduced feed intake in the case of soybean meal diet. Myrosinase might have affected the animal gut and its content activities which in turn reflected on digestion of the diet components, an explanation, however, could be suggested. Moreover, correlation between oxygen and feed intake ($r = 0.089$) and regression coefficient ($b = 0.121$) were calculated. Since feed intake differences among treatments were not statistically significant, and regression coefficient was low and non-significant, the calculation of adjusted means was not attempted.

In summary, oxygen consumption by mice in response to certain meals of Brassica species was measured. Oxygen uptake

¹Duncan's multiple range test was employed to compare differences

Table 24. Means of oxygen consumption by mice fed several meals with and without myrosinase

Meal	Myrosinase		Average
	nil	0.3 percent	
Soybean meal	3.231	3.257	3.244
Bronowski	3.074	2.980	3.027
Target	2.713	2.586	2.649
Nugget	2.630	2.585	2.739
Echo	2.994	2.806	2.900
Yellow sarson	2.814	2.798	2.806
Oriental mustard	2.871	2.721	2.796
Oriental mustard + FeSO ₄	2.972	2.953	2.972
LSR min. 5 percent max.		0.156 ^A 0.196	0.111 ^B 0.129
All meals	2.915	2.83	
LSD 5 percent 1 percent		0.057 0.075	
Error mean square (159 df)	0.032		

* Expressed as ml/g body weight/hour

A = LSR
min. 1 percent 0.205
max. 0.245

B = LSR
min. 1 percent 0.146
max. 0.166

Table 25. Means of feed intake/100g body weight/day
(on the basis of dry matter)

Meal	Myrosinase		Average
	nil	0.3 percent	
Soybean meal	11.6	9.8	10.6
Eronowski	11.4	11.0	11.2
Target	11.1	10.4	10.7
Nugget	10.6	10.9	11.1
Echo	11.7	11.3	11.2
Yellow sarson	11.6	11.1	11.4
Oriental mustard	11.5	11.2	11.4
Oriental mustard + FeSO ₄	12.1	11.7	11.9
All meals	11.5	10.8	
LSD 5 percent		0.600	
1 percent		0.787	
Error mean square (64 df)	0.15		

was minimal by mice receiving Target and Nugget meals followed by those having other Brassica and soybean meals. It appeared that, among studied meals, Argentine-type (Brassica napus L.) meals were more deleterious to test animals.

3.4 Experiment 4

Effects of glucosinolates from Brassica napus L. and Brassica campestris L., fed with and without myrosinase, on metabolic rate of mice.

3.4.1 Objective

The previous experiment (Experiment 3) showed impaired oxygen consumption by mice fed meals of different Brassica species both with and without inclusion of myrosinase. Furthermore, the results of this investigation also made it apparent that the factors responsible for depression of the metabolic rate of mice must be attributed not only to the glucosinolate(s) content but also to the nature of thioglucosides. This was shown by the fact that the reduction in oxygen uptake was inversely related to the level of oxazolidinethione in the meals, particularly when myrosinase was added. It thus became evident that the adverse effect on myrosinase may be significantly influenced by the type of glucosinolates as well as by the total amount of these compounds.

The objective of this experiment was to study the variability existing among commercially prepared rapeseed meals in terms of effects upon oxygen metabolism with and without myrosinase.

3.4.2 Materials and methods

3.4.2.1 Experimental design. This experimental design was a 12 x 2 factorial with twelve different meals (eleven rapeseed meals and one soybean meal) and two (zero and 0.3 percent) levels of myrosinase, employing five mice per treatment group.

3.4.2.2 Diet composition. Twenty-four isonitrogenous diets (contained 17 percent crude protein) were prepared according to table 26. Constituents of the basal diet, the mineral and vitamin pre-mixes are given in Appendix F. Rations containing 35 (± 2) percent levels of the various commercial rapeseed meals or soybean meal control were formulated so as to provide adequate levels of all nutrients requirements of mice. Bell, (unpublished), employed similar rations for evaluating the digestible energy and metabolizable energy values of these rapeseed meals. Formulation was on the basis of dry matter in both experiments.

Eleven commercial rapeseed meals from several locations across Canada with different (1) processing (Expeller, pre-press solvent extraction and direct solvent extraction) and (2) from different varieties (Brassica campestris L. and Brassica napusL.) were utilized in this experiment (table 26). The analytical measurements of the hydrolyzed products of glucosinolate contents of these meals are shown in table 27.

Animal management and oxygen uptake measurement were similar to that of Experiment 3.

3.4.3 Results

The results may be conveniently divided into two parts:

Table 26. Ration formulation

Diet No.	Name of meal	Basal percent	Meal percent	Myrosinase percent	ITC + OZT* mg/g diet
1	A	67.37	32.63	0.0	3.426
2	A	67.37	32.63	0.3	3.426
3	B	65.10	34.90	0.0	1.501
4	B	65.10	34.90	0.3	1.501
5	C	64.16	35.84	0.0	2.616
6	C	64.16	35.84	0.3	2.616
7	D	63.39	36.61	0.0	4.503
8	D	63.39	36.61	0.3	4.503
9	E	66.09	33.91	0.0	3.059
10	E	66.09	33.91	0.3	3.052
11	F	66.02	33.98	0.0	3.330
12	F	66.02	33.98	0.3	3.330
13	H	63.88	36.12	0.0	2.637
14	H	63.88	36.12	0.3	2.637
15	L	64.56	35.44	0.0	2.516
16	L	64.56	35.44	0.3	2.516
17	N	63.78	36.22	0.0	2.318
18	N	63.78	36.22	0.3	2.318
19	P	65.01	34.99	0.0	3.149
20	P	65.01	34.99	0.3	3.149
21	S	63.99	36.01	0.0	5.437
22	S	63.99	36.01	0.3	5.437
23	soybean	73.03	29.97	0.0	0.000
24	soybean	73.03	29.97	0.3	0.000

* ITC = isothiocyanates and OZT = oxazolidethione

Table 27. Glucosinolate contents of commercial rapeseed meals

Rapeseed meal	Butenyl ITC		Thioglucosides*		Total
	Butenyl ITC	Pentenyl ITC	Pentenyl ITC	OZT	
A	2.1	0.5	0.5	7.9	10.50
B	1.6	1.0	1.0	1.7	4.30
C	3.0	2.2	2.2	2.1	7.30
D	2.9	1.0	1.0	8.4	12.30
E	3.6	2.8	2.8	2.6	9.00
F	4.1	3.1	3.1	2.6	9.80
H	2.9	2.4	2.4	2.0	7.30
L	2.6	2.2	2.2	2.3	7.10
N	2.5	1.9	1.9	2.0	6.40
P	3.6	3.0	3.0	2.4	9.00
S	3.1	0.9	0.9	11.1	15.10

* Expressed as mg of the released aglycones per g oil free meal.

ITC = isothiocyanate

OZT = (S)-5-vinylloxazolidine-2-thione (goitrin)

Thioglucosides measured by C.G. Youngs, National Research Council, Saskatoon, Sask.

(i) Oxygen uptake. Data pertaining to the responses of mice fed meals with and without myrosinase in this experiment are shown in tables 28 and 29. Statistical analysis of variance and of co-variance revealed that the response of animals to different treatments were significantly ($P < 0.01$) variable (Appendix G). This variation from control (soybean meal) in terms of oxygen uptake ranged from 14 to 20 percent below soybean meal ($P < 0.01$) (table 28). The greatest oxygen uptakes were observed by the groups receiving A and N rapeseed meals. The lowest values were obtained for S, C and B rapeseed meals in the same order. Duncan's new multiple range test (Steel and Torrie, 1960) was utilized to compare differences due to treatments. Dietary myrosinase supplementation of rapeseed meals consisting depressed both oxygen uptake and feed intake ($P < 0.01$). This finding confirmed our previous determinations in Experiment 3.

The interaction between meals and myrosinase was significant at ($P < 0.01$) except in the case of oxygen uptake, where the co-variance analysis indicated that this interaction was significant at the 5 percent level of probability (Appendix G). It was noticed that the interaction between Brassica species meals and myrosinase was, however, not significant in the Experiment 3 and as co-variance analysis is dealing with residual effect of these mentioned toxic factors. Thus, on the basis of these two facts it might be concluded that this deviation is a sign of existence of complex substance(s) in these meals, which affected the animal response

due to the presence of dietary myrosinase supplementation. The means of oxygen consumption of mice fed commercial rapeseed meals with no myrosinase supplementation are given in table 28. Among these meals H, A and N meals showed a superior meals B and S an inferior effect on oxygen consumption. However, the superior meals were still lower than soybean meal control in terms of oxygen metabolism. It is noticeable that this deviation was highly significant ($P < 0.01$). It is of particular interest that B meal contains the lowest level of glucosinolates, but it ranked poor in quality in this experiment. Furthermore, dietary myrosinase did not have an effect on B meal (table 28). Moreover, the results of this experiment were quite in agreement to gain values of growing mice that were fed these rations (Bell, unpublished data). Nevertheless, there was a discrepancy between gain value and oxygen measurement for B meal. The reason for this difference is less clear. There is no readily obvious parallel between the oxygen consumption, growth effects and the glucosinolate contents of the meals. (Tables 27 and 28). Thus it might be concluded that rapeseed meals contained other factor(s) than glucosinolates or their glucosinolates have the potential to release other toxic compounds besides isothiocyanates and oxazolidinethione in vitro as well as in vivo.

Myrosinase supplementation of rapeseed meal diets consistently depressed oxygen intake but there was a wide range of response (0.4 to 18 percent depression). The rapeseed

meal sample that was most affected by added myrosinase was H meal, but it is also of interest that it was one of the best meals in the absence of myrosinase. Furthermore, by referring to tables 27 and 28 with respect to the interaction between meals and myrosinase, in general, it is evident that thioglucosidase activity markedly enhanced the deleterious effects of rapeseed meals particularly in those containing high levels of oxazolidinethione. For instance, in A, D, and S meals this effect is very obvious. This fact was quite in agreement with that found in Experiment 3. The addition of dietary myrosinase did not have any effect either positive or negative on P meal which contains a low level of oxazolidinethione.

From these observations the conclusion probably can be drawn that changing the number of carbon molecules in the aglycones of glucosinolates changes the animal reaction to the addition of thioglucosidase. It must be kept in mind, however, that this comparison is not made on the basis of equal amounts of compared factors. It is worthwhile to note that myrosinase supplementation did not affect mice fed soybean diets significantly in terms of oxygen consumption, even though it reduced feed intake ($P > 0.01$). To explain this finding more investigations are required. In addition similar improvement occurred in an earlier investigation (Experiment 3) with regard to soybean meal plus thioglucosidase. It is difficult to properly interpret these effects due to lack of information about specificity of substrate for myrosinase. It is, however, possible that myrosinase stim-

ulates the motility of the gastrointestinal tract and its glands, and organism to secrete more hydrolyzing nutrient factor(s) which results in improving digestibility of diet. Mice fed diets containing soybean meal with and without myrosinase supplementation have different feed intake but are similar with regard to oxygen consumption (tables 28 and 29). Bell¹ found that dietary myrosinase supplementation improved digestibility of diets fed to mice.

(ii) Feed intake. Significant differences ($P < 0.01$) existed in feed intakes due to (1) treatment, (2) supplementation of myrosinase and (3) interaction between treatments and thioglucosidase. The diets containing C, P, S, E, and H meals resulted in significantly poorer feed intakes than other meals. The type and amount of glucosinolates in the rapeseed meals possibly influences feed intake.

The addition of 0.3 percent myrosinase to rapeseed meals resulted in a general depression of feed intake, the reduction was highly significant ($P < 0.01$) with a wide variation. Furthermore, there was a significant interaction effect between supplementation myrosinase and meal source. For example, in the presence of added myrosinase the diets containing D, S and P meals were more markedly affected and resulted in lower feed intake.

Correlation between oxygen uptake and feed intake was low and non-significant ($r = 0.093$). The regression coefficient for oxygen consumption and feed intake was calculated ($b = 0.123$), and it was not statistically significant.

¹Bell, unpublished data

Table 28. Means of oxygen consumption by mice fed rapeseed meals with and without myrosinase*

Meal	Myrosinase		Average	Gain ^a (g)
	nil	0.3%		
A	2.938	2.696	2.817	10.60
B	2.678	2.673	2.676	9.21
C	2.725	2.607	2.666	7.90
D	2.832	2.626	2.729	7.21
E	2.828	2.547	2.687	9.54
F	2.791	2.676	2.734	8.08
H	2.987	2.452	2.719	7.93
L	2.750	2.636	2.693	6.06
N	2.934	2.815	2.875	10.12
P	2.776	2.711	2.744	8.70
S	2.700	2.533	2.617	5.20
Soybean	3.272	3.285	3.278	10.15
LSR min.		0.162 ^A	0.115 ^B	
LSR max.		0.195	0.138	
All meals	2.851	2.688		
LSD 5 percent		0.039		
LSD 1 percent		0.055		
Error mean square (216 df)		0.034		

* Expressed as ml/g body weight/hour

a - quoted from Bell, J.M. Department of Animal Science, Univ. of Saskatchewan.

A - LSR min. 0.176
max. 0.249

B - LSR min. 0.151
max. 0.176

Table 29. Means of feed intake/100g body weight/day

Meal	Myrosinase		Average
	nil	0.3 percent	
A	11.40	12.35	11.87
B	13.77	9.65	11.18
C	10.28	9.97	10.15
D	10.76	7.26	9.01
E	10.25	10.13	10.20
F	10.72	10.08	10.41
H	10.76	12.22	11.48
L	12.77	9.65	11.21
N	13.06	10.66	11.86
P	10.36	8.68	9.53
S	11.32	8.41	9.98
Soybean	11.76	9.61	10.67
LSR min. 5 percent		0.400 ^A	1.400 ^B
LSR max.		1.675	1.775
All meals	11.35	9.88	
LSD 5 percent			
LSD 1 percent			
Error mean square (96 df)	0.2		
A = LSR 1 percent	min. 1.850		
	max. 2.175		
B = LSR 1 percent	min. 1.850		
	max. 2.237		

3.5 Experiments 5 and 6

Effects of in vitro treatment of rapeseed meal with and without myrosinase in buffered solutions of various pH values on growth rates, liver and kidney weights of weanling mice.

The results of the previous metabolic rate studies indicated that toxic factor(s) such as organic nitrile(s) might be released upon in vivo hydrolysis of glucosinolates from inactivated rapeseed meal. This means that variations in animal response, in terms of respiration, were related not only to the variety of rapeseed meal (glucosinolate contents) but to the possibility of hydrolyzing glucosinolates by an alternative pathway possibly as a result of processing method. These findings led to the initiation of these two experiments.

3.5.1 Experiment 5

3.5.1.1 Objective

This experiment was designed to study:

- (1) The effects on growth rate, liver and kidney weights of rapeseed meal with and without myrosinase pre-treatment and
- (2) The effect of acidic and natural pH (buffered solution) on the inactivated rapeseed meal on mentioned criteria.

3.5.1.2 Materials and methods

(i) Experimental design. The experiment was of a 4 x 2 factorial randomized complete block design involving four levels of pH (natural, 7, 5, and 3.6) and two levels of

myrosinase (zero and 0.3%). Five weanling male mice were allotted to each treatment.

(ii) Meal treatment. A commercially prepared, pre-press solvent type meal (Brassica napus L.) was utilized in this experiment. The treatment of meal with different pH buffer solutions (citric acid and sodium phosphate dibasic) was conducted in the following manner: one hundred parts by weight of meal were mixed in a glass beaker with 500 of the buffer solution and the slurry was stirred mechanically for two hours at room temperature. In preparation of the treated meal, natural pH distilled water was used instead of the buffer solution. The pH of the slurry was measured with a pH meter (radio-meter, Model 26) at three equal intervals during stirring.¹ Where required, freeze-dried myrosinase was added to the meal and mixed thoroughly; then the meal was subjected to buffer treatment. After two hours of stirring the wet rapeseed meal mash was thinly spread on a stainless steel tray and air-dried at room temperature with the aid of a fan. It was found necessary to take precautions (reducing relative humidity surrounding the tray) during drying of the meal, otherwise microbial growth would occur while the meal was drying. The amount of each of the buffered compounds in 100g of dried meal was calculated as follows:

pH	Citric acid (g)	Sodium phosphate dibasic (g)
3.6	6.50	4.57
5	4.65	7.26
7	1.69	11.69
natural (5.8)	0.0	0.0

¹pH of meal as natural was 5.8

(iii) Diet formulation. All diets were made up to contain 17 percent of protein. The protein sources were prepared rapeseed meal (19 percent of total diets) and soybean meal. The basal that was employed in this experiment was similar to Experiment 4 (Appendix F).

(iv) Animal management. Male mice (originally Carworth Farms No. 1, maintained as a closed colony since 1949) were weaned at a weight of 8 ± 0.5 g and group-fed a weanling diet. Forty weanling mice were then allotted at random within 4 days after weaning at weights of 10.5 ± 0.5 g and under 18 days of age, to their respective diets and individual cages. The environmental temperature was thermostatically controlled to 25°C. Feed and tap water were provided ad libitum for a 14-day feeding trial. At the end of the trial the animals were weighed and killed. Weight gain, weight of fresh liver, kidney and feed intake were recorded.

(v) Results. Weight gains: Animal response, in terms of weight gains, were affected by myrosinase supplementation ($P < 0.01$), by the pH of the buffered solution used ($P < 0.01$) and it was also evident that the effects of the myrosinase pre-feeding treatment of the meal was influenced by the pH used, as indicated by the interaction effect ($P < 0.05$) (table 30) (Appendix H). The lowest gain was observed in the mice fed meal treated at pH5. There were no significant differences between test animals fed meal of natural pH or buffered at pH7, in terms of gains. However, the effect of pH3.6 on gains was better than the pH5

(table 30). From this observation it might be concluded that pH5 was nearest the optimum condition for release of isothiocyanate, oxazolidinethione or other harmful substance(s) such as organic nitrile(s).

Organ weights: The average weight of mice liver weights was significantly ($P < 0.05$) smaller in test animals which were fed pH5 - buffered meal than other meals (Appendix H). In contrast, this difference was reversed when liver weight was calculated in proportion to the weight gain (g of liver per 100g of weight gain) (table 30). Thus one could interpret that pH had an effect on the chemical decomposition of the thioglucosides of rapeseed meal, which in turn resulted in enlargement of the liver on the basis of percentage of gain (adjusted weight) and in slower gains of mice relative to control animals fed natural meal. The initial weights of weanling mice in this experiment were almost equal (10 ± 0.5 g), thus liver and kidney weights were calculated in proportion to the weight gain instead of final weight.

The animals fed pH5 - buffered meal had the largest kidney (on the basis of 100g of weight gain) weights ($P < 0.01$). However, kidney weight with respect to absolute value of test animals and feed consumption were not significant from each other (table 30) (Appendix H). The error between absolute and corrected (g kidney/100g gain) kidney weights might be interpreted that animal gain was not proportional to the kidney weight. In the case of feed intake, it could be concluded that there was no indication of a severe

palatability problem in regard to this level of rapeseed meal.

The addition of dietary myrosinase (0.3% of meal) to the meals resulted in a general depression of gain, feed intake and highly ($P < 0.01$) enlargement of the relative weights of livers and kidneys (table 30) (Appendix H). However, the effect of myrosinase supplementation on the absolute weights of kidneys and livers was not significant.

The significant ($P < 0.05$) interaction between treated meals and dietary myrosinase was observed with respect to gain and feed intake (table 30) (Appendix H). These observations would be an indication that these factors affected animal response in different ways under conditions of this experiment. It was noticeable that the mice fed pH5 - buffered meal plus myrosinase showed the poorest gain and the largest liver weight (table 30) with regard to percentage of gain.

3.5.2 Experiment 6

3.5.2.1 Objective

The object of this study was to obtain data on the responses of weanling mice to higher levels of glucosinolates by maintaining the conditions of the previous experiment (Experiment 5) constant.

3.5.2.2 Materials and methods

The rapeseed meals were prepared as described previously for Experiment 5. The experimental design, animal management and diet composition were also as reported

Table 30. Effects on growth, liver, kidney weight and feed intake of mice fed diets containing rapeseed meal with and without myrosinase and prepared with buffered solutions of various pH's

Treatment	No. of mice	Gain (g)	Liver weight (g)	Liver ¹ weight percent	Kidney weight (g)	Kidney weight percent	Feed In-take (D.M) (g)
Meal + no myrosinase	20	9.75	1.23	12.61	0.29	2.97	42.2
Meal + myrosinase	20	6.38	1.30	20.37	0.29	4.54	36.0
LSD 1 percent		1.29	N S	2.47	N S	0.69	4.21
pH natural	10	9.56	1.26	13.17	0.31	3.24	38.3
pH3.6	10	8.02	1.28	15.96	0.31	3.86	40.1
pH5	10	5.64	1.02	18.08	0.25	4.43	37.2
pH7	10	9.06	1.49	16.44	0.28	3.09	40.9
LSR min.		1.81	0.20 ²	3.49		0.97	5.96
max.		1.94	0.21	3.73	N S	1.04	6.37
pH natural x no myrosinase	5	11.44	1.27	11.01	0.34	2.97	39.2
pH3.6 x no myro.	5	9.50	1.33	11.00	0.27	2.94	42.6
pH5 x no myro.	5	6.27	0.98	15.63	0.25	3.98	38.7
pH7 x no myro.	5	11.44	1.33	11.62	0.31	2.71	48.5

Table 30. Continued

pH natural x myrosinase	5	7.68	1.26	16.40	0.29	3.77	37.5
pH3 x myrosinase	5	6.54	1.23	18.80	0.35	5.35	37.6
pH5 x myrosinase	5	5.02	1.06	21.11	0.26	5.18	35.7
pH7 x myrosinase	5	6.30	1.66	26.34	0.26	4.12	33.3
LSR* min.		1.91					6.24
5 percent max.		2.19	N S	N S	N S	N S	7.17
Error mean square (32 d.f.)		2.19	0.047	8.06	0.34	0.63	23.49

¹Liver and kidney weights adjusted on the basis of 100g of weight gain

* and 2

Least significant range, minimum and maximum values required for significant differences at $P > 0.05$ or $P > 0.01$, respectively.

N.S., non-significant

in Experiment 5. In this experiment prepared rapeseed meal was incorporated into diets at a level of 28 percent instead of the 19 percent meal which was included in the diets in the previous feeding trial.

3.5.2.3 Results

Buffered meals, either acidic or basic pH caused a highly significant ($P < 0.01$) reduction in gains and feed intakes of mice relative to the control (natural meal) (table 31) (Appendix I). In general, the adverse effects of buffered meals were quite in agreement with previous findings (Experiment 5), but there was a great difference between animal response in this trial and the first trial in terms of gains. In Experiment 6, test animals gained almost 50 percent of those in Experiment 5. This drastic reduction could be due to higher utilization level of rapeseed meal in the second experiment (tables 30 and 32). It was of particular interest that the meal buffered with pH5 showed similar effect in both experiments. In addition the effect of buffered meals on adjusted weights (g organ/100g of gain weight) of liver and kidney was significant (Appendix I).

Inclusion of dietary myrosinase consistently affected all criteria which were evaluated (table 31) (Appendix I). This means thioglucosidase supplementation not only severely depressed gain and feed consumption and the absolute weight of liver and kidney, but also caused an enlargement of the liver and kidney in relation to percent of gain weight ($P < 0.01$). This observation confirmed the previous finding in the case of organ weights, that means in both trials animals fed pH5

buffered meal, had the largest liver weight in terms of percent gain weight (adjusted weight). The liver and kidney enlargement might be attributed to cyanogenetic (nitriles) material rather than goitrogenic materials.

The combination (interaction) of myrosinase with different meals increased significantly the liver weight either as an absolute value or as gain in weight as a percentage of gain weight. Furthermore, the livers of mice that received buffered meals (at pH5 and 3.6) were dark in color and firmer in texture as was also evident in the previous experiment.

The meals treated at different levels of pH without myrosinase supplementation were subjected to chemical assay and the glucosinolate products were determined (table 32). In contrast to the biological responses, there were no differences between meals prepared in different ways, with respect to thioglucoside products.

3.6 Experiment 7

Effects of treating rapeseed meal with buffered solutions containing various levels of cystine and with and without filtration.

In the previous studies (Experiments 5 and 6) it was found that the biological value of the rapeseed meal (Brassica napus, commercially processed by pre-press solvent) was

Table 31. Effects on growth, liver, kidney and feed intake of mice by feeding diets containing rapeseed meal with and without myrosinase and prepared with buffered solutions of various pH's

Treatment	No. of mice	Gain (g)	Liver weight (g)	Liver ¹ weight percent	Kidney weight (g)	Kidney weight percent	Feed In-take (g)
Meal + no myrosinase	20	7.37	1.12	15.19	0.28	3.80	38.9
Meal + myrosinase	20	3.31	0.93	28.10	0.23	6.94	29.9
LSD 1 percent		1.09	0.12	4.85	0.03	1.45	4.43
pH natural	10	6.77	1.07	15.80	0.28	3.60	39.0
pH3.6	10	4.97	1.05	21.12	0.25	5.03	31.9
pH5	10	5.29	1.03	19.47	0.25	4.72	32.4
pH7	10	4.32	0.95	21.99	0.25	5.78	34.2
min. LSR		1.43		6.87		2.05	6.27
max.		1.53	N S	7.35	N S	2.19	6.71
pH natural x no myrosinase	5	8.36	1.20	14.35	0.30	3.58	44.7
pH3.6 x no myro.	5	7.11	1.22	17.15	0.30	4.21	38.6
pH5 x no myro.	5	7.86	1.01	18.12	0.28	3.56	37.8
pH7 x no myro.	5	6.13	1.05	18.14	0.27	4.40	34.0

Table 31. Continued

pH natural x myrosinase	5	0.94	31.14	0.26	5.01	33.3
pH3.6 x myro.	5	0.88	31.20	0.21	7.44	25.3
pH5 x myro.	5	1.05	38.74	0.23	8.49	26.9
pH7 x myro.	5	0.85	33.86	0.24	9.56	34.00
LSR* min.		0.19	9.71 ²		2.15	
5 percent max.		0.22	11.01	N S	2.47	N S
Error mean square(32 df)		0.022	31.21	0.0016	2.77	26.02

¹Liver and kidney weights adjusted on the basis of 100g of weight gain

* and ²Least significant range, minimum and maximum values required for significant differences at $P > 0.05$ or $P > 0.01$, respectively.

N.S. non-significant

Table 32. Thioglucosinolate contents of buffered meals*

PH	Butenyl ITC	Pentenyl ITC	OZT	OH-nitrile	Total
3.6	2.44	2.44	9.21	0.51	13.12
5.0	2.50	0.97	9.32	0.20	12.99
7.0	2.30	1.38	8.85	0.20	12.73
Natural	2.90	1.00	8.40	0.05	12.35

* Expressed as mg of the released aglycones per g of meal

ITC = isothiocyanate

OZT = oxazolidinethione

severely impaired when it was buffered at pH5. It was concluded that the buffer might (1) have accelerated hydrolysis of glucosinolate contents of the meal and (2) have changed the pathway of hydrolysis to give rise to nitrile(s) in addition to goitrogenic compounds.

The toxic action of nitriles on animal organs (liver, kidney, lung, etc.) is well known. It has been accepted that the limiting factor in detoxifying cyanide in vivo is sulfur donor compounds such as cystine, glutathione, thiosulfate, etc. The following studies were undertaken to determine whether cystine supplementation will affect rapeseed meal toxicity.

This investigation included two feeding trials (experiments A and B) with cystine-treated rapeseed meal, then processed with different procedures.

3.6.1 Experiment 7A

Effects of treating rapeseed meal with buffered solution containing various levels of L-cystine.

3.6.1.1 Objective

The purpose of this investigation was to study:

- (1) The combination effect of various levels of cystine supplementation and pH5 - buffered solution on rapeseed meal detoxification and
- (2) Both water soluble and insoluble non-enzymatic hydrolyzed glucosinolate products (goitrogenic and cyanogenetic).

3.6.1.2 Materials and methods

(i) Experimental design. The factorial experiment was a completely randomized design of five levels (0.0, 0.26, 0.72, 1.44 and 2.88/100g of protein source) of L-cystine, three different protein sources (Brassica napus meal, Brassica napus meal (Bronowski) and casein) and extraction against no extraction. Each treatment had five replications.

(ii) Meal preparation. The protein sources were mixed thoroughly with various levels of L-cystine then buffered at pH5 in the manner of that of Experiment 5. Fifteen of the prepared mash slurries were subjected to filtration. The extract was then separated by filtration of the slurry in a double-cotton cheese cloth, and the residue dried at room temperature by the aid of a fan. The extracted soluble material (from meals and casein) was collected and dried in a drying oven and the dried material was weighed.

(iii) Diet formulation. Three isonitrogenous diets were formulated to contain 15 percent crude protein and be nutritionally adequate for the growing mice (Appendix J). These protein sources were added to the basal ration as dictated in the experimental design. The vitamins and minerals and content of basal diet were similar to those of Experiment 5.

Amino acid analysis of the untreated casein and rapeseed meals (Appendix K) was performed on a technicon auto-analyzer.

(iv) Animal management and procedure was similar to that of Experiment 5.

(v) Results. Feeding 0.26, 0.72 and 2.88 percent cystine slightly improved rate of weight gain of growing mice (table 33). Supplementary cystine at both zero or higher than 0.26 percent level caused the enlargement of liver and kidney weights in terms of percent of weight gain (adjusted weight). The animals fed diets containing the lowest level (0.26g cystine/100g of protein source) of cystine had the smallest liver ($P < 0.01$) and kidney weights ($P < 0.05$) with respect to adjusted weight (table 33) (Appendix L). Inclusion of cystine to the ration did not show any effect on feed intake. Protein source significantly affected gain, adjusted weights of liver and kidney, and feed intake. Under conditions of this experiment, rapeseed (Brassica napus) meal commercially prepared by pre-press solvent extraction was inferior ($P < 0.01$) to casein and Bronowski meal on the basis of growth and enlarged liver and kidney (adjusted weight) of growing mice. Test animals fed commercial rapeseed meal gained 74 percent and 84 percent of those receiving casein and Bronowski meal, (for 14 days feeding trial) in the same order. Moreover, commercial rapeseed meal caused increase in liver and kidney weights (adjusted weight). Furthermore, casein was superior to Bronowski meal on the basis of mouse growth and adjusted weight of mouse liver and kidney. This inferiority might be due to glucosinolate content, imbalanced amino acids (Appendix K), protein quality, and crude fiber contents, of rapeseed meals compared with casein.

It was of particular interest to note that extraction

of the slurry had no effect on those parameters that were measured.

Statistical analysis of the data showed several significant interactions (Appendix L). Incorporation of 0.26 percent of L-cystine improved the quality of commercial rapeseed meal in terms of growth rate and adjusted liver and kidney weights. Addition of cystine to Bronowski was also beneficial. However, high levels of supplementary cystine showed adverse effects (table 33). Protein source x extraction did not improve the quality of protein source with regard to evaluated criteria in this experiment.

Diets containing buffered casein (at pH5) x cystine and not filtered improved rate of gains (table 34). This improvement could be due to cystine supplementation as casein already contained trace amounts of cystine. In the case of liver and kidney weights this interaction effect was small even though it was statistically significant ($P < 0.05$).

Diets containing Bronowski meal plus cystine either filtered or not filtered affect animal responses ($P < 0.01$). Animals fed 0.72 percent cystine had the largest weight gains ($P > 0.05$) and the smallest adjusted weights of liver and kidney (table 35). However, supplementation with cystine did not influence feed intake.

Filtration of pre-treated commercial rapeseed meal with cystine significantly ($P < 0.01$) improved the quality of the meal compared with no cystine treated x non-filtered meal (table 36). Bell¹ fed the same level of the

¹Unpublished data

commercial rapeseed meal to growing mice under similar conditions and found they gained 8 ± 0.5 g during 14 days on test. This improvement might be due to filtration, removing buffer soluble glucosinolate products, as addition of cystine levels in this case did not have effect.

(Table 36) (Appendix L).

It was of particular interest that, incorporation of cystine at 0.26 percent level into commercial rapeseed meal without filtration improved the quality (157%) over the non-filtered meals which supplemented with either zero or higher levels of cystine (table 36). The responses of animals fed diets containing non-filtered commercial rapeseed meal plus 0.26g cystine per 100g of the meal were almost equal to those fed casein 0.26 percent (or zero percent) cystine unfiltered (tables 34 and 36). From these observations one might conclude that under conditions of this experiment the 0.26g of supplementary cystine detoxified (probably through combination with organic nitrile(s) and isothiocyanates) rapeseed (Brassica napus) meal without affecting the metabolic pathways of other dietary constituents. However, the animal which received commercial meal plus cystine (except the 0.26 percent level of cystine) had a larger liver with dark color and firmer texture. Similar observations were made in previous studies, (Experiments 5 and 6), when rapeseed meal was buffered at pH5. Thus these abnormalities (low gain, large and colored liver and kidney) might be an indication of deleterious effects of products (organic nitriles and goitro-

genic compounds) of glucosinolate hydrolysis under these conditions.

In summary, the results from this investigation indicated that (1) the chemical analysis showed that filtration of buffered rapeseed meal removed approximately 50 percent of hydrolyzed glucosinolate products (cyanogenic and goitrogenic) (table 37)*. The addition of cystine did not affect the removal of glucosinolate products (table 37) and (2) filtration of buffered meal pre-treated with cystine improved the biological responses of test animals in terms of growth rate and conditions of liver and kidneys (table 36). The results of chemical and biological assays are in agreement with each other. Furthermore, the addition 0.26g L-cystine to 100g rapeseed (Brassica napus) meal by buffering at pH5 without filtration improved the meal quality by 42 percent in terms of gains of growing mice.

In short, under conditions of this experiment it seems that cystine(0.26 percent)counteracted toxicants present in rapeseed meal.

3.6.2 Experiment 7B

Effects of treating rapeseed meal with buffered solutions containing various levels of L-cystine with and without myrosinase.

3.6.2.1 Objective

In the previous part of this experiment it was

* Performed by Youngs, National Research Council, Saskatoon, Saskatchewan.

Table 33. Effects on growth rate, liver, kidney weights and feed intake of mice by feeding diets containing buffered (at pH5) casein and rapeseed meals plus cystine with and without filtration

Percent Cystine	No. of mice	Gain (g)	Liver weight (g)	Liver weight percent	Kidney weight (g)	Kidney weight percent	Feed Intake (g)
0.00	30	10.81	1.27	11.98	0.33	4.08	45.33
0.26	30	11.18	1.7	11.39	0.33	3.12	46.19
0.72	28	11.56	1.29	12.06	0.35	4.53	45.67
1.44	30	10.80	1.35	13.19	0.33	5.33	45.57
2.88	30	11.19	1.36	12.51	0.35	4.07	46.01
min. 5 percent				1.07		0.32	
max.				1.03		0.36	
<u>Protein Source</u>							
Casein	50	12.69	1.35	10.67	0.35	2.85	47.23
Bronowski	50	11.21	1.33	12.17	0.34	3.12	46.78
C-meal ¹	48*	9.43	1.24	13.84	0.32	3.69	43.25
min. 1 percent		0.90		1.08		0.25	
max.		0.94		1.14		0.28	
Filtration	73	11.56	1.35	11.99	0.33	3.01	46.81
Not-filtration	75	10.63	1.25	12.46	0.34	3.41	44.69
LSD							

¹C-meal = Commercial rapeseed meal.

Table 33. Continued

Cystine x Protein Source												
percent cystine x casein												
0.00	10	12.49	1.32	10.17	0.37	2.94	48.03					
0.26	10	11.61	1.25	10.84	0.34	2.99	48.50					
0.72	10	13.26	1.42	10.37	0.37	2.85	48.04					
1.44	10	13.46	1.35	10.54	0.33	2.56	46.80					
2.88	10	12.44	1.42	11.47	0.36	2.99	44.78					
percent cystine x Bronowski												
0.00	10	10.94	1.35	12.65	0.35	3.27	46.17					
0.26	10	11.12	1.27	11.39	0.32	2.93	46.38					
0.72	10	12.63	1.31	11.10	0.36	2.27	45.99					
1.44	10	9.74	1.29	13.34	0.31	3.27	46.67					
2.88	10	11.78	1.44	12.37	0.37	3.20	48.19					
percent cystine x C-meal												
0.00	10	9.00	1.15	13.13	0.29	3.50	41.20					
0.26	10	10.83	1.30	11.95	0.33	3.10	43.70					
0.72	8	8.79	1.14	14.75	0.30	3.81	42.97					
1.44	10	9.20	1.42	15.66	0.36	4.23	43.26					
2.88	10	9.35	1.22	13.70	0.33	3.73	45.15					
LSR												
min.	1	2.01		2.45		0.74						
max.	1	2.38		2.90		0.87						

Table 33. Continued

<u>Cystine x Filtration</u>									
percent cystine x filtration									
0.00	15	11.95	1.41	11.64	0.36	3.00	46.70		
0.26	15	10.80	1.26	11.68	0.31	2.93	45.98		
0.72	13	12.28	1.34	11.86	0.34	2.92	47.15		
1.44	15	11.31	1.40	11.43	0.34	3.12	46.78		
2.88	15	11.49	1.41	12.35	0.35	3.12	47.45		
percent cystine x not filtration									
0.00	15	9.67	1.13	12.33	0.32	3.38	43.97		
0.26	13	11.56	1.28	11.10	0.35	3.08	46.41		
0.72	15	10.83	1.23	12.28	0.34	3.49	44.18		
1.44	15	10.29	1.30	13.93	0.33	3.59	44.37		
min.		1.65			0.04				
LSR		1 percent			0.047				
max.		1.92							

Table 33. Continued

<u>Protein Source x Filtration</u>									
Casein	25	12.86	1.41	10.88	0.35	2.84	49.38		
Bronowski	25	11.13	1.35	12.37	0.34	3.09	47.19		
C-meal	23	10.71	1.33	12.76	0.32	3.12	43.87		
<u>Protein Source x Filtrate</u>									
Casein x Filt.	25	12.44	1.29	10.47	0.35	2.86	45.08		
Bronowski	25	11.33	1.31	11.99	0.35	3.16	46.38		
C-meal	25	8.15	1.16	14.92	0.32	4.22	42.64		
LSR	min.								
	5 percent								
	max.								
Error mean square	(118 d f)	3.068	24.288	4.549	0.310	0.407	48.101		

* missing data = 2

Table 34. Effects on growth rate, liver, kidney weights and feed intake of mice by feeding diets containing buffered casein plus cystine with and without filtration

Treatment	No. of mice	Gain (g)	Liver wt. (g)	Filtered after buffered treatment		Kidney wt. (g)	Kidney wt. percent	Feed intake (g)
				Liver wt. percent	Kidney wt. percent			
0.00	5	13.61	1.48	10.06	0.39	2.94	50.33	
0.26	5	11.25	1.27	11.35	0.33	2.99	51.36	
0.72	5	13.87	1.44	10.41	0.37	2.74	50.54	
1.44	5	13.80	1.50	10.91	0.36	2.64	50.82	
2.88	5	11.79	1.38	11.67	0.34	2.90	43.88	
Not Filtered after buffered treatment								
0.00	5	11.37	1.16	10.29	0.35	2.94	45.74	
0.26	5	11.97	1.23	10.33	0.35	2.99	45.65	
0.72	5	12.66	1.40	10.33	0.37	2.97	45.55	
1.44	5	13.12	1.21	10.17	0.30	2.49	42.78	
2.88	5	13.10	1.46	11.27	0.38	2.95	45.69	
LSR min.	5 percent	2.17						
max.		2.57						
Error mean square (118df)		3.068	24.288	4.549	0.310	0.407	48.101	

wt. = weight

Table 35. Effects on growth rate, liver, kidney weights and feed intake of mice by feeding diets containing buffered Bronowski meal plus cystine with and without filtration

Treatment	No. of mice	Gain (g)	Liver wt. (g)	Liver wt. percent	Kidney wt. (g)	Kidney wt. percent	Feed intake (g)
Percent Cystine			Filtered after buffered treatment		Not filtered after buffered treatment		
0.00	5	11.49	1.51	13.31	0.37	3.15	47.95
0.26	5	10.93	1.23	11.19	0.30	2.72	44.53
0.72	5	12.63	1.29	11.08	0.36	2.93	46.63
1.44	5	9.00	1.22	13.60	0.32	3.59	46.28
2.88	5	11.61	1.44	12.57	0.35	3.07	50.56
0.00	5	10.40	1.20	11.99	0.34	3.40	45.59
0.26	5	11.31	1.31	11.59	0.35	3.14	48.24
0.72	5	12.63	1.23	11.13	0.36	2.99	45.35
1.44	5	10.48	1.37	13.09	0.31	2.96	47.06
2.88	5	11.96	1.44	12.17	0.39	3.33	45.69
min.		2.17					
LSR 5 percent max.		2.57					
Error mean square (118d.f.)		3.068	24.288	4.549	0.310	0.407	48.101

Table 36. Effects on growth rate, liver, kidney weights and feed intake of mice by feeding diets containing buffered commercial rapeseed meal plus cystine with and without filtration

Treatment	No. of mice	Gain (g)	Liver wt. (g)	Liver wt. percent	Kidney wt. (g)	Kidney wt. percent	Feed in-take (g)
Percent Cystine			Filtered after buffered treatment				
0.00	5	10.75	1.25	11.56	0.31	2.93	41.82
0.26	5	10.24	1.29	12.52	0.31	3.08	42.07
0.72	5	10.36	1.21	14.11	0.30	3.09	44.29
1.44	5	11.11	1.50	12.79	0.34	3.13	43.26
2.88	5	11.09	1.41	12.82	0.37	3.39	47.92
			Not filtered after buffered treatment				
0.00	5	7.25	1.05	14.71	0.27	4.08	40.58
0.26	5	11.42	1.31	11.38	0.35	3.12	45.34
0.72	5	7.22	1.08	15.39	0.31	4.53	41.66
1.44	5	7.27	1.34	18.54	0.38	5.33	43.27
2.88	5	7.61	1.04	14.59	0.29	4.07	42.37
min. 5 percent LSR		2.17		2.64		0.79	
max. Error mean square (118d.f.)		2.57		3.13		0.94	
		3.068	24.288	4.549	0.310	0.407	48.101

Table 37. Glucosinolate contents of commercial rapeseed meal treated at different conditions.¹

Treatment	Glucosinolate ²			Total
	Butenyl	Pentenyl	OZT	
Percent Cystine				
0.00 x Filt.	1.76	0.70	4.39	6.85
0.26 x Filt.	1.60	0.66	3.73	6.00
1.44 x Filt.	1.39	0.66	4.31	6.30
2.88 x Filt.	1.36	0.59	3.68	5.63
0.00 x nat.pH x Filt.	2.72	1.06	6.21	10.00

¹Cystine thoroughly mixed with meal then buffered at pH₅

²Expressed as mg of the released aglycones per gram of meal

found that the addition of a certain level of L-cystine to rapeseed meal at a certain pH improved the meal quality.

This experiment was designed to study the effects of pre-treated Brassica napus meal, commercially prepared by pre-press solvent extraction with various levels of cystine, myrosinase, and treated at different pH values.

3.6.2.2 Materials and methods

(i) A 3x2x2 factorial completely randomized design was used involving three levels (0.26, 0.52, and 1.04 percent) of L-cystine*, two levels (0.0 and 0.3 percent) of myrosinase* and the natural pH of the meal against the pH5 buffered meal. Five weanling mice were allotted to each treatment.

(ii) Meal preparation and animal management. Meal preparation, animal management and procedures were similar to those of the first part of this experiment.

(iii) Results. The average gains, liver and kidney weights of test animals are shown in table 38. Mice which received pH5 buffered rapeseed meal plus 0.26 percent L-cystine gained satisfactorily. This observation was close to that found in the previous experiment (table 36). The livers and kidneys of these mice were normal in comparison to those fed casein (previous part). However, under conditions of this experiment, supplementation with cystine was generally harmful and lethal for test animals (table 38). The reasons

* = percent of meal

Table 38. Average gains and organ weights of mice fed prepared rapeseed meal for 14 days

pH	Myrosinase percent	Criterion	Cystine percent		
			0.26	0.54	
Natural	0.01	Weight gain	4.12	4.71	died
		Liver weight	0.78	0.97	died
		Kidney weight	0.24	0.24	died
Natural	0.3	Weight gain	4.59	2.44	died ⁴
		Liver weight	1.08	0.94	died
		Kidney weight	0.27	0.25	died
5	0.0	Weight gain	10.95	1.33	-2.66 ²
		Liver weight	1.36	0.92	0.85
		Kidney weight	0.33	0.20	0.19
5	0.3	Weight gain	0.72	2.75	-0.79 ²
		Liver weight	0.83	1.75	0.77
		Kidney weight	0.19	0.28	0.20

⁴ and ²The number of mice died within 14 days of trial.
gain or organs expressed in g.

were not clear. Furthermore, it was also hard to interpret deleterious effects of myrosinase in this experiment because of the high toxicity of the meals with and without myrosinase supplementation. The livers of the test animals (except those fed 0.26g cystine, no myrosinase and pH5 buffered meal) were dark in color and of firm consistency.

On the basis of the data obtained from these two parts of this experiment, one might conclude that the proper level of cystine and a pH5 buffer solution would detoxify either glucosinolate contents of rapeseed meal or their hydrolyzed products, probably nitrile(s) and isothiocyanates.

3.7 Experiment 8

Effects of treatment of rapeseed meal with various levels of cystine and iron sulphate and of dietary supplementation with myrosinase on the responses of growing mice.

The use of cystine has been effective in alleviating the effects of the present toxic factors in rapeseed (Brassica napus) meal in terms of (1) the mouse growth depression and (2) the diminishing enlargement of the liver and kidney (Experiments 5, 6, and 7). This improvement (detoxification) was probably attributed to the chemical ability of cystine to combine with released organic nitrile(s) and isothiocyanates from glucosinolate contents. However, it seems that the need for more investigations in this regard is justified.

3.7.1 Objective

The present investigation was undertaken to study the following purposes:

- 1 - treatment of rapeseed (Brassica napus) meal with various levels of ferrous sulfate and L-cystine.
- 2 - the effect of myrosinase supplementation of the diet on the feeding value of rapeseed meal.
- 3 - the effect of acidic pH on rapeseed meal quality.
- 4 - the effects of the various combinations of ferrous, cystine, myrosinase and pH treatments on growth rate and selected organs of growing mice.

3.7.2 Materials and methods

This experiment of factorial design involved three (0.0, 0.13, and 0.26 percent in meal) L-cystine levels, two (pH5 and natural pH) levels of pH, myrosinase (0.3 percent, in meal) versus no myrosinase supplementation, four (0.0, 0.35, 0.7, and 8 percent in meal) ferrous sulfate levels and five replications per treatment (Appendix M).

The details regarding preparation of the rapeseed meal samples, the diets (contained 28 percent treated rapeseed meals), animal management and other requiremental procedures were as described for Experiments 5 and 6.

3.7.3 Results and discussion

Analysis of variance revealed that cystine incorporation into rapeseed meal improved ($P < 0.01$) gains and feed intakes of mice (tables 39 and 40) (Appendix N). This finding supported our previous demonstration. Acidic (pH5) buffered meal depressed gain and feed intake but increased liver and kidney weights in terms of adjusted weight, (tables 41 and 42). Enlargement of the liver was also in agreement with that

found for the acidic pH in Experiments 5 and 6. Depression of gain and liver enlargement could be a reflection of cyanogenetic and goitrogenic products of hydrolyzed glucosinolates of rapeseed meal treated in acidic media. Adverse effects of myrosinase supplementation were very severe on all of the criteria which were evaluated (tables 39, 40, 41, 42, 43, and 44), (Appendix N). The mode of action of myrosinase on glucosinolate cleavage has been discussed previously, (Experiments 4, 5 and 6).

Benefits from chemical treatment of rapeseed meal with iron sulfate were not evident in this experiment. This might be due to the low level use of iron sulfate with respect to the level of glucosinolate content. However, a high level (8 percent) of ferrous sulfate supplementation showed a harmful effect on the biological activity of mice with regard to gain, inspected organs and feed intake. (Appendices O,P,Q,R,S, and T). We might conclude that the high level of ferrous iron caused an adverse effect through the imbalanced absorption of other minerals in diet, for instance Cu, Zn, Mg, etc., and utilization of nutrients by making complex compounds in the body; for example, chelation with amino acids (Mahler and Cordes, 1967). Furthermore, the high level of ferrous iron has probably given rise to organic nitriles, which are harmful to animals. Decomposition of the predominant glucosinolate contents of rapeseed meal chemically and enzymatically has been investigated by Youngs,^{*} who observed that utilization of higher than

* Private communication

1 percent of ferrous sulfate and lower than 8 percent of this chemical compound (depending on the level of glucosinolate contents) is useful for improving the quality of rapeseed meal.

Incorporation of increasing levels of cystine was in general beneficial, but the effects were largely restricted to rapeseed meal treated at pH5 and treated with no myrosinase ($P > 0.01$). Diets containing meal treated with cystine at pH other than 5 resulted in poor gain, poor feed intake, and liver and kidney enlargement of mice. These observations were due apparently to the use of 0.0 percent and 0.13 percent cystine. Furthermore, the inclusion of myrosinase to pre-treated meal with cystine drastically damaged the quality of the rapeseed meal with regards to the animal responses (tables 39, 40, 41, 42, 43, and 44) (Appendix N).

The pH5 buffered solution had definite adverse effects on enzyme-inactivated rapeseed meal (tables 45, 46, 47, 48, 49, and 50) (Appendices N, O, P, Q, R, S, and T). It was thought that these adverse effects were due to the release of organic nitriles as well as goitrogenic compounds. It is of particular interest that this acidic pH lies within the range of the gastrointestinal tract pH in animals. Thus it is important and necessary to try to find a satisfactory method to either entirely remove or neutralize glucosinolate contents of rapeseed meal.

We have been able to remove these contents by buffering (at pH5) plus filtering the rapeseed (Brassica napus) meal

(Experiment 7) and to neutralize them by cystine supplementation of upto 60 percent glucosinolate products (table 60).

Incorporation of increasing levels of cystine was in general beneficial, but the effects were largely restricted to rapeseed meal treated at pH5 and to meal treated with 0, 0.35 or 0.70 percent FeSO_4 (tables 45, 46, 47, 48, 49, and 50). Results were rather erratic with FeSO_4 at 8 percent and were especially poor when 0.13 or 0.26 percent cystine was added. Treatment of rapeseed meal with the acidic pH solution was deleterious with the exception of samples treated with 8 percent and 0.13 or 0.26 percent cystine. The use of FeSO_4 at 0.35 percent or 0.7 percent resulted in no change in growth rate but at 8 percent depressed gains, due apparently to the very low gains made by mice fed 0.13 percent cystine, natural pH-treated rapeseed meal.

Inclusion of ferrous sulfate to rapeseed meal showed in general no beneficial effect. 8.0 percent FeSO_4 resulted in a reduction in gain, feed intake, absolute weight of livers and kidneys ($P > 0.01$), and enlargement of adjusted weight of livers and kidneys. However, the opposite results were observed, when rapeseed meal was treated with 8.0 percent FeSO_4 at the acidic pH (pH5) and with 0.70 percent FeSO_4 at the natural pH. Myrosinase supplementation damaged the quality of the meal with respect to weight gain, feed intake and inspected organs. This adverse effect was very severe when the meal was pre-treated with myrosinase buffered at pH5. Treatment of the meal at pH5 with ferrous sulfate caused gain depression, lower

feed intake, smaller livers and kidneys with regard to absolute weights, and enlargement of liver and kidney in terms of corrected weights. This evidence is probably an indication of the production of nitriles in addition to goitrogenic compounds. It is known that nitriles are more harmful than goitrogenic compounds to animals. Furthermore, animals fed diets containing meal treated with ferrous sulfate and myrosinase had poor gain, lower feed intake and abnormal livers and kidneys (tables 51, 52, 53, 54, 55, and 56) (Appendix N).

Test animals fed treated meals with cystine, FeSO_4 and dietary myrosinase did not generally show significant responses with regard to gain weights and adjusted weight of liver and kidney (Appendix N). However, incorporation of 0.26 percent or 0.13 percent cystine into meals pre-treated with 0.0 percent, 0.35 percent or 0.70 percent FeSO_4 and 0.0 percent myrosinase improved the meal quality with respect to evaluated criteria (tables 57, 58, 59). From these observations one might conclude that cystine supplementation has counteracted the growth depressing and goitrogenic factors in rapeseed meal.

The quality of five meals treated with cystine or myrosinase at various pH's was evaluated in vitro and in vivo (table 60). Results in vitro indicated that incorporation of increasing levels of cystine was beneficial in terms of reducing goitrogenic compounds. Similar beneficial results were also found in vivo in terms of animal responses (i.e.

weight gain, and liver and kidney weights). However, these findings could not be fully explained due to lack of measurements of other glucosinolate products such as organic nitriles, thioniamide, etc.

The relationship between feed intake and animal responses was revealed by correlation studies (table 61). Results indicated that feed intake and liver enlargement, and feed intake and kidney enlargement were significantly ($P < 0.01$) correlated.

Briefly this experiment demonstrated that:

- (1) chemical treatment of rapeseed meal with ferrous sulfate was beneficial when used at a proper level
- (2) incorporation of cystine at a certain level into buffered meal was encouraging (this encouragement confirmed our earlier findings of Experiment 7)
- (3) enzyme-inactivated meal buffered at pH5 caused drastic reduction in gains and enlargement of livers and kidneys and
- (4) in this experiment, the effects of all factors on animal responses were statistically significant.

The co-efficient of variation among observations was 12.86 percent. The lower variability among data might have caused these differences to be significant. It was of particular interest that most of the interaction effects in this investigation were also significant. Those differences due to combination of various factors are not clear. The environmental factor, supplementation of different compounds and both other unknown factors could, however, be an explanation.

Table 40. Average feed intakes obtained for the interaction effects of myrosinase levels, cystine levels and pH treatments (g)*

pH	Myrosinase in meal %	Cystine, % in meal			pH	Myrosinase %, in meal	
		0.0	0.13	0.26		0.0	0.3
5	0.0	39.40	41.30	43.65	36.05	40.28	34.15
	0.3	28.91	28.50	34.53			
Natural	0.0	38.96	39.80	38.58	38.38	38.20	38.38
	0.3	38.56	36.23	38.20			
Cystine		36.46	36.46	38.74			
pH x Cystine	5	34.25	34.90	39.09	36.05		
	Natural	38.76	38.01	38.39	38.38		
Cystine x Myrosinase	0.0	39.18	40.55	41.11	40.28		
	0.3	38.73	32.65	36.36	34.15		

* LSR values for minimum and maximum: myrosinase 1.73; pH1.73, cystine 2.21, 2.31; pH x cystine 2.99, 3.31; cystine x myrosinase 2.27, 2.31g. pH x myrosinase x cystine are not significant.

Table 41. Average liver weights obtained for the interaction effects of myrosinase levels, cystine levels and pH treatments (g)*

pH	Myrosinase in meal %	Cystine, % in meal			pH	Myrosinase %, in meal	
		0.0	0.13	0.26		0.0	0.3
5	0.0	1.01	1.15	1.16	0.99	1.10	0.94
	0.3	0.77	0.77	1.08			
Natural	0.0	1.04	1.02	1.20	1.05	1.10	0.94
	0.3	1.07	0.94	1.00			
Cystine							
pH x Cystine	5	0.98	0.97	1.11	1.11	0.99	1.05
	Natural	0.89	0.96	1.12			
Cystine x Myrosinase	0.0	1.02	1.08	1.18	1.18	1.10	0.94
	0.3	0.92	0.85	1.04			

* LSR values for minimum and maximum: myrosinase 0.05; pH0.05; cystine 0.06, 0.07;

pH x cystine 0.09, 0.1; cystine x myrosinase 0.40, 0.45 and pH x myrosinase x cystine 0.13, and 0.15g.

Table 42. Average adjusted liver weights obtained for interaction effects of myrosinase levels, cystine levels and pH treatments (g)*

pH	Myrosinase in meal %	Cystine, % in meal			pH	Myrosinase %, in meal	
		0.0	0.13	0.26		0.0	0.3
5	0.0	17.90	15.05	13.09	20.62	15.60	24.47
	0.3	58.33	58.33	26.93			
Natural	0.0	14.68	17.02	16.85	17.26	15.60	24.47
	0.3	15.94	21.17	19.12			
Cystine		18.18	10.08	17.61			
pH x Cystine	5	25.57	21.42	17.41			20.62
	Natural	15.31	18.91	17.82			17.26
Cystine x Myrosinase	0.0	16.03	15.92	14.76			15.60
	0.3	22.88	29.51	22.51			24.47

* LSR values for minimum and maximum: myrosinase 1.48, pH01.48; cystine 1.84, 1.93; pH x cystine 2.57, 2.86; cystine x myrosinase 2.57, 2.86 and pH x myrosinase x cystine 3.64 and 4.26g.

Table 43. Average kidney weights obtained for interaction effects of myrosinase levels, cystine levels and pH treatments (g)*

pH	Myrosinase in meal %	Cystine, % in meal			pH	Myrosinase %, in meal	
		0.0	0.13	0.26		0.0	0.3
5	0.0	0.28	0.33	0.30	0.26	0.29	0.25
	0.3	0.20	0.33	0.27			
Natural	0.0	0.27	0.27	0.28	0.27	0.29	0.25
	0.3	0.28	0.25	0.27			
Cystine		0.26	0.26	0.28			
pH x Cystine	5	0.24	0.26	0.28	0.29		
	Natural	0.27	0.26	0.28	0.25		
Cystine x Myrosinase	0.0	0.27	0.30	0.29	0.29		
	0.3	0.24	0.23	0.27	0.25		

* LSR values for minimum and maximum: myrosinase 0.01, pH non-significant; cystine 0.01, 0.02; pH x cystine 0.02, 0.03; cystine x myrosinase 0.02, 0.03 and pH x myrosinase x cystine 0.03 and 0.04g.

Table 44. Average adjusted kidney weights obtained for interaction effects of myrosinase levels, cystine levels and pH treatments (g)*

pH	Myrosinase in meal %	Cystine, % in meal			pH	Myrosinase %, in meal	
		0.0	0.13	0.26		0.0	0.3
5	0.0	4.96	4.31	3.38	5.41	4.11	6.51
	0.3	15.15	25.00	6.73			
Natural	0.0	3.81	4.50	3.93	4.44	4.11	6.51
	0.3	4.17	5.63	5.16			
Cystine							
pH x Cystine	5	5.00	5.38	4.44	4.44	5.41	6.51
	Natural	6.89	5.80	4.35			
Cystine x Myrosinase	0.0	3.91	5.01	4.53	4.44	4.11	6.51
	0.3	4.24	4.42	3.62			
		5.97	7.98	5.84			

* LSR values for minimum and maximum: myrosinase 0.56; pH 0.56; cystine 0.69, 0.72;
 pH x cystine 0.97, 1.08; cystine x myrosinase 0.97, 1.08 and pH x myrosinase
 x cystine 1.38 and 1.62g.

Table 45. Average growth responses obtained for the interaction effects of cystine levels, FeSO₄ levels and pH treatments (g)*

pH	Cystine % in meal	FeSO ₄ in meal, %				pH x cystine	pH
		0.0	0.35	0.70	8.0		
5	0	3.60	3.23	3.61	3.47	3.48	4.80
	0.13	4.50	4.60	4.41	4.40	4.48	
	0.26	6.47	5.84	5.06	8.36	6.43	
Natural	0	6.47	5.98	8.14	6.99	6.89	6.08
	0.13	6.51	6.35	7.61	0.25	5.18	
	0.26	8.19	5.35	7.41	3.78	6.17	
FeSO ₄		5.95	5.23	6.04	4.54		
Cystine	0	5.04	4.61	5.87	5.23	5.19	6.08
	0.13	5.51	5.47	6.01	2.32	4.83	
	0.26	7.31	5.59	6.23	6.07	6.30	
pH	5	4.86	4.55	4.36	5.41	4.80	6.08
	Natural	7.04	5.89	7.72	3.67	6.08	

* LSR values for minimum and maximum: pH x 0.23; FeSO₄ 0.33, 0.35; cystine 0.27, 0.30; pH x cystine 0.40, 0.45; pH x FeSO₄ 0.46, 0.53; FeSO₄ x cystine 0.57, 0.60 and pH x FeSO₄ x cystine 0.80 and 0.97g.

Table 46. Average feed intakes obtained for the interaction effects of cystine levels, FeSO₄ levels and pH treatments (g)*

pH	Cystine % in meal	FeSO ₄ in meal, %				pH x cystine	pH
		0.0	0.35	0.70	8.0		
5	0	34.77	29.05	35.19	37.61	34.15	36.05
	0.13	35.82	33.00	36.63	34.16	34.90	
	0.26	38.92	41.12	36.04	40.21	39.09	
Natural	0	38.81	38.72	40.53	36.94	38.76	38.38
	0.13	40.98	40.31	43.55	27.21	38.01	
	0.26	42.09	40.50	40.41	30.54	38.39	
FeSO ₄		38.57	37.12	38.73	34.45		
Cystine	0	36.79	33.88	37.86	37.28	36.46	36.05
	0.13	38.40	36.65	40.09	30.69	36.46	
	0.26	40.51	40.81	38.23	35.37	38.74	
pH	5	36.60	34.39	35.95	37.33	36.05	38.38
	Natural	40.63	39.40	41.50	31.57	38.38	

* LSR values for minimum and maximum: pH 1.73; FeSO₄ 2.44, 2.61; cystine 2.21, 2.31; pH x cystine 2.99, 3.31; pH x FeSO₄ 3.45, 3.92; FeSO₄ x cystine 4.22, 4.99 and pH x FeSO₄ x cystine non-significant g.

Table 47. Average liver weights obtained for the interaction effects of cystine levels, FeSO₄ levels and pH treatments (g)*

pH	Cystine % in meal	FeSO ₄ in meal, %				pH x cystine	pH
		0.0	0.35	0.70	8.0		
5	0	0.90	0.90	0.87	0.88	0.89	0.99
	0.13	1.03	0.93	0.94	0.91	0.96	
	0.26	1.16	1.13	1.00	1.18	1.12	
Natural	0	1.08	1.01	1.00	1.12	1.06	1.05
	0.13	1.11	1.07	1.11	0.63	0.98	
	0.26	1.37	1.02	1.11	0.90	1.10	
FeSO ₄		1.11	1.01	1.01	0.94		
Cystine	0	1.01	0.95	0.93	1.00	0.98	1.11
	0.13	1.07	1.00	1.02	0.77	0.97	
	0.26	1.24	1.07	1.05	1.04	1.11	
pH	5	1.03	0.99	0.94	0.99	0.99	1.05
	Natural	1.19	1.03	1.07	0.88	1.05	

* LSR values for minimum and maximum: pH 0.05; FeSO₄ 0.07, 0.08; cystine 0.06, 0.07; pH x cystine 0.09, 0.10; pH x FeSO₄ 0.10, 0.12; FeSO₄ x cystine 0.13, 0.15 and pH x FeSO₄ x cystine 0.18 and 0.22g.

Table 48. Average adjusted liver weights obtained for the interaction effects of cystine levels, FeSO₄ levels and pH treatments (g)*

pH	Cystine % in meal	FeSO ₄ in meal, %			pH x cystine	pH
		0.0	0.35	0.70		
5	0	25.00	20.86	24.09	25.36	20.62
	0.13	22.88	20.21	21.32	20.68	
	0.26	17.92	19.34	19.76	14.11	
Natural	0	16.69	18.88	12.28	16.02	20.62
	0.13	17.05	16.85	14.58	252.00	
	0.26	16.72	19.04	14.97	23.80	
FeSO ₄		18.65	19.31	16.72	20.70	
Cystine	0	20.03	20.60	15.84	19.12	20.62
	0.13	19.41	18.28	16.97	33.18	
	0.26	16.96	19.14	16.85	17.13	
pH	5	21.19	21.75	21.55	18.29	17.26
	Natural	16.19	17.48	13.86	23.97	

* LSR values for minimum and maximum: pH1.48; FeSO₄ 2.10, 2.25; cystine 1.85, 1.93; pH x cystine 2.57, 2.86; pH x FeSO₄ 2.97, 3.38; FeSO₄ x cystine 3.64, 4.26 and pH x FeSO₄ x cystine 5.15 and 6.24g.

Table 49. Average kidney weights obtained for the interaction effects of cystine levels, FeSO₄ levels and pH treatments (g)*

pH	Cystine % in meal	FeSO ₄ in meal, %			pH x cystine	pH
		0.0	0.35	0.70		
5	0	0.23	0.26	0.23	0.24	
	0.13	0.31	0.26	0.25	0.26	
	0.26	0.29	0.28	0.25	0.28	0.26
Natural	0	0.27	0.28	0.28	0.27	
	0.13	0.26	0.27	0.33	0.26	
	0.26	0.36	0.24	0.28	0.28	0.27
FeSO ₄		0.29	0.27	0.27	0.25	
Cystine	0	0.25	0.27	0.25	0.25	0.26
	0.13	0.29	0.26	0.29	0.21	0.26
	0.26	0.30	0.28	0.26	0.27	0.28
pH	5	0.27	0.26	0.24	0.26	0.26
	Natural	0.29	0.26	0.29	0.22	0.27

* LSR values for minimum and maximum: pH non-significant; FeSO₄ 0.01, 0.02; cystine 0.01, 0.02; pH x cystine 0.02, 0.03; pH x FeSO₄ 0.03, 0.03; FeSO₄ x cystine 0.03, 0.04 and pH x FeSO₄ x cystine 0.05 and 0.06g.

Table 50. Average adjusted kidney weights obtained for interaction effects of cystine levels, FeSO₄ levels and pH treatments (g)*

pH	Cystine % in meal	FeSO ₄ in meal, %				pH x cystine	pH
		0.0	0.35	0.70	8.0		
5	0	6.38	8.04	6.37	6.62	6.89	5.41
	0.13	6.88	5.65	5.66	6.13	5.80	
	0.26	4.46	4.79	4.94	3.82	4.35	
Natural	0	4.17	4.68	3.43	3.71	3.91	4.44
	0.13	3.09	4.25	4.33	72.72	5.01	
	0.26	4.39	4.48	3.77	6.08	4.53	
FeSO ₄		4.87	5.16	4.47	5.50		
Cystine	0	4.96	5.85	4.25	4.78	5.00	5.41
	0.13	5.26	4.74	4.82	9.05	5.38	
	0.26	4.10	5.00	4.17	4.44	4.44	
pH	5	5.55	5.71	5.50	4.80	5.41	4.44
	Natural	4.11	4.41	3.75	5.59	4.44	

* LSR values for minimum and maximum: pH0.56; FeSO₄ 0.79, 0.85; cystine 0.69, 0.72; pH x cystine 0.97, 1.08; pH x FeSO₄ 1.13, 1.28; FeSO₄ x cystine 1.38, 1.62 and pH x FeSO₄ x cystine 1.95, 2.37g.

Table 51. Average growth responses obtained for the interaction effects of myrosinase levels, FeSO₄ levels and pH treatments (g)*

pH	Myrosinase % in meal	FeSO ₄ in meal, %			pH X myrosinase	pH X FeSO ₄
		0.0	0.35	0.70		
5	0.0	7.21	3.55	6.78	7.38	
	0.3	2.51	2.01	1.94	2.22	4.79
	0.0	7.76	5.61	8.62	6.70	
	0.3	6.35	6.84	6.82	5.46	6.08
FeSO ₄		5.95	5.23	6.04	4.54	
pH X FeSO ₄	5	4.86	4.55	4.36	5.41	4.79
	Natural	7.04	5.89	7.72	3.67	6.08
FeSO ₄ X Myrosinase	0.0	7.47	6.36	7.70	6.63	6.97
	0.3	4.43	4.09	4.38	2.45	3.83

* LSR values for minimum and maximum: FeSO₄ 0.33, 0.35; pH x FeSO₄ 0.46, 0.53; pH x myrosinase 0.33, 0.35 and pH x myrosinase x FeSO₄ 0.65 and 0.78g.

Table 52. Average feed intakes obtained for the interaction effects of myrosinase level, FeSO_4 levels and pH treatments (g)*

pH	Myrosinase % in meal	FeSO_4 in meal, %				pH X myrosinase	pH X FeSO_4
		0.0	0.35	0.70	8.0		
5	0.0	40.98	41.39	40.93	42.50	41.45	36.06
	0.3	32.03	27.39	30.98	32.15	30.64	
	0.0	41.40	38.35	43.49	33.45	39.11	
	0.3	40.11	41.33	39.51	29.69	37.66	
FeSO_4		38.57	37.12	38.73	34.45		
pH X FeSO_4	5	36.60	34.39	35.95	37.33	36.06	38.27
	Natural	40.63	39.40	41.50	31.57		
FeSO_4 X Myrosinase	0.0	41.06	39.87	42.20	37.97	40.27	34.14
	0.3	36.07	34.36	35.24	30.92		

* LSR values for minimum and maximum: FeSO_4 2.44, 2.61; pH x FeSO_4 3.45, 3.92; pH x myrosinase 2.44, 2.61 and pH x myrosinase x FeSO_4 non-significant g.

Table 53. Average liver weights obtained for the interaction effects of myrosinase levels, FeSO₄ levels and pH treatments (g)*

pH	Myrosinase % in meal	FeSO ₄ in meal, %				pH X myrosinase	pH X FeSO ₄
		0.0	0.35	0.70	8.0		
5	0.0	1.11	1.18	1.08	1.05	1.12	
	0.3	0.95	39.80	0.79	0.76	0.87	0.98
Natural	0.0	1.23	1.02	1.13	0.96	1.10	
	0.3	1.14	1.04	1.01	0.80	1.00	1.04
FeSO ₄		1.11	1.01	1.01	0.94		
pH X FeSO ₄	5	1.03	0.99	0.94	0.99	0.98	
	Natural	1.19	1.03	1.07	0.88	1.04	
FeSO ₄ X Myrosinase	0.0	1.17	1.10	1.11	1.01	1.09	
	0.3	1.05	0.92	0.91	0.87	0.93	

* LSR values for minimum and maximum: FeSO₄ 0.07, 0.08; pH x FeSO₄ 0.10, 0.12; pH x myrosinase 0.07, 0.08 and pH x myrosinase x FeSO₄ 0.15 and 0.17g.

Table 54. Average adjusted liver weights obtained for the interaction effects of myrosinase levels, FeSO₄ levels and pH treatments (g)*

pH	Myrosinase % in meal	FeSO ₄ in meal, %			pH X myrosinase	pH X FeSO ₄
		0.0	0.35	0.70		
5	0.0	15.39	33.23	15.92	12.47	15.17
	0.3	37.84	29.80	40.72	31.53	39.18
Natural	0.0	15.85	18.18	13.10	19.79	16.41
	0.3	17.95	15.20	14.80	32.12	18.31
FeSO ₄		18.65	19.31	16.72	20.70	
pH X FeSO ₄	5	21.19	21.75	21.55	18.29	20.69
	Natural	16.19	17.48	13.86	23.97	17.87
FeSO ₄ X Myrosinase	0.0	15.66	17.29	14.41	15.23	15.64
	0.3	23.17	22.49	20.77	35.51	25.48

* LSR values for minimum and maximum: FeSO₄ 2.10, 2.25; pH x FeSO₄ 2.97, 3.38; pH x myrosinase 2.10, 2.25 and pH x myrosinase x FeSO₄ 4.21 and 5.20g.

Table 55. Average kidney weights obtained for the interaction effects of myrosinase levels, FeSO₄ levels and pH treatments (g)*

pH	Myrosinase % in meal	FeSO ₄ in meal, %			pH X myrosinase	pH X FeSO ₄
		0.0	0.35	0.70		
5	0.0	0.32	0.31	0.29	0.27	
	0.3	0.23	0.21	0.20	0.25	0.25
Natural	0.0	0.31	0.24	0.31	0.23	
	0.3	0.28	0.29	0.27	0.21	0.26
FeSO ₄		0.29	0.27	0.27	0.25	
pH X FeSO ₄	5	0.27	0.26	0.24	0.26	0.25
	Natural	0.29	0.26	0.29	0.22	0.26
FeSO ₄ X Myrosinase	0.0	0.31	0.28	0.30	0.25	0.28
	0.3	0.25	0.25	0.24	0.23	0.24

* LSR values for minimum and maximum: FeSO₄ 0.01, 0.02; pH x FeSO₄ 0.03, 0.03; pH x myrosinase 0.01, 0.02 and pH x myrosinase x FeSO₄ 0.18 and 0.21g.

Table 56. Average adjusted kidney weights obtained for the interaction effects of myrosinase levels, FeSO₄ levels and pH treatments (g)*

pH	Myrosinase % in meal	FeSO ₄ in meal, %			pH X myrosinase	pH X FeSO ₄
		0.0	0.35	0.70		
5	0.0	4.43	8.73	4.27	3.65	
	0.3	9.16	10.44	10.30	11.26	5.39
Natural	0.0	3.99	4.27	3.59	3.43	
	0.3	4.40	4.23	3.95	3.84	4.41
FeSO ₄		4.87	5.16	4.47	5.50	
pH X FeSO ₄	5	5.55	5.71	5.50	4.80	5.39
	Natural	4.11	4.41	3.75	5.39	4.41
FeSO ₄ X Myrosinase	0.0	4.14	4.40	3.89	3.77	4.05
	0.3	5.64	6.11	5.47	9.38	6.65

* LSR values for minimum and maximum: FeSO₄ 0.79, 0.85; pH x FeSO₄ 1.13, 1.28; pH x myrosinase 0.79, 0.85 and pH x myrosinase x FeSO₄ 1.59 and 1.90g.

Table 57. Average growth responses and feed intake obtained for the interaction effects of myrosinase levels, cystine levels and FeSO_4 levels (g)*

Myrosinase in meal %	Cystine in meal %	FeSO_4 in meal, %			
		0.0	0.35	0.70	8.0
GAIN					
0.0	0.0	6.41	4.54	7.49	6.99
	0.13	7.94	6.14	8.28	4.75
	0.26	8.07	8.39	7.33	8.15
0.3	0.0	3.66	4.67	4.26	3.47
	0.13	3.08	4.80	3.74	0.10
	0.26	6.55	2.79	5.13	3.99

FEED INTAKE*

0.0	0.0	38.12	34.74	42.04	41.81
	0.13	42.67	40.23	45.20	34.09
	0.26	42.38	44.65	39.39	38.03
0.3	0.0	35.46	33.03	33.68	32.76
	0.13	34.13	33.08	34.97	27.29
	0.26	38.63	36.97	37.07	32.72

* LSR values for minimum and maximum : Myrosinase x cystine x FeSO_4 4.55 and 5.69g.

Table 58. Average absolute and adjusted kidney weights obtained for the interaction effects of myrosinase levels, cystine levels and FeSO₄ levels (g)

Myrosinase in meal %	Cystine in meal %	FeSO ₄ in meal, %			
		0.0	0.35	0.70	8.0
KIDNEY *					
0.0	0.0	0.27	0.26	0.28	0.27
	0.13	0.34	0.28	0.33	0.25
	0.26	0.34	0.29	0.29	0.24
0.3	0.0	0.22	0.28	0.23	0.22
	0.13	0.24	0.25	0.24	0.17
	0.26	0.31	0.23	0.24	0.31
KIDNEY % *					
0.0	0.0	4.21	5.72	3.73	3.86
	0.13	4.28	4.56	3.90	5.26
	0.26	4.21	3.45	3.95	2.74
0.3	0.0	6.01	5.97	5.39	6.34
	0.13	7.79	5.20	6.41	1.70 ¹
	0.26	4.73	8.24	4.67	7.76

* LSR values for minimum and maximum : Myrosinase x
cystines x FeSO₄ 0.05, 0.06 and 1.95 and 2.37g.

¹lost weight; final weight used instead of weight gain.

Table 59. Average absolute and adjusted liver weights obtained for the interaction effects of myrosinase level, cystine level and FeSO_4 level(g)

Myrosinase in meal %	Cystine in meal %	FeSO_4 in meal, %			
		0.0	0.35	0.70	8.0
LIVER *					
0.0	0.0	1.05	0.97	0.99	1.08
	0.13	1.23	1.07	1.13	0.89
	0.26	1.24	1.25	1.20	1.04
0.3	0.0	0.93	0.94	0.88	0.92
	0.13	0.92	0.93	0.92	0.65
	0.26	1.29	0.90	0.91	1.04
LIVER % *					
0.0	0.0	16.38	21.36	13.21	15.45
	0.13	15.49	17.42	13.64	18.73
	0.26	15.36	14.89	16.37	12.67
0.3	0.0	25.40	20.12	20.65	26.51
	0.13	29.87	19.37	24.59	16.50
	0.26	19.66	32.25	17.73	26.06

* LSR values for minimum and maximum : Myrosinase x
cystine x FeSO_4 0.18, 0.22, and 5.48 and 6.24g.

Table 60. Effects on chemical and biological assay responses of rapeseed meal treated with cystine and myrosinase at various pH's.

pH	Myrosinase percent in meal	Cystine percent in meal	Chemical assay response glucosinolate*				Total
			Butenyl	Pentenyl	OZT	Nitrile	
Natural	0.0	0.0	2.9	1.0	8.4	0.05	12.35
5	0.0	0.0	1.8	0.7	6.1	0.20	8.80
5	0.3	0.0	0.3	0.2	6.5	0.1	7.10
5	0.0	0.13	2.0	0.8	5.9	0.15	8.85
5	0.0	0.26	1.7	0.7	3.3	0.15	5.85

Biological assay response

	Gain (g)	Feed in-take (g)	Liver wt. percent		Kidney wt. percent	
			percent	percent	percent	percent
Natural	7.32	38.18	15.98	3.82	4.90	11.11
5	5.50	37.07	17.09	4.90	4.62	4.02
5	1.71	32.48	50.29	11.11	4.62	4.02
5	8.43	43.82	15.80	4.62	4.02	4.02
5	7.7	42.05	14.54	4.02	4.02	4.02

* Expressed as mg of released aglycones per g of meal(DM): Butenyl, butenylisothiocyanate
 Pentenyl, pentenylisothiocyanate
 OZT, (S)-5-vinylloxazolinethione

Table 61. Correlation coefficients between various biological responses of mice

	Gain (g)	Feed Eff.	Liver percent	Kidney percent	Feed Cons. (g)	Liver wt. (g)	Kidney wt. (g)
Gain	1						
Feed Eff.	-0.364**						
Liver/percent	0.028	-0.050	1				
Kidney/percent	-0.080	0.045**	0.111*	1			
Feed Cons.	0.693**	-0.224**	0.141*	-0.028	1		
Liver wt.	0.704**	-0.307**	0.455**	0.045	0.591**	1	
Kidney wt.	0.595**	-0.233**	0.151*	0.316**	0.565**	0.687**	1

Correlations between the variables after removal of all main effects, interaction effects, and regression effects.

	Gain (g)	Feed Eff.	Liver percent	Kidney percent	Feed Cons. (g)	Liver wt. (g)	Kidney wt. (g)
Gain	1						
Feed Eff.	-0.133**						
Liver/100gm B.wt.	0.002	0.061	1				
Kidney/100gm B.wt.	-0.175**	0.000	0.052	1			
Feed Cons.	0.327**	0.052	0.165**	0.033	1		
Liver wt.	0.460**	0.072	0.501**	-0.019	0.295**	1	
Kidney wt.	0.281**	-0.029	0.123	0.264**	0.254**	0.453**	1

* or ** Statistically significant at $P < 0.05$ or 0.01

3.8 Experiment 9

Detoxification of rapeseed meal with buffered solutions, temperature, filtration, cystine and ferrous sulfate.

Among the various methods that have been studied for the detoxification of rapeseed meal, the following methods were observed with satisfactory results: autoclaving (Belzile et al. 1963), steam and aqueous extraction (Bell, 1965) and chemical modification (Youngs and Perlin, 1967). However, these authors observed gradual deterioration in protein quality by heat or production of nitriles by chemical hydrolysis. We found that both the filtered pH5-buffered rapeseed (Brassica napus) meal at room temperature (Experiment 7A) (table 36) and the unfiltered pH5-buffered plus L-cystine (Experiment 7A) (table 36), and (Experiment 7B) (table 38), resulted in a satisfactory growth rate, prevention of the enlargement of liver and kidneys of mice, and reduction by 55 percent of isothiocyanates, goitrin and probably of the all nitriles (table 38). However, we observed that mice fed rapeseed meal thus treated still showed slightly lower gains than animals fed casein as the source of protein. This experiment was designed to test our methods further to evaluate the quality of treated meals by biological and chemical assays and to study the effects of temperature on glucosinolate hydrolysis.

3.8.1 Materials and methods

(1) Experimental design. The feeding experiment was of a 2x3x4 factorial design involving three degrees of temperature, 25° (room), 45°C and 100°C (boiling), filtration versus

non-filtration and four ferrous sulfate (1, 2, 3, and 4 percent in meal) levels were utilized. Five weanling white mice were allotted to each treatment.

(ii) Meal treatments. The rapeseed meal which was utilized in this experiment was the same commercial rapeseed meal used in the previous five experiments. The meal was treated in the following manner:

(1) L-cystine was added to the rapeseed meal at 0.26 percent of the meal, then mixed in a twin-shell mixer for about one hour. This meal was divided into four parts and each part in turn was mixed with a certain level of ferrous sulfate as dictated by the experimental design.

(2) 1,250ml of pH5 buffered solution and 250g of the above prepared meal were placed in a glass beaker and mechanically stirred for two hours to allow the ferrous salt and/or pH5 buffered solution to hydrolyze the thioglucoside contents. In each case in which the hot buffered solution was employed, the beaker was maintained in a water bath; its temperature was thermostatically controlled and the buffered solution was preheated to either 45°C or 100°C (boiling). Then the meal was added to the pre-heated buffered solution. The beaker and its contents were then kept at room temperature (25°C), 45°C and 100°C for two hours to obtain complete hydrolysis of the glucosinolate contents. It must be noted that in the case of the 100°C sample, some water evaporated and hence the ratio of meal to buffered solution would change. Therefore, it was necessary to keep this ratio

constant by adding hot distilled water during heating to maintain the constant volume.

(3) After two hours of stirring the slurry at room, 45°C and 100°C temperature, the slurried meals were either filtered in a double cotton bag or thinly spread on a stainless steel tray for drying (as unfiltered meal) according to the design of the experiment.

(4) The residues were dried at room temperature, in a drying oven at 40°C and 100°C, similar to the temperature at which the meal was previously treated. After drying the residues were ground and stored in a cold room until chemical and biological assays were performed.

(iii) Diet formulation. The composition of the basal diet employed in this experiment is given in Appendix F. The remainder was provided by 28 percent of treated rapeseed meal and 5 percent of soybean meal to achieve 17 percent protein levels in the final diets. All diets except for the daily allowances, were kept refrigerated during the course of the experiment in order to prevent any changes in diet composition prior to ingestion of the feed.

Experimental procedure and animal management were followed in the manner of the previous experiments.

3.8.2 Results

(i) Chemical assay. The isothiocyanates, oxazolidinethione and hydroxy nitrile contents of the treated rapeseed meals (Brassica napus) were measured according to the methods of Youngs and Wetter (1967)*. The determined values of

* Chemical analysis performed in National Research Council, Saskatoon, Saskatchewan.

(S)-5-vinyl-2-oxazolidinethiones ((S) goitrin), 3-butenyl, isothiocyanate, 4-pentenylisothiocyanate and 1-cyano-2-hydroxy-3-butene are presented in table 62. The data show that hydrolytic breakdown had taken place by action of either ferrous sulfate, buffer, temperature or under the influence of combinations of these treatments.

The chemical analysis of meals (residues) revealed that when supplementation of ferrous sulfate into the meal was increased, the amount of isothiocyanates and oxazolidinethiones (goitrin) recovery was decreased. It was of interest that the formation of hydroxynitrile did not increase with each increment of ferrous salt. It might be concluded that released hydroxynitrile (or other toxic factors besides this one) was removed either by filtration or neutralization with supplementary cystine. It seems that hydroxynitrile is less soluble at higher (100°C) temperatures (table 62).

In general, temperature showed less effect on the non-enzymatic degradation of glucosinolate contents of residues than did ferrous sulfate. However, when the meal was treated with 3 percent or 4 percent FeSO_4 and boiled for two hours, the resulting meal contained 0.0mg/g of isothiocyanates and goitrin, and 2.03 to 3.38mg/g of hydroxynitrile.

Filtration had substantial effects upon detoxifying rapeseed meal. This means 73.6 percent of goitrogenic substances, with regard to the untreated meal, produced by non-enzymatic hydrolysis were either soluble and removed by filtration or converted to hydroxynitrile and other unmeasured factor(s).

(Table 62 and its footnote). Non-filtration also had an effect on reducing the isothiocyanates and goitrin contents of the treated meal. Non-filtered meals analyzed for released goitrogenic factors show that 34 percent of the compounds with respect to the untreated meal was recovered. Furthermore, unfiltered meals contained twice the amount of hydroxynitrile as that of the filtered meals.

In summary, we might conclude that the proportion of goitrin plus isothiocyanates recovered from treated meals varied from zero to about 34 percent of the glucosinolate contents of the original meal. However, it seems that treated meals contained higher amounts of hydroxynitrile than the original meal. Furthermore, it is possible to obtain zero g/kg of isothiocyanates, goitrin, trace amounts of nitrile and other toxic compounds by treating rapeseed (Brassica napus) meal with the method which was employed (pH x 4 percent FeSO_4 x filtration at room temperature) in this experiment. (Table 62).

(ii) Biological assay. Chemical assay showed that glucosinolate products were either soluble or made into insoluble complexes with supplied compounds (i.e. cystine), or other nutrients contents of the meal in different degrees. The feeding test was carried out to relate the quality of the meals in terms of chemical assay with the biological responses on the basis of measurements of gain in weight; feed intakes and liver and kidney weights of mice. The results of the feeding test revealed that wet heating, supplementary FeSO_4

Table 62. Glucosinolate products released by myrosinase following various FeSO₄, temperature, filtration treatments of rapeseed meal (mg)¹ *

Glucosino late products	Temp. FeSO ₄ 7H ₂ O	Room temp.		45°C temp.		100°C temp.		Total
		Filt.	Unfilt.	Filt.	Unfilt.	Filt.	Unfilt.	
Butenyl		0.95	1.63	0.82	1.43	0.26	0.83	
Pentenyl	1%	0.44	0.57	0.26	0.42	0.18	0.67	
OZT		2.24	2.76	2.41	2.38	1.88	1.68	
OH-Nitrile		0.68	2.30	0.84	2.43	1.57	2.57	
Total		4.31	7.26	4.33	6.66	3.90	5.75	32.21
Butenyl		0.77	1.11	0.80	0.37	0.11	0.20	
Pentenyl	2%	0.22	0.19	0.43	0.98	0.00	0.11	
OZT		2.37	2.63	3.45	5.39	0.16	1.33	
OH-Nitrile		0.43	1.88	0.46	1.08	2.06	2.47	
Total		3.79	5.81	5.14	7.82	2.33	4.11	28.90

Table 62. Continued

Butenyl	0.54	0.80	0.50	0.50	0.00	0.00	0.00
Pentenyl	0.20	0.20	0.21	0.14	0.00	0.00	0.00
OZT	1.45	1.79	1.89	1.28	0.00	0.00	0.00
OH-Nitrile	0.96	2.14	0.87	2.43	2.03	3.38	
Total	3.21	4.93	3.47	4.35	2.03	3.38	21.37
Eutenyl	0.35	0.50	0.07	0.42	0.00	0.00	0.00
Pentenyl	0.11	0.09	0.09	0.04	0.00	0.00	0.00
OZT	0.28	2.76	1.52	0.73	0.00	0.00	0.00
OH-Nitrile	0.93	1.66	1.25	2.19	2.21	2.93	
Total	2.67	5.01	2.93	3.38	2.21	2.93	19.13
TOTAL	13.98	23.01	15.87	22.21	10.47	16.17	
TOTAL		36.99		38.08		26.64	

1 Pentenyl, Butenyl, OZT and OH-Nitrile released by myrosinase of meal without treatment were 2.9, 1.0, and 8.4, 0.05mg/g respectively.

* Expressed as mg of the released aglycones/g of meal.

and filtration all significantly ($P < 0.01$ and $P < 0.05$) affected animal responses (tables 63, 64, 65, 66, 67, and 68) (Appendix U). The results of the feeding experiments may be conveniently divided into four parts:

(1) Temperature effect: It was apparent from the mouse responses in the feeding trial that there was significantly ($P < 0.01$) better gain, higher feed intake ($P < 0.05$) and slightly lower adjusted liver weight for test animals fed meals treated either at room temperature or 45°C than for mice that received meals treated at 100°C (tables 63, 64, and 66) (Appendix U). In general, the biological responses in the feeding test were in agreement with the chemical assay with respect to the amount of hydroxy nitrile formed however, were in disagreement with regards to the production of isothiocyanates and oxazolidinethione of the wet heated meal in the buffered solution containing cystine (table 69). The beneficial effect of low temperature on the adjusted weight of liver (with comparison to mice fed casein in Experiment 7) was slightly evident. This small difference between means was not significant in terms of adjusted liver weight but in the instance of absolute liver weight it was significant ($P < 0.05$) (tables 65 and 66) (Appendix U). This abnormality might be due to higher nitrile content of the treated meal at 100°C .

(2) Filtration effects: Feeding unfiltered treated rapeseed meal significantly ($P < 0.01$) depressed gain and feed intake and, in contrast, it caused significant

($P < 0.01$) enlargement of liver and kidney weights in terms of either absolute weight or percentage of gain of mice. A macroscopic examination of the organs (liver, kidney) of these animals showed that livers and kidneys were dark in color and firm in texture. In contrast, filtered meals gave satisfactory results in terms of growth and normal color and texture of organs of mice in comparison with the original meal (see previous experiments). In the case of the filtration effect full agreement was found between biological activity and chemical analysis, (tables 62, 63, 64, 66, and 68).

(3) Ferrous sulfate effects: Treatment with ferrous salt under conditions of this experiment led to a little improvement of meal quality with respect to gain and feed consumption. High levels of ferrous salt gave higher gains and better feed intake ($P < 0.05$). The failure of ferrous sulfate to completely counteract the toxicity of glucosinolate products which were formed by chemical degradation was expected, in view of the analytical data in table 62. The data indicated that ferrous sulfate gave rise to hydroxy nitrile which is highly toxic to mice. A comparison of animals that received meals treated with ferrous sulfate with those fed either casein or Bronowski (Experiment 7), revealed that these treated meals caused an enlargement, dark color, and firmness of the liver and kidney of mice.

(4) Interaction effects: A comparison of the gains, organ (liver and kidney) weights and feed intake of growing mice fed twenty-four different treated meals (table 62)

suggested that the test animals fed meal treated at room temperature with 4 percent ferrous sulfate in buffered solution (pH5) and filtered had gains, liver and kidney conditions and feed intake (tables 63 to 68) equivalent to those expected of animals fed casein as a source of protein in Experiment 7A. Furthermore, it was of particular interest that this meal contained the minimum amount of toxic material. So from the chemical and biological test results one might conclude that this method for detoxification of rapeseed (Brassica napus) meal is effective. Moreover, it was evident that mice fed diets containing treated rapeseed (Brassica napus) meal either with 2 percent ferrous salt at room temperature, at 45°C and filtered, or 4 percent ferrous sulfate at room temperature and filtered in this experiment showed similar results with regard to gain, feed intake and organ (liver and kidney) weights to test animals which received diets containing Bronowski meal (a natural free-thioglucoside contents) in Experiment 7A.

In brief, the glucosinolate product content was modified in meals treated by a combination of cystine, ferrous sulfate supplementation, pH5 buffered solution, temperature and filtration treatments. Quality of the meals was evaluated by feeding it to growing mice. Estimation of the values of the tested meals was based on the measurements of gain and liver and kidney weights.

On the basis of the analytical and animal response data, it might be concluded that we have been able to develop an

Table 63. Average growth responses obtained for the interaction effects of FeSO_4 levels, temperatures and filtration treatments (g)*

FeSO_4 in meal %	Filtration	Temperature, °C			FeSO_4 X Filtration	FeSO_4
		25	45	100		
1	Filtered	10.40	9.92	10.51	10.27	8.32
	Non-filtered	6.07	6.94	6.09	6.36	
2	Filtered	11.36	11.40	9.14	10.63	9.25
	Non-filtered	8.70	7.60	7.29	7.86	
3	Filtered	9.99	10.30	9.64	9.97	8.85
	Non-filtered	6.71	8.93	7.55	7.73	
4	Filtered	12.11	11.07	10.70	11.29	9.53
	Non-filtered	8.27	8.42	6.60	7.76	
Temperatures		9.21	9.32	8.44		
Temp. X FeSO_4	1% FeSO_4 2 3 4	8.23	8.43	8.30	8.32	
		10.03	9.15	8.21	9.25	
		8.35	9.61	8.59	8.85	
		10.19	9.74	8.65	9.53	
Filtration X FeSO_4	Filtered	10.97	10.67	10.00	10.55	
	Non-filtered	7.44	7.97	6.88	7.43	

* LSR values for minimum and maximum: FeSO_4 0.75, 0.81; temperature 0.65, 0.68; filtration 0.53; temperature x filtration 0.92, 1.04; FeSO_4 x filtration 1.06, 1.26; FeSO_4 x temperature 1.30, 1.56 and FeSO_4 x temperature x filtration 1.84 and 2.28g

Table 64. Average feed intakes obtained for the interaction effects of FeSO_4 levels, temperature and filtration treatments (g)*

FeSO_4 in meal %	Filtration	Temperature, °C			FeSO_4 X Filtration	FeSO_4
		25	45	100		
1	Filtered	44.70	45.00	45.80	45.16	39.77
	Non-filtered	36.20	35.60	31.30	34.36	
2	Filtered	45.20	51.20	41.30	45.90	42.67
	Non-filtered	41.40	37.60	39.30	39.43	
3	Filtered	44.50	46.90	44.00	45.13	42.58
	Non-filtered	37.70	42.30	40.10	40.03	
4	Filtered	46.70	46.00	44.90	45.86	42.65
	Non-filtered	41.60	40.10	36.60	39.43	
Temperatures		42.26	43.10	41.42		
Temp. X FeSO_4	1% FeSO_4	40.45	40.30	38.55	39.77	
	2	43.30	44.40	40.30	42.67	
	3	41.10	44.60	42.05	42.58	
	4	44.15	43.05	40.75	42.65	
Filtration X FeSO_4	Filtered	45.28	47.30	44.00	45.53	
	Non-filtered	39.23	38.90	38.83	38.99	

* LSR values for minimum and maximum: FeSO_4 1.68, 1.83; temperature 1.92, 2.03; filtration 2.07, temperature x filtration 2.72, 3.08; FeSO_4 x filtration 3.14, 3.66; FeSO_4 x temperature 3.85, 4.62 and FeSO_4 x temperature x filtration 5.44 and 6.76g

Table 65. Average liver weights obtained for the interaction effects of FeSO₄ levels, temperature and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄
		25	45	100		
1	Filtered	1.27	1.20	1.29	1.25	1.10
	Non-filtered	0.91	0.98	0.94	0.94	
2	Filtered	1.30	1.34	1.19	1.27	1.16
	Non-filtered	1.06	1.08	0.96	1.03	
3	Filtered	1.13	1.22	1.11	1.15	1.11
	Non-filtered	0.96	1.18	1.06	1.06	
4	Filtered	1.37	1.28	1.22	1.29	1.18
	Non-filtered	1.08	1.16	0.96	1.06	
Temperatures		1.14	1.18	1.09		
Temp. X FeSO ₄	1% FeSO ₄	1.09	1.09	1.11	1.10	
	2	1.18	1.21	1.07	1.16	
	3	1.04	1.20	1.08	1.11	
	4	1.22	1.22	1.09	1.18	
Filtration X FeSO ₄	Filtered	1.27	1.26	1.20	1.24	
	Non-filtered	1.00	1.10	0.98	1.03	

* LSR values for minimum and maximum:

FeSO₄ 0.08, 0.09; temperature 0.07, 0.08; filtration 0.05; temperature x filtration 0.10, 0.12; FeSO₄ x filtration 0.12, 0.14; FeSO₄ x temperature 0.15, 0.18 and FeSO₄ x temperature x filtration 0.21 and 0.26g.

Table 66. Average adjusted liver weights obtained for the interaction effects of FeSO₄ levels, temperatures and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄
		25	45	100		
1	Filtered	12.34	12.14	12.34	12.26	
	Non-filtered	18.31	14.25	15.93	16.16	14.21
2	Filtered	11.47	11.84	13.10	12.13	
	Non-filtered	12.34	14.48	13.44	13.42	12.71
3	Filtered	11.57	11.81	11.87	11.75	
	Non-filtered	14.80	13.42	14.29	14.17	12.95
4	Filtered	11.25	11.60	11.44	11.43	
	Non-filtered	13.06	13.80	15.45	14.10	12.76
Temperatures		13.13	12.91	13.47		
Temp. X FeSO ₄	1% FeSO ₄	15.32	13.19	14.12	14.21	
	2	11.90	12.98	13.27	12.71	
	3	13.18	12.61	13.08	12.95	
	4	12.15	12.70	13.44	12.76	
Filtration X FeSO ₄	Filtered	11.65	11.84	12.18	11.89	
	Non-filtered	14.62	13.98	14.77	14.44	

* LSR values for minimum and maximum: FeSO₄ 1.57, 1.71; temperature 1.36, 1.43; filtration 1.11; temperature x filtration 1.92, 2.18; FeSO₄ x filtration 1.76, 2.05; FeSO₄ x temperature 2.72, 3.96 and FeSO₄ x temperature x filtration 3.84 and 4.78g.

Table 67. Average kidney weights obtained for the interaction effects of FeSO₄ levels, temperatures and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄
		25	45	100		
1	Filtered	0.38	0.32	0.36	0.35	0.33
	Non-filtered	0.29	0.34	0.27		
	Filtered	0.35	0.37	0.34		
	Non-filtered	0.31	0.32	0.27		
	Filtered	0.30	0.32	0.30		
	Non-filtered	0.30	0.34	0.30		
	Filtered	0.34	0.35	0.37		
	Non-filtered	0.30	0.31	0.28		
Temperatures		0.33	0.33	0.31		
Temp. X FeSO ₄	1% FeSO ₄	0.33	0.33	0.31	0.33	0.33
	2	0.33	0.34	0.30		
	3	0.31	0.33	0.30		
	4	0.32	0.33	0.32		
Filtration X FeSO ₄	Filtered	0.35	0.34	0.34	0.34	0.30
	Non-filtered	0.33	0.33	0.28		

* LSR values for minimum and maximum: FeSO₄ 0.02, 0.02; temperature 0.07, 0.08; filtration 0.02; temperature x filtration 0.03, 0.03; FeSO₄ x filtration 0.03, 0.04; FeSO₄ x temperature 0.04, 0.05 and FeSO₄ x temperature x filtration 0.05 and 0.07g.

Table 68. Average adjusted kidney weights obtained for the interaction effects of FeSO₄ levels, temperatures and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ x Filtration	FeSO ₄
		25	45	100		
1	Filtered	3.82	3.22	3.41	3.48	4.30
	Non-filtered	5.76	4.98	4.64		
2	Filtered	3.04	3.26	3.74	3.34	4.30
	Non-filtered	3.64	4.16	3.75		
3	Filtered	1.61	3.08	3.25	3.19	3.59
	Non-filtered	4.70	3.79	4.09		
4	Filtered	2.76	3.16	3.49	3.13	3.61
	Non-filtered	3.65	3.71	4.32		
Temperatures		3.82	3.67	3.85		
Temp. x FeSO ₄	1% FeSO ₄	4.79	4.10	4.02	4.30	4.30
	2	3.34	3.71	3.74		
	3	3.97	3.43	3.66		
	4	3.20	3.43	3.94		
Filtration x FeSO ₄	Filtered	3.21	3.11	3.47	3.28	3.28
	Non-filtered	4.43	4.16	4.21		

* LSR values for minimum and maximum:
 FeSO₄ 0.47, 0.51; temperature 0.41, 0.43;
 filtration 0.33; temperature x filtration 0.58,
 0.65; FeSO₄ x filtration 0.73, 0.85; FeSO₄ x
 temperature 0.81, 0.98 and FeSO₄ x temperature
 x filtration 1.15 and 1.43g.

effective method to detoxify rapeseed (Brassica napus) meal. It must be pointed out however, that this technique has been tested only on a laboratory scale. The applicability of this method to commercial production requires further studies.

3.9 Experiment 10

Detoxification of rapeseed meal with cold or hot water, filtration and L-cystine.

3.9.1 Objectives

In Experiment 9 the effects of filtration, temperature and ferrous sulfate in the presence of L-cystine (0.26g/100g meal) and pH5 buffered solution on detoxification of Brassica napus rapeseed meal was investigated. One purpose of the current study was to determine if cystine has any effect on improvement of rapeseed meal quality when it is treated with distilled water. Another objective was to find out when rapeseed meal is treated with distilled water instead of pH5 buffered solution what role filtration plays in the overall view of the biological toxicity of rapeseed.

3.9.2 Materials and methods

A 2x3x3(filtration, temperature and cystine levels) type of design was used, employing five weanling mice per treatment group.

Various (0.0 percent, 0.26 percent or 0.4 percent in meal) levels of L-cystine thoroughly mixed with rapeseed meal, then subjected to different wet heating (the ratio of distilled water to the meal was 5:1), and filtration versus unfiltration.

Meal preparation, ration formulation and experimental procedure were as described in Experiment 9.

3.9.3 Results

Analysis of variance of growth rate, selected organs (kidney and liver) and feed consumption (Appendix V) of mice fed the various meals revealed that high temperature (100°C) improved gain ($P < 0.01$) feed intake and reduced liver and kidney weights in terms of percentage of gain (tables 70, 71, 72, 73, 74, and 75). However there was no difference between meals treated with water at room temperature or 45°C.

The enlargement of liver and kidney of test animals that received filtered meal residues was probably due to hydroxy nitrile or other toxic factor(s) formulation (tables 72 and 74). It is possible that to conclude supplemented cystine was extracted by filtration and did not counteract nitriles or other toxic factor(s) formation. However, residues were not subjected to chemical analysis for hydrolyzed glucosinolate products in this experiment. In this experiment, it was observed that when the level of cystine increased the gain of mice also increased. However, this improvement was not statistically significant ($P < 0.05$).

It was of interest that in contrast to the experiment 9 filtration did not show a favourable effect in this experiment (tables 70 to 75).

On the basis of animal responses we might interpret that this contradictory observation indicates that glucosinolate contents of the rapeseed meal and their hydrolyzed products (nitrile(s) and other harmful factor(s)) are less soluble in

water (except in the case of boiling water) than pH5 buffered solution. Furthermore, it might be added that mice that received unfiltered rapeseed meal (as source of protein) and treated with cystine at room temperature had better gain ($P < 0.01$) and feed intake ($P < 0.05$) and smaller ($P < 0.05$) kidney in terms of adjusted weight than test animals fed filtered meal (tables 70 to 75). This improvement could be attributed to the chemical ability of cystine to counteract toxic factor contents of rapeseed meal.

The effects of temperature x filtration were highly significant ($P < 0.01$) on gains, liver weights and feed consumption of test animals. Both filtration x 0.26g cystine x hot water (100°C) and filtration x 0.4g cystine x hot water treated meal gave the best gains and lowest liver and kidney weight in terms of percentage gain in this trial. From these observations, we might suggest that high temperature caused higher nitrile(s) production which in turn was either neutralized with cystine or extracted along hot water (tables 70 to 75). The poorest gains and largest organs were obtained from mice fed filtered meals with and without cystine treated at room and 45°C temperatures. This observation constitutes additional support for the combining ability of cystine with nitrile(s) and isothiocyanates.

In order to obtain additional evidence regarding the fate of the glucosinolates, the filtered solutions of the slurried rapeseed meal treated with cystine (0.4g level) and hot water (100°C) were collected and the water-soluble materials

were removed. The recovered fractions were subjected to chemical analysis, then compared with original meal composition (table 75). The residual toxicity in the aqueous extraction was slightly higher than original meal.

Filtration and supplementary cystine effects on animal responses were not statistically significant ($P < 0.05$). Moreover, most of the interaction effects in this investigation were not significant. The coefficient of variation among observations was 24.27 percent. The high variability among data might cause these differences to be non-significant.

3.10 Experiment 11

Detoxification of rapeseed meal with ferrous sulfate, cold or hot water and filtration.

3.10.1 Objective

It was shown in Experiment 9 of this investigation that the combination of ferrous sulfate, L-cystine, buffered solution and filtration considerably detoxified Brassica napus rapeseed meal. In Experiment 10, the effects of L-cystine in the absence of ferrous salt and buffered solution were investigated. Thus this was a study for evaluating the effects of ferrous sulfate in cold or hot water meal treatment and filtration on meal quality.

3.10.2 Materials and methods

Experimental design. The experimental design was a $2 \times 3 \times 4$ factorial with two types (filtration and unfiltration), three different temperatures and four ferrous (1 percent, 2 percent, 3 percent, and 4 percent) sulfate levels. Five weanling

Table 70. Average growth responses obtained for the interaction of cystine temperature and filtration treatments (g)*

Cystine in meal %	Filtration	Temperature, °C			Cystine X Filtration	Cystine
		25	45	100		
1	Filtered	7.28	5.94	8.26	7.16	7.66
	Non-filtered	8.54	6.90	8.43	7.95	
2	Filtered	6.30	7.15	10.71	8.05	7.92
	Non-filtered	8.88	8.09	6.41	7.79	
3	Filtered	6.18	8.51	11.23	8.64	8.50
	Non-filtered	8.55	7.89	7.87	8.10	
Temperatures		7.57	7.55	8.97		
Temp. X Cystine	1% Cystine	7.91	6.42	8.34	7.66	
	2	7.59	7.62	8.56	7.92	
	3	6.91	8.59	10.00	8.50	
Filtration X Cystine	Filtered	6.59	7.20	10.07	7.95	
	Non-filtered	8.55	7.89	7.87	8.10	

* LSR values for minimum and maximum: temperature 1.27, 1.32 and temperature x filtration 1.87 and 2.08g.

Table 71. Average liver weights obtained for the interaction effects of cystine, temperature and filtration treatments (g)*

Cystine in meal %	Filtration	Temperature, °C			Cystine X Filtration	Cystine
		25	45	100		
1	Filtered	0.84	1.04	1.23	1.03	1.09
	Non-filtered	1.22	1.12	1.06	1.13	
2	Filtered	1.09	1.06	1.20	1.11	1.13
	Non-filtered	1.20	1.15	1.05	1.13	
3	Filtered	1.02	1.21	1.43	1.22	1.22
	Non-filtered	1.26	1.32	1.08	1.22	
Temperatures		1.08	1.15	1.14		
Temp. X Cystine	1% Cystine	1.03	1.08	1.14	1.09	
	2	1.14	1.10	1.12	1.13	
	3	1.14	1.26	1.25	1.22	
Filtration X Cystine	Filtered	0.92	1.10	1.28	1.10	
	Non-filtered	1.23	1.20	1.06	1.16	

* LSR values for minimum and maximum: temperature x filtration 0.16 and 0.17g.

Table 72. Average adjusted liver weights obtained for the interaction effects of cystine, temperature and filtration treatments (g)*

Cystine in meal %	Filtration	Temperature, °C			Cystine X Filtration	Cystine
		25	45	100		
1	Filtered	15.80	18.10	15.20	16.36	15.48
	Non-filtered	14.39	16.29	13.12	14.60	
2	Filtered	17.80	15.53	11.19	14.84	15.09
	Non-filtered	13.63	14.86	17.58	15.35	
3	Filtered	17.97	15.30	13.11	15.46	14.69
	Non-filtered	14.02	15.45	19.35	13.94	
Temperatures						
Temp. X Cystine	1% Cystine	15.60	15.92	13.75		
	2	15.09	17.19	14.16	15.48	
	3	15.71	15.19	14.38	15.09	
Filtration X Cystine	Filtered	17.19	16.31	13.16	15.15	
	Non-filtered	14.01	15.53	14.35	14.63	

* ISR values for minimum and maximum: temperature 1.57, 1.64 and temperature x filtration 2.22 and 2.50g.

Table 73. Average kidney weights obtained for the interaction effects of cystine, temperature and filtration treatments (g)*

Cystine in meal %	Filtration	Temperature, °C			Cystine X Filtration	Cystine
		25	45	100		
1	Filtered	0.25	0.28	0.32	0.28	0.30
	Non-filtered	0.32	0.28	0.31	0.30	
2	Filtered	0.28	0.31	0.36	0.31	0.30
	Non-filtered	0.29	0.30	0.26	0.28	
3	Filtered	0.24	0.30	0.34	0.29	0.30
	Non-filtered	0.32	0.30	0.28	0.31	
Temperatures		0.29	0.40	0.31		
Temp. X Cystine	1% Cystine	0.28	0.28	0.31	0.30	0.30
	2	0.28	0.30	0.31	0.30	
	3	0.29	0.31	0.31	0.30	
Filtration X Cystine	Filtered	0.28	0.30	0.34	0.30	0.30
	Non-filtered	0.32	0.30	0.28	0.30	

* LSR values for minimum and maximum: non-significant

Table 74. Average adjusted kidney weights obtained for the interaction effects of cystine, temperature and filtration treatments (g)*

Cystine in meal %	Filtration	Temperature, °C			Cystine X Filtration	Cystine
		25	45	100		
1	Filtered	4.23	5.18	4.11	4.38	4.16
	Non-filtered	3.66	4.08	3.74	3.82	
2	Filtered	4.51	4.63	3.34	4.16	3.94
	Non-filtered	3.28	3.77	4.17	3.74	
3	Filtered	4.24	3.75	3.13	3.70	3.76
	Non-filtered	4.58	3.73	3.18	3.83	
Temperatures						
Temp. X Cystine	1% Cystine	3.94	4.63	3.92	4.16	4.12
	2	3.89	4.20	3.75	3.94	
	3	4.41	3.74	3.15	3.76	
Filtration X Cystine	Filtered	4.50	4.16	3.17	4.12	3.79
	Non-filtered	3.82	3.74	3.83	3.79	

* LSR values for minimum and maximum: non-significant

Table 75. Average feed intake obtained for the interaction effects of cystine, temperature and filtration treatments (g)*

Cystine in meal %	Filtration	Temperature, °C			Cystine X Filtration	Cystine
		25	45	100		
1	Filtered	39.33	41.97	42.59	38.34	38.64
	Non-filtered	45.60	43.11	36.84		
2	Filtered	40.85	35.92	44.00	37.39	37.41
	Non-filtered	39.89	42.26	38.45		
3	Filtered	35.59	45.95	47.49	40.09	40.06
	Non-filtered	41.79	42.45	44.63		
Temperatures		40.51	41.95	42.33		
Temp. X Cystine	1% Cystine	39.59	39.42	36.91	41.57	41.52
	2	37.39	36.27	38.57		
	3	35.97	41.10	43.10		
Filtration X Cystine	Filtered	38.59	41.28	44.69	41.52	41.67
	Non-filtered	42.43	42.61	39.97		

* LSR values for minimum and maximum: temperature x filtration 4.02 and 4.55g.

Table 76. Comparisons of nutrient contents and glucosinolate products in rapeseed meal and in dissolved solids recovered from the filtrate (D M basis)*

Constituent	Original Rapeseed Meal	Filtrate Residue
<u>Nutrients Percent</u>		
Crude protein	37.78 ¹	42.43
Crude fiber	14.22	0.56
Ether extract	2.15	
Ash	7.5	12.5
Nitrogen free extract	38.35	44.51
Ca		1.3
P		0.55
Energy Kcal/g	4.67	4.40
<u>Glucosinolate Products(mg/g)</u> ²		
Butenylisothiocyanate	3.22	2.84
Pentenylisothiocyanate	1.11	1.03
Oxazolidinethione	9.33	7.60
OH nitrile	0.05	2.93
Total	13.71	14.40

*The solid losses from the aqueous extraction were determined by drying collected extracts. Samples of dried filtrate which made 6 to 7 percent of the meal were used for chemical analysis of nutrient and glucosinolate products.

¹ Protein and non-protein nitrogen was not distinguished.

² Glucosinolate product contents of the residual meal were not determined

mice were used in each cell of the design.

The experimental procedure has been described already in Experiments 9 and 10.

3.10.3 Results

In general, feeding the various rapeseed (Brassica napus) meals at 28 percent level resulted in no improvement compared with the results of the experiment 9. This means a comparison of twenty-four treated meals revealed that feeding these meals as source of protein to growing mice resulted in a lower gain and feed intake and in increased (in terms of adjusted weight) both liver and kidney weights (tables 77 to 82) with respect to the results of Experiment 9. The poor results in this experiment might be due to unsupplemented cystine to the meals.

Statistical analysis of gains, feed intakes and organs (liver and kidney) weights indicated that growth rate and feed intake were significantly depressed and liver and kidney weights were enlarged ($P < 0.01$) when rapeseed meal was treated with hot water (100°C) (tables 77, 78, 80, and 82) (Appendix W). In addition to enlargement, these organs were abnormal in color and structure. However, in previous experiment (Experiment 10) when cystine pre-treated rapeseed meal was boiled, it gave better results than an unboiled one ($P < 0.01$).

Filtration improved ($P < 0.01$) the quality of the meals with regards to gain, feed intake and liver and kidney weights (tables 77, 78, 80, and 82) (Appendix W). However, mice fed the filtered meals under conditions of this experiment gained

7.53 grams during 14 days, whereas mice that received the filtered meals under conditions of Experiment 9 gained 10.55 grams. This finding might be an indication that toxic products of decomposed glucosinolate contents of the rapeseed meal are not readily removeable by distilled water. It must be mentioned that glucosinolate product content of the treated meals in this experiment were not chemically determined.

Ferrous sulfate in this experiment adversely affected the rapeseed meal value when it was used at higher levels (tables 77 to 82) (Appendix W). From these observations, on the basis of responses of mice, it might be concluded that, under conditions of this experiment, the use of higher (more than 2 percent) levels of ferrous sulfate possibly caused promotion of non-enzymatic degradation of glucosinolate products and formation of hydroxy nitrile(s) and other toxic substance, for example, thionamide.

It was found that treating rapeseed meal with 3 percent or 4 percent ferrous sulfate gave rise to a high level of hydroxy nitrile and quality of the meal was poor in terms of animal responses (Experiment 9). Other investigators have shown that ferrous salt attacks glucosinolate to form hydroxy nitrile and thionamide (Austin et al. 1968) and (Youngs and Perlin, 1967).

When the meals were filtered the mice results were ($P < 0.01$) inferior in the case of meal treated at 100°C . When meals were not filtered, all growth responses were

depressed and organs were enlarged regardless of processing temperature. Filtration improved meal values treated at room and 45°C, but it was ineffective when meal was treated at 100°C. (Tables 77 to 82).

Mice fed the meals treated with 1 percent ferrous sulfate salt and treated at room or 45°C temperature gave better results than test animals that received the treated meals with 2 percent or 4 percent ferrous salts at room, 45°C or 100°C temperature. Furthermore, the mice growth results were inferior in the cases of the meal which were treated with 3 percent or 4 percent ferrous salts and boiled.

When meal treated with 1 percent ferrous sulfate at 45°C temperature and filtered, resulted in maximum growth responses and lower organs. The meal values were also improved when treated with 4 percent ferrous salt at room temperature and filtered. However, mice fed treated meals with 3 percent or 4 percent ferrous sulfate at 100°C had lowest gain regardless of filtration in this experiment (tables 77 to 82).

Table 77. Average growth responses obtained for the interaction effects of FeSO₄ levels, temperatures and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄
		25	45	100		
1	Filtered	7.82	10.74	9.37	9.31	7.99
	Non-filtered	6.07	7.02	6.95	6.68	
2	Filtered	7.76	8.92	5.22	7.30	6.89
	Non-filtered	7.70	6.58	5.19	6.69	
3	Filtered	7.47	7.42	4.08	6.32	6.23
	Non-filtered	7.90	4.65	5.87	6.16	
4	Filtered	9.48	7.89	4.19	7.18	6.07
	Non-filtered	5.48	4.14	5.25	4.95	
Temperatures		7.46	7.17	5.76		
Temp. X FeSO ₄	1% FeSO ₄	2	8.88	8.16	7.99	7.99
		3	7.75	5.20	6.89	
		4	7.03	4.97	6.23	
			6.01	4.72	6.07	
Filtration X FeSO ₄	Filtered	8.13	8.72	5.71	7.53	7.53
	Non-filtered	6.79	5.60	5.81	6.07	

* LSR values for minimum and maximum: FeSO₄ 1.11, 1.18; temperature 0.96, 0.99; filtration 0.78; FeSO₄ x temperature 1.46, 1.76; FeSO₄ x filtration 1.56, 1.78 and temperature x filtration 1.35 and 1.50g.

Table 78. Average feed intakes obtained for the interaction effects of FeSO_4 levels, temperatures and filtration treatments (g)*

FeSO_4 in meal %	Filtration	Temperature, °C			FeSO_4 X Filtration	FeSO_4
		25	45	100		
1	Filtered	50.90	47.98	46.15	48.34	43.88
	Non-filtered	39.50	40.90	38.20	38.93	
2	Filtered	43.88	45.84	33.99	41.23	39.88
	Non-filtered	39.75	40.24	35.60	38.53	
3	Filtered	47.87	40.86	34.05	40.92	39.93
	Non-filtered	44.34	33.37	39.09	38.93	
4	Filtered	50.44	43.69	37.65	43.92	39.78
	Non-filtered	35.67	35.56	35.67	35.63	
Temperatures		43.99	41.05	37.54		
Temp. X FeSO_4	1% FeSO_4	45.02	44.44	42.17	43.88	
	2	41.81	43.04	34.71	39.88	
	3	46.10	37.11	36.54	39.93	
	4	43.05	39.62	36.66	39.78	
Filtration X FeSO_4	Filtered	48.27	44.59	37.96	43.60	
	Non-filtered	39.73	37.51	37.13	38.12	

* LSR values for minimum and maximum: filtration 0.86; temperature x filtration 1.13, 1.29 and filtration x FeSO_4 4.74 and 7.07g.
 FeSO_4 1.22, 1.30; temperature 1.05, 1.10;

Table 79. Average liver weights obtained for the interaction effects of FeSO₄ levels, temperatures and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄	
		25	45	100			
1	Filtered	1.28	1.42	1.12	1.27	1.15	
	Non-filtered	0.99	1.04	1.06			
2	Filtered	1.10	1.05	0.84	0.99	0.97	
	Non-filtered	1.04	1.00	0.79			
3	Filtered	1.01	0.94	0.79	0.91	0.95	
	Non-filtered	1.06	0.95	0.96			
4	Filtered	1.18	1.08	0.90	1.05	1.01	
	Non-filtered	1.00	0.96	0.95			
Temperatures		1.08	1.05	0.92			
Temp. X FeSO ₄	1% FeSO ₄ 2 3 4	1.13	1.23	1.09	1.15	1.15	
		1.07	1.02	0.81			0.97
		1.03	0.94	0.87			0.95
		1.09	1.02	0.92			1.01
Filtration X FeSO ₄	Filtered	1.14	1.12	0.91	1.06	1.06	
	Non-filtered	1.02	0.98	0.94			0.98

* LSR values for minimum and maximum: FeSO₄ 0.09, 0.1; temperature 0.08, 0.09; filtration 0.07 temperature x filtration 0.09, 0.10 and filtration x FeSO₄ 0.09 and 0.15g.

Table 80. Average adjusted liver weights obtained for the interaction effect of FeSO₄ levels, temperatures and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄
		25	45	100		
1	Filtered	13.20	13.24	12.70	13.04	14.79
	Non-filtered	17.26	15.59	16.77	16.54	
2	Filtered	15.05	12.10	16.72	14.62	14.70
	Non-filtered	13.80	15.27	15.29	14.78	
3	Filtered	14.26	13.75	19.83	15.94	16.43
	Non-filtered	13.57	20.31	16.89	16.92	
4	Filtered	12.49	13.90	22.10	16.16	18.35
	Non-filtered	18.53	23.94	19.18	20.55	
Temperatures		14.77	16.00	17.43		
Temp. X FeSO ₄	1% FeSO ₄	15.23	14.41	14.73	14.79	
	2	14.42	13.68	16.00	14.70	
	3	13.91	17.03	18.36	16.43	
	4	15.51	18.92	20.64	18.35	
Filtration X FeSO ₄	Filtered	13.75	13.24	17.83	14.94	
	Non-filtered	15.79	18.77	17.03	17.19	

* LSR values for minimum and maximum: FeSO₄ 0.49, 0.53; temperature 0.81, 0.85; filtration x temperature 0.60, 0.67; FeSO₄ x temperature 0.65, 0.78; filtration x FeSO₄ 0.69, 0.79 and FeSO₄ x temperature x filtration 0.92 and 1.24g.

Table 81. Average kidney weights obtained for the interaction effects of FeSO₄ levels, temperature and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄
		25	45	100		
1	Filtered	0.34	0.36	0.33	0.34	0.30
	Non-filtered	0.25	0.27	0.26		
2	Filtered	0.30	0.31	0.23	0.28	0.26
	Non-filtered	0.27	0.24	0.22		
3	Filtered	0.27	0.26	0.21	0.24	0.25
	Non-filtered	0.27	0.23	0.27		
4	Filtered	0.34	0.29	0.23	0.28	0.26
	Non-filtered	0.24	0.22	0.24		
Temperatures		0.29	0.28	0.25		
Temp. X FeSO ₄	1% FeSO ₄	2	0.29	0.31	0.29	0.30
		3	0.28	0.27	0.22	
		4	0.27	0.24	0.24	
			0.29	0.25	0.29	
Filtration X FeSO ₄	Filtered	0.31	0.31	0.25	0.29	0.25
	Non-filtered	0.26	0.24	0.25		

* LSR values for minimum and maximum: FeSO₄ 0.03, 0.03; temperature 0.02, 0.03; filtration 0.02; temperature x filtration 0.03, 0.04 and filtration x FeSO₄ 0.04 and 0.05g.

Table 82. Average adjusted kidney weights obtained for the interaction effects of FeSO₄ levels, temperature and filtration treatments (g)*

FeSO ₄ in meal %	Filtration	Temperature, °C			FeSO ₄ X Filtration	FeSO ₄	
		25	45	100			
1	Filtered	3.47	3.34	3.51	3.44	3.77	
	Non-filtered	4.19	3.98	4.08			
2	Filtered	3.92	3.50	4.59	4.33	3.88	
	Non-filtered	3.53	3.59	4.22			
3	Filtered	3.70	3.91	5.41	3.34	4.28	
	Non-filtered	3.36	5.01	4.29			
4	Filtered	3.57	3.68	5.83	4.36	4.58	
	Non-filtered	4.38	5.41	4.67			
Temperatures		3.76	4.04	4.57			
Temp. X FeSO ₄	1% FeSO ₄ 2 3 4	3.83	3.71	3.79	3.77		
		3.72	3.54	4.40			3.88
		3.53	4.46	4.85			4.28
		3.97	4.54	5.25			4.58
Filtration X FeSO ₄	Filtered	3.66	3.60	4.83	4.03		
	Non-filtered	3.86	4.49	4.31	4.22		

* LSR values for minimum and maximum: FeSO₄ 2.37, 2.54; temperature 2.05, 2.14; filtration 1.68 and temperature x filtration 2.90 and 3.22g.

4. GENERAL DISCUSSION

4.1 Oxygen metabolism

Feeding experiments with meals of Brassica species as natural goitrogen sources and synthetic goitrogenic and thyroactive compounds to mice showed a wide variation in animal response with regard to metabolic rate. The results of these experiments have revealed that allyl-(in Oriental mustard sarson), 3-butenyl (in yellow sarson), 4-pentenylisothiocyanate and (S)-5-vinyl-2-oxazolidinethione (in rapeseed) as single glucosinolate products or as one of the released aglycones of the thioglucoside had deleterous effects on oxygen uptake of the mouse. Langer (1964) and Langer and Stolc (1965), by giving allylisothiocyanate to rats demonstrated that this compound significantly depressed radioiodine uptake by the thyroid. Turner (1946), Blakely and Anderson (1947a, 1948b) reported that when rapeseed meal was fed to an animal as a main source of protein the thyroid gland became enlarged. From data obtained in Experiments 3 and 4, it seems that the toxicity of goitrin (oxazolidinethione) was more severe than isothiocyanates (allyl and butenylisothiocyanates). Clandinin et al. (1966) fed racemic goitrin to chicks as 0.15 percent of the ration, which caused depression of growth rate, hyperplasia and hypertrophy of thyroid gland. The amount of progoitrin content of the rapeseed meals utilized in these experiments ranged from 0.6 to 4.9 percent of the ration, Bronowski meal which provided 0.04 percent in the ration resulted in no effect on the bio-

logical response of the mouse. However, Bronowski meal affected the animal response when myrosinase was supplied. Myrosinase supplementation of meal diets consistently depressed oxygen consumption. It was of particular interest that the adverse effects of myrosinase on diets paralleled the level of (S)-5-vinyl-oxazolidine-2-thione (its precursor) in the meals. This fact appeared in both experiments (Experiments 3 and 4). There are several possible explanations for this observation: (a) The rate of hydrolysis of glucosinolates by thioglucosidases which may be produced by microflora of intestine might be slower than the thioglucosidase accompanying thioglucosides in plant. (b) The degree of progoitrin conversion could depend upon the concentration of this enzyme; this means the rate of passage is another possible point in which in turn will change the media of hydrolysis. So the specific activity or velocity of myrosinase could be proportional to its conversion. Moreover, all these conditions might result in changing the amount of glucosinolate products absorbed per unit of time. It is known that (Greer, 1962) the duration of inhibition of radioiodine uptake seems to be correlated well with goitrin content in the blood and urine.

From present investigation might speculate that the addition of myrosinase could cause a change in the slope of gradient accumulation of iodine, which in turn will lead to hypofunction of the thyroid gland. Hypofunction of the thyroid gland results in a lower level of thyroxine. The effect would be reflected in reduced oxygen consumption of animals.

The thyroid inhibiting effect of goitrin was first detected by measuring the reduced uptake by the thyroid of radioiodine (I^{131}) after test animals were fed this compound (Astwood et al. 1949a, 1949b, and Carroll 1949). Greer and Deeney (1959) reported that progoitrin was converted to goitrin by myrosinase in the body.

Administration of progoitrin and myrosinase inhibited the thyroid uptake of radioiodine in man and rats. Greer (1969) administered orally progoitrin and myrosinase together to man and determined the accumulation gradient and radioiodine uptake. This author performed his experiment with progoitrin only and found similar results, but the latent interval from ingestion of progoitrin to a detectable change in the slope of the accumulation gradient appeared to be somewhat longer than with progoitrin plus myrosinase. In addition, he mentioned that the serum goitrin concentration reached at maximum approximately 35 hours after progoitrin administration, whereas maximum was reached within the first half-hour after giving goitrin. It was of interest that acid hydrolysis in the stomach and duodenal secretion were not responsible for the conversion of progoitrin to goitrin.

In the present study, the expected effect of dietary myrosinase on P meal (Experiment 4) did however, not appear whereas its progoitrin level was similar to that of other meals that did respond. This result might be interpreted to mean that there is a possibility that some of the meals of rapeseed contained free oxazolidinethione in addition to their glucosinolate contents.

Feed intake of diets containing meals of Brassica species was very variable, particularly in the presence of dietary myrosinase. One might develop the conclusion that these meals containing goitrogenic factor(s) after ingestion and absorption caused the hypofunction of thyroid gland. The change of thyroid function could cause reduction of thyroxine level in the body, which in turn is reflected in lowered appetite of the animals. Belzile and Bell (1963) reported that gains of mice fed enzyme active meal remained significantly ($P < 0.05$) inferior relative to treated meal. Jat (1967) stated that increasing or decreasing function of thyroid gland caused the changes in appetite for carbohydrate. Workentin et al. (1943) found that feed intake (starch diet) and caloric intake was reduced in rats after thyroidectomy. Methylthiouracil had the same effects as thyroidectomy. Soularac (1967) from his experimental results demonstrated that administration of large doses of thyroxine to mice increased the feed intake by 33 percent and glucose by 25 percent. The thyroidectomized rat slowed standard food consumption by 30 percent and that of carbohydrate by 90 percent. Brody (1945) states that in fact, all anabolic processes are retarded by thyroidectomy including the rates of digestion, peristalsis, assimilation, especially of carbohydrates, etc. In 1968 Bass reported that the degree of correlation was high between the frequency of the basic electrical rhythm (slow wave) of gastrointestinal and thyroid function and hypothyroidism was associated with slower wave rate than normal. This indicates that cellular

metabolic processes which are influenced by the thyroid gland govern the frequency of motility of intestine.

Evidence for goitrogenic effects of Brassica species meals has been repeatedly reported (see literature review). The presence of goitrogenic principles in some of the Brassica species meals is confirmed in this study. The variation of the inferior oxygen uptake obtained (table 28) for Brassica meals under the experimental conditions is probably associated with several factors. The level of glucosinolate contents and the nature of this content may have influenced the oxygen uptake in this study. Enzyme systems and bacteria in the digestive tract could be another factor. The crude fiber content of these meals and nature of this fiber content may have influenced the oxygen uptake in this study as well.

4.2 Meals treated at different pH's

Analysis of organ weights per se (as absolute value) was chosen because the smaller size of test animals (with respect to normal size) leaves the comparison of relative organ weight open to question. Furthermore, the ratio of organ weight to body weight (relative organ weight) is not used in this investigation for comparing experimental groups because prerequisites ((a) that the two quantities can be assumed positively and linearly related, (b) that the regression line intersects the ordinate at the origin and (c) that the variance in body weight (y) is proportional to organ weight (x)) for using such ratio were not satisfied. Thus, the organ weights proportional to the final body weight must be used for comparing

test animals in one standard as well. The initial weights of weanling mice were almost equal ($10 \pm 0.5g$) in these experiments; thus the weight gains were arbitrarily used instead of final body weights for adjusting liver and kidney weights in one standard.

Under conditions of these experiments (Experiments 5 and 6), rapeseed meal prepared with different buffered solutions resulted in a significant reduction in gain, feed intake and enlarged liver and kidney weights (adjusted gain weight) relative to the control (natural meal). There are reports in the literature of the enlargement of liver and kidney in rats and swine fed Brassica meal (Bowland et al., 1963 and Nordfeldt et al., 1954). In addition to growth and feed intake, depression and enlarged organs, a marked liver congestion was often detected in this experiment, particularly in the mice fed pH5 - buffered meal. VanEtten et al., (1969) observed dark and firm livers in rat fed nitriles. Belzile (1963) showed that the pH(3, 6 and 9) of buffers without dietary enzyme being present produced neither an effect on glucosinolate products in vitro nor on growth and feed intake. However, this author found significant growth depression when thioglucosidase was added to the meal and buffered at pH6 and 9. This author used rapeseed meal with lower glucosinolate contents than that used in this investigation.

The addition of myrosinase to enzyme-inactivated (commercial) meal buffered at various pH's consistently de-

pressed the growth rate and feed intake and caused enlargement of livers and kidneys in terms of percentage of weight gain. The latter phenomenon was more evident in the second trial. Furthermore, liver congestion was observed in animals that received myrosinase supplementation. Interaction effects involving pH5 - buffered meal and myrosinase were more severe than other pH buffered meals x myrosinase in terms of biological evaluation (gains and adjusted liver and kidney weights) of rapeseed meal in both experiments (tables 30 and 31). Such observations indicate the presence of toxic substances in buffered meal(s) other than those measured by chemical assay. Moreover, the results of this feeding experiment suggest that the predominant thioglucosides (gluconapin, glucobrassicinapin and progoitrin) of rapeseed meal might (i) be degraded non-enzymatically by various pH's in vitro to form goitrogenic and/or cyanogenetic plus goitrogenic products. (ii) myrosinase supplementation should accelerate the hydrolysis of these glucosinolates in vitro and in vivo. It is also possible that the addition of thioglucosidase supports animal myrosinase to hydrolyze these potential toxic compounds in the intestinal tract. The latter suggestion supports our previous finding (metabolic rate studies).

From our finding, we could be reasonably certain that acid hydrolysis in the stomach was not responsible for the non-enzymatic hydrolysis of thioglucosinolate by considering the rate of passage of this compound and a high acidity

of gastric juice. For further illustration following data is cited (Altman and Dittmer, 1968).

Table 83. Gastrointestinal pH of the rat and mouse

Gastro-intestinal tract. Part	Animal	Level of pH
Gastric juice	rat	2 - 4
duodenum	normal rat and germ free rat	6.3 - 6.4
jejunum	normal rat and germ free rat	5.8 - 6.3
upper ileum	normal rat and germ free rat	6.5 - 7.0
lower ileum	normal rat and germ free rat	6.9 - 9.0
cecum	normal mice and germ free mice	6.6 - 7.5
colon	normal mice and germ free mice	6.5 - 6.5

Furthermore, Wetter (1955) reported that the optimum conditions for release of isothiocyanates from rapeseed meal in vitro are at pH4. However, Appelqvist and Josefsson (1965) from their experimental results and literature (Scwimmer's work) stated that the pH-optimum of the thioglucosidase reaction is 6 to 7.0. Daxenbichler et al. (1966) showed that less than 5 percent of mustard myrosinase hydrolysis of purified epi-progoitrin occurred at pH2.3 and 12 on standing for 24 hours. Greer (1962) demonstrated that progoitrin did not

convert to goitrin when incubated with widely varying concentrations of HCl. The questions that should be raised are (a) in what part of the body (mouth, small intestine, cecum or colon) hydrolysis of glucosinolates (with and without myrosinase) takes place

(b) how conversion is accomplished and

(c) what parts of the gastro-intestinal tracts are absorption sites. Satisfactory answers could be found by feeding labelled glucosinolate.

The major problem for full explanation in this study appears to lie in the experimental procedure to determine the existence and types of organic cyanogenetic (nitrile) compounds. Histological examination of organs would be useful in this regard. We might, therefore, only conclude that this study provides no information about separate effects of cyanogenetic or goitrogenic compounds on biological response of mice.

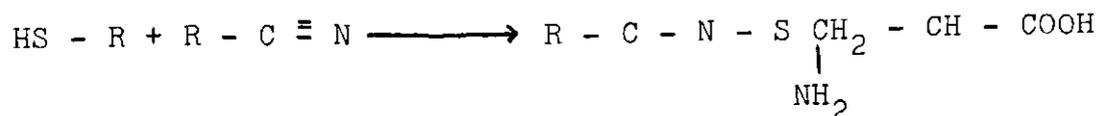
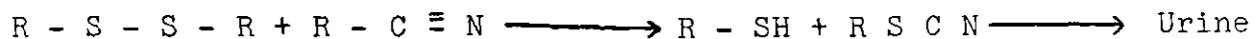
By referring to observed gains and feed intake of mice fed natural and buffered meals at pH 3.6 and 7 (Experiment 5) the differential mouse responses demonstrated in these feeding experiments cannot be ascribed to the presence of buffer pH salts in the diets containing the prepared meals. Belzile (1963) stated that buffer salt did not cause a depression of growth and feed intake of mice.

In summary this experiment indicated that the occurrence of undesirable substances other than isothiocyanates and oxazolidinethione could be speculated. However, in the absence of chemical analysis of the buffered prepared meals

(with and without myrosinase inclusion) in Experiment 5 and with myrosinase supplementation in Experiment 6 (table 32) it is difficult to ascertain the toxicity effects of either unknown compounds or of individual goitrogenic compound, which might be obtained from splitting glucosinolate under different conditions. Thus it appears that major attention should be given to toxic products in general from obtained factors and find out a proper solution to neutralize, decompose or remove these unfavorable materials to make an acceptable protein supplement source.

4.3 Effects of treated meal with the addition of cystine and myrosinase

Results of Experiment 6 made it evident that the addition of a certain level (0.26 percent of meal) of L-cystine to Brassica napus meal improved the growth and prevented both the enlargement and abnormality of livers and kidneys of mice. It must be noticed that this improvement was observed when the meal was not pre-treated with myrosinase, but when it was buffered (tables 36 and 38). It was thought that cystine in acidic media reacted with nitrile(s) (an organic compound containing the group $C\equiv N$) and isothiocyanates to yield a non-toxic product compound as shown by the following equations:



A report of similar investigation was not found in literature reviews. However, Blakely and Coop (1949) reported that a rat poisoned by cyanide was prevented from death by intravenous injection of sulfur donor compounds (see literature review).

The addition of higher levels of cystine to rapeseed meals did not have a beneficial effect (Experiment 6) (tables 36 and 38). In the instance of casein the addition of higher levels (0.72 and 1.44 percent) of cystine slightly improved the gain rate of growing mice. Probably supplementary cystine improved casein quality (Appendix K).

Filtration of buffered rapeseed meal resulted in the significant reduction by 50 to 55 percent of total amounts of isothiocyanates, oxazolidinethione and OH-nitrile. This observation was supported by Belzile's finding (1963) in the case of goitrogenic compounds. Furthermore, the biological and chemical assays were in agreement with each other in this investigation.

In the second part of Experiments 5 and 6, it was observed that when higher levels of cystine were incorporated into rapeseed meal, it caused either reduction in gain rate or death of test animals. The author was not able to interpret this observation. Under conditions of this experiment, the excess supplementation of cystine to create a mineral or malonaldehyde complex however, might be an explanation. Kay and Mitch (1968) reported that cystine plus molybdenum made a constant complex at room temperature. It has been proved that among amino acids cystine and histamine are most capable to make amino acid chelates with heavy metal ions (Mahler and Cordes, 1967).

4.4 A study of natural goitrogenic compounds and OH-nitrile

Three experiments (Experiments 9, 10 and 11) involving feeding trials and chemical tests were designed to study the biological activity of naturally-occurring (S) goitrin, isothiocyanates, hydroxynitrile and possibly identified toxic compounds rather than goitrogenic compounds.

By referring to the reports which have been reviewed (section 2, page 48 to 59), it could be claimed that much of the prior literature, in the nutritional field, on species of Brassica which contain progoitrin (in rapeseed) or epi-progoitrin (in Crambe seed) relates to the goitrogenic properties of the seed meals from these plants. This could probably be due to the early recognized (S) goitrin or (R) goitrin formed from progoitrin or epi-progoitrin, respectively. However, Youngs and Perlin (1967) have been able to show that when ferrous sulfate was added to rapeseed (Brassica campestris) the thioglucosinolate contents decomposed to yield nitriles and thionamide. Daxenbichler et al. (1968, 1967 and 1965) found that autolyzed Brassica napus and Crambe seed meals yielded either goitrin, or unsaturated nitrile and diastereomeric episulfide (containing nitriles), or a mixture of all these toxic compounds.

We have been able to show that by controlling the conditions of hydrolysis of glucosinolate contents of rapeseed (Brassica napus) meals either by supplementing chemical compounds

(FeSO₄, buffer solution, cystine) or by different methods of process, the glucosinolate products would be varied and possibly interchanged (tables 32, 37, and 62). For instance, oxazolidinethione, isothiocyanates and hydroxynitrile contents of the treated meals ranged from 0.0 to 5.3mg/g, 0.0 to 2.2mg/g and 0.43 to 3.38mg/g of meal in the same order. It should be noted that original meal (control) contained 8.4mg/g goitrin, 3.9mg/g isothiocyanates and 0.05mg/g hydroxynitrile (tables 32 and 62).

On the basis of our observations it might be concluded that nitrile becomes less soluble in the hot buffer (pH5) solution in the presence of ferrous sulfate (table 62). This possibility could be due to a complex formation. Special experiments should be carried out to find a full explanation for observations. However, when meal was treated with cystine in hot water, hydroxynitrile was completely soluble (table 76).

In these experiments, in order to compare the results of the chemical assay with biological assay, mice feeding trials were performed (Experiments 9, 10 and 11). The results of biological assays (Experiment 9) were closely in agreement with chemical assays.

When meals were treated at either room or 45°C temperature, the mouse growth was at maximum, whereas mice fed meals treated at 100°C had poor gains and enlarged organs (Experiments 9, 10 and 11). It was suggested that growth depression and enlargement of organs (liver and kidney) were due to greater formation of, and less solubility of, hydroxynitrile (Experiment 9). There are reports in the literature of enlarged

livers and kidneys in rats and swine when fed rapeseed meal (Bell, 1955 and Carroll, 1949).

Under conditions of Experiment 9, test animals that received meals treated with ferrous sulfate and cystine at pH5-buffer solution performed better than mice fed meals treated with ferrous sulfate in distilled water (Experiment 11). This improvement might be attributed to the presence of cystine and acidic pH media.

Filtration significantly improved the meal quality in these experiments. This improvement was due to removal of toxic factor contents of the meals (table 62). It must be pointed out that the best results were obtained in Experiment 9. However, in the case of Experiment 10 (meals treated only with cystine in distilled water), test animals performed better when fed unfiltered meals.

Several interaction effects were significant in these experiments. The meals treated with 3 percent or 4 percent ferrous sulfate, 0.26 percent cystine and pH5 solution at 100C regardless of filtration contained 0.0mg/g and 2.64mg/g goitrogenic (goitrin and isothiocyanates) and hydroxynitrile, respectively. When these meals were fed to mice, responses were poorer than animals fed treated meals (containing 1.78, 0.61 and 1.3 mg/g goitrin, isothiocyanates and hydroxynitrile respectively) at either room or 45^oC temperature. This difference could be due to a higher level of hydroxynitrile probably other unknown toxic factor(s) in the boiled meals. It was of particular interest that among experimental (Experi-

ment 9, 10 and 11) diets, the diet containing meal treated with 4 percent FeSO_4 , cystine, pH5-buffer solution and filtered (Experiment 9) resulted in maximum growth (12.11g).

On the basis of observations of these experiments (particularly Experiment 9), it was concluded that hydroxynitrile was almost four fold as toxic as isothiocyanates and goitrin. Furthermore, it was thought that unfiltered meal contained other toxic materials (i.e., thionamide, nitriles, tannin, etc.), in addition to those determined.

VanEtten, et al. (1969) fed either isolated or an unisolated nitrile mixture (hydroxy and epi sulfide) at levels of 1 and 2mg nitrile per g of diet to rats and found that 1mg of nitrile mixture severely affected gain, liver, kidney and thyroid gland of rats and 2mg of nitrile caused the death of animals. Lo (1969) fed synthetic hydroxynitrile to mice and demonstrated that animals received 0.8mg nitrile per g diet gained 20 percent less than control.

We found that mice growth responses fed unisolated hydroxynitrile at level of 0.88mg/g diet (Experiment 9) were lower by 50 percent when compared with animals fed casein (Experiment 7). While an equal gain was obtained when the mouse was fed unisolated hydroxynitrile at level 0.26mg/g diet. The results of feeding trials indicated that isothiocyanate (3-butenyl and 4-pentenylisothiocyanates) contents of the meals treated by our methods had no influence on biological activity of mice. This ineffectiveness could be due to lower concentration of isothiocyanate contents than

the tolerance level of mice. Furthermore, the performance of test animals fed diet containing 1.5mg unisolated goitrin plus a total of 0.62mg isothiocyanates and hydroxynitrile per gram diet was severely affected. This finding might be an indication of severe harmful effect of goitrin.

Lo (1969) demonstrated that mice that received orally 2.64mg of synthetic 3-butenylisothiocyanate per g diet did not show responses in terms of gain, but feed intakes were slightly lower than control animals. VanEtten (1969) fed either isolated, unisolated epi-progoitrin or (R) goitrin at the levels of 15mg and 2.3mg/g diet to rat respectively, and found both compounds resulted in a reduction of gain and in enlargement of liver and kidney.

On the basis of data obtained from our experiments, we would suggest that by changing the method of the process, the amount and type of glucosinolate products can be changed. Furthermore, our results have shown that meals treated by our technique contained other soluble toxic material which were chemically estimated.

5. SUMMARY

Meals of certain Brassica species were used in twelve feeding experiments with male white mice. The effects of the synthetic thyroid-active iodinated-casein, the synthetic goitrogenic thiouracil and natural glucosinolates on the metabolic rate, as measured by oxygen consumption ($\dot{V}O_2$) was studied in four of the experiments. The remaining eight experiments involved studying the effects of commercial rape-seed (Brassica napus) meal treated by different methods, on biological responses of growing male white mice in terms of weight gain, feed intake, and liver and kidney weights. The results are summarized as follows:

A - Oxygen metabolism

(1) A simple automatic respirometer for measuring $\dot{V}O_2$ was designed and tested. Its reliability, accuracy and simplicity were confirmed by over 700 individual tests, using different diets at different times of the year.

(2) The oxygen uptake of individual mice fed thiouracil, iodinated-casein and control diets were measured. Feeding thiouracil resulted in a reduction of $\dot{V}O_2$ whereas iodinated-casein caused an increase. Similar results from in vitro studies have been extensively reported.

(3) Oxygen consumption by mice fed certain meals of Brassica species was estimated (Experiment 3). There was significant variation in $\dot{V}O_2$ by mice due to the different meals. This variation attributed to differences in levels and types of glucosinolates in the different meals. Oxygen

consumption was at a minimum with mice receiving diets containing Brassica napus and $\dot{V}O_2$ increased progressively with the following species of Brassica and the control diets, Brassica campestris, Brassica juncea, SSP sarson and soybean meal. Furthermore, feeding dietary myrosinase indicated that inclusion of supplementary thioglucosidase in diets containing thioglucosides depressed oxygen consumption. It is noteworthy that myrosinase supplementation had no effect on yellow sarson and ferrous treated oriental mustard oil meals. However, the mildly antithyroid effect of Bronowski meal became more pronounced in the presence of myrosinase.

(4) In another experiment, the oxygen uptake by mice fed eleven samples of rapeseed (Brassica napus and Brassica campestris) meals, commercially prepared (expeller, pre-press solvent extraction and direct solvent extraction) with and without myrosinase were measured. There was no obvious parallel between $\dot{V}O_2$ and glucosinolate contents of the meals. It was concluded that the rapeseed meals contained other harmful factor(s) than thioglucosides which have the potential to release other toxic compounds (organic nitriles and thionomide) besides isothiocyanates and oxazolidinethione in vivo and in vitro.

Myrosinase supplementation of rapeseed meal diets consistently depressed oxygen uptake, but there was a wide range of response (0.4 to 18 percent depression). However, dietary myrosinase had no effect on mice fed soybean meal, even though feed intake was reduced significantly (Experiments 3 and 4). The reason for these differences is not

immediately apparent; however, gastrointestinal motility, secretory, absorptive power, etc., might be beneficially affected.

Results from Experiments 3 and 4 indicated that goitrin or its progoitrin had a more deleterious effect than either allyl or butenylisothiocyanates.

Under conditions of these experiments oxygen uptake and feed intake were lower for all Brassica meals used, particularly those which contained more than one type of glucosinolates than standard meal. The inferiority of $\dot{V}O_2$ is probably associated with several factors, including the level and nature of glucosinolate contents, intestine bacterial secretion, motility and secretion of gastrointestinal tract, hydrolysis, absorption rate and absorption site of glucosinolates or their products.

B - Treatment of a commercial rapeseed meal
(Brassica napus)

(1) Effects of pH and myrosinase

Buffered meals, of either acidic or basic pH caused a highly significant reduction in rate of gain and feed intakes. The livers and kidneys of growing mice fed these meals were enlarged, dark and firm (Experiments 5, 6 and 7). In both feeding trials (Experiment 5 and 6) when growing mice which were fed meal buffered at pH 5 had the poorest gains and largest liver and kidney weights with dark color and consistency. Furthermore, the addition of myrosinase caused a reduction in gain, feed intake and organ abnormality. On the basis of these observations, it was concluded that the extent of the growth

rate depression and abnormality of livers and kidneys of test animals may be dependent on both glucosinolate contents and the pathway of glucosinolate hydrolysis.

- (2) Comparison of the value treated rapeseed meals with casein

Brassica napus meal, Bronowski meal and casein were supplemented with various levels of cystine and slurried with pH5 buffer solution (1:5 ratio). The prepared meals and casein (as a source of protein) were fed to growing mice for 14 days. Estimation of the values of the tested protein sources were based on the measurement of gain, feed intake and weights of fresh livers and kidneys (Experiment 7).

From the results of this experiment the following conclusions were made:

- (a) the addition of cystine at 0.26 percent in Brassica napus meal was beneficial. However, high levels of cystine supplementation showed adverse effects.
- (b) Under conditions of this experiment Brassica napus meal, commercially prepared by pre-press solvent extraction was inferior ($P < 0.01$) to casein and Bronowski meal. Furthermore, casein was superior to Bronowski meal. It was thought that the inferiority might be due to glucosinolate contents, protein quality (amino acid imbalance, ratio of protein nitrogen to non-protein nitrogen) and probably crude fiber contents of rapeseed meals when compared to casein.

- (c) Filtration of Brassica napus meal significantly improved quality of the meal.
- (d) Incorporation of cystine at 0.26 percent into Brassica napus meal with filtration improved the quality of the meal (157 percent) over unfiltered meal and almost equal to casein.
- (e) Addition of cystine to rapeseed meal at natural pH had no beneficial effects (Experiment 7B).

(3) Treatment of rapeseed meal with FeSO_4 , cystine pH and myrosinase

Rapeseed meals treated with various chemical compounds were incorporated into the basal diet and fed to growing mice (Experiment 8). The following inferences could be made from the experimental results:

- (a) Cystine supplementation improved ($P < 0.01$) the responses of the test animals.
- (b) Acidic (pH5) buffered meal depressed gain, and feed intake but caused enlargement of livers and kidneys of growing mice.
- (c) Adverse effects of dietary myrosinase were severe on all of the criteria that were evaluated.
- (d) The benefits of ferrous sulfate were not evident in this experiment. It was possibly due to the wide range levels of the compounds used.
- (e) The beneficial effects of cystine were largely restricted to pH5 buffered solution.

It was concluded that further experiments should be carried out to find the influence of cystine, ferrous

sulfate and pH on the detoxifying of rapeseed.

(4) Treating rapeseed meal with different methods

Studies were conducted to investigate the effects of chemical compounds (FeSO_4 , cystine and pH) and the methods processing (filtration and temperature) on detoxifying Brassica napus meal. The investigations included demonstration of the chemical composition of glucosinolate products (isothiocyanates, goitrin and hydroxynitrile) of the treated meals and animal responses in terms of weight gain and organ (liver and kidney) weights. Particular attention was given to the formation of hydroxynitrile and its toxicity (Experiment 9).

The contents of isothiocyanates and goitrin were decreased while hydroxynitrile production was increased with increasing ferrous sulfate supplementation. The process also resulted in some apparent alteration of the toxic constituents. These observations were more noticeable when the pre-treated meals were boiled in acidic pH.

The extraction of the treated meals with buffer solution resulted in about 74 percent of the glucosinolate products of the slurried meal going into solution. Furthermore, oxazolidinethione was less soluble than isothiocyanates at low temperature.

Among the various procedures devised to improve the feeding value of rapeseed meal, three methods have been successful. The effectiveness of these methods were verified by fact that the gains of mice receiving the diet

containing the untreated meal were only about 60 percent of that of mice fed either meals which were treated by our technique or fed casein as source of protein.

Macroscopic examination of the organs (liver and kidney) showed that test animals fed un-isolated hydroxynitrile in excess of 0.50mg/g diet were dark in color and firm in texture. These pathological signs were more obvious when higher levels of the hydroxynitrile were fed. In contrast, filtered meal when previously treated with buffer solution gave satisfactory results in terms of organ conditions when compared with feeding Casein.

The beneficial effects of certain levels of L-cystine were observed in our investigations (Experiments 7,8,9 and 10). The addition of this amino acid had no advantages when the pre-treated meals were filtered (Experiments 7 and 10). However, when meals pre-treated with cystine were boiled in distilled water and filtered, this resulted in improvement of the meal.

In brief, studies were conducted (1) to find whether glucosinolate contents of meal of Brassica species affect metabolic rate of animals and how large is the magnitude of their effects with and without dietary myrosinase, and (2) to develop an effective technique to detoxify rape-seed (Brassica napus) meal without damaging its nutrient contents.

The experimental results showed that glucosinolate contents of the meals adversely affected metabolic

rate of mice. The magnitude of $\dot{V}O_2$ varied widely, depending type, amount of glucosinolate contents, and depression of $\dot{V}O_2$ was more severe when dietary myrosinase was added. Oxygen uptake was minimal by mice receiving Target and Nugget meals followed by those having other Brassica and soybean meals. It appeared that among studied meals Argentine-type (Brassica napus) meals were more deleterious to test animals. Furthermore, myrosinase effects on different meals were not similar, that means, type of glucosinolate (substrate) apparently had effect on activity of supplementary myrosinase.

The results of chemical and biological assays suggest that the predominant thioglucosides (gluconapin, glucobrassica-napin and progoitrin) of meal of Brassica species might be degraded by different pathways, enzymatically and non-enzymatically. Thus, the amount of glucosinolate products depend on the glucosinolate hydrolysis pathway: these in turn, can change the extent of toxicity of the meals. For instance, it was found that meals containing hydroxy nitrile are more toxic than those containing goitrin. The latter in turn, are more toxic than those containing isothiocyanates.

Among methods used to detoxify rapeseed meal the following two proved to be very effective:

- (1) Addition of a certain quantity of L-cystine to rapeseed (Brassica napus) meal improved growth and prevented abnormality of livers and kidneys of mice. It was postulated that cystine in acidic (pH) media reacted with

nitriles and isothiocyanates to yield a non-toxic compound. (Section 2.8, literature review).

(2) The filtration of buffered (pH) rapeseed meal resulted in a significant reduction of total amount of isothiocyanates, goitrin and unknown toxic material(s).

Our observations indicated that the techniques developed for detoxifying rapeseed meal were effective. The value of the meals treated by our methods were equal to the value of casein. It should be mentioned that the effectiveness of these techniques is based on laboratory results. Thus, the industrial application of these techniques requires further investigation.

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APPENDIX A

Diet composition for Experiment 2

Diet No.	Basal percent	Soybean meal percent	Iodinated-Casein percent	Thio-uracil* percent
1	73.03	26.97	0.0	0.0
2	73.03	26.97	0.12	0.0
3	73.03	26.97	0.0	0.075

*6-n-propyl-2-thiouracil

Basal composition

Composition	Percent
Barley	34.4
Sucrose	15.0
Lard (Stab.)	10.0
Cellulose (B.W.200)	5.0
Starch	26.6
Vitamin pre-mix	5.0
Mineral pre-mix	4.0

APPENDIX BFormula of vitamin mixture and mineral
mixture for Experiment 2

Vitamin pre-mix	
Element	Percent
Thiamin hydrochloride	0.010
Riboflavin	0.020
Pantothenic acid (85 percent)	0.100
Niacin	0.100
Biotin (D)	0.002
Pyridoxine chloride	0.010
Vitamin B12	0.050
Folacin (Folic acid)	0.005
Inositol	0.004
Choline chloride	8.000
Vit. A (325,000 IU/gm)	0.030
Vit. D ₃ (200,000 IU/gm)	0.013
Vit. E (125,000/lb)	1.00
Vit. K (menadine)	0.400
Corn starch	90.300

Mineral pre-mix	
Element	Percent
NaCl	4.55
CaH PO ₄	78.13
KHCO ₃	14.29
Mg	2.04
MnSO ₄ ·H ₂ O	0.41
FeSO ₄ ·2H ₂ O	0.41
CuSO ₄ ·5H ₂ O	0.11
ZnO	0.11

APPENDIX C

Analysis of variance of oxygen uptake by mice
fed goitrogenic and thyroactive drugs

Source of variance	df	MS
Treatment	2	2.095**
error	27	0.123
total	29	0.26

Analysis of variance of feed intake by mice
fed goitrogenic and thyroactive drugs

Source of variance	df	MS
Treatment	2	29.95
error	13	16.52
total	14	19.62

** Statistically significant at $P < 0.01$

APPENDIX D

Formula of diets, mineral mixture and
vitamin mixture for Experiment 3

Diet composition	
Ingredient	Percent
Oatmeal, 100 mesh	64.41
Lard stabilized	3.39
Alpha protein (soybean)	1.00
Casein	1.00
Vitamin-mineral pre-mix	5.00
Cellulose (BW 100)	5.00
Lysine-1	0.10
Methionine OH-analogue	0.10
Source of protein	20.00

Ingredients of vitamin-mineral mixture	
Elements	Amount (g)
NaCl (iodized)	11.11
Calcium - phosphate (20.20)	22.22
ZnO	0.049
Vitamin A (325,000 IU/g)	0.013
Vitamin D ₃ (200,000 IU/g)	0.011
Vitamin E (125,000 IU/lb)	0.033
Thiamine-HCl	5.0mg
Riboflavin	6.0mg
Pantothenic acid (58 percent)	1.3mg
Niacin	3.3mg
Biotin (D)	2.0mg
Pyridoxine HCl	2.0mg
Folic acid	6.0mg
Vitamin B ₁₂	9.0mg
Cerelose	33.15g
Starch	33.33g

APPENDIX E

Variance analyses and co-variance of oxygen consumption by mice fed different meals of Brassica species (Experiment 3)

Analysis of variance			Analysis of co-variance		
Source of variance	df	MS	df	MS	
Meal	7	0.865**	7	0.408*	
Myrosinase	1	0.250**	1	0.162*	
Meal x Myrosinase	7	0.026	7	0.012	
Error	144	0.032	63	0.021	

Variance analyses and co-variance of feed intake of mice fed different meals of Brassica species

Analysis of variance			Analysis of co-variance		
Source of variance	df	MS	df	MS	
Meal	7	0.125	7	0.125*	
Myrosinase	1	0.787**	1	0.862*	
Meal x Myrosinase	7	0.075	7	0.062	
Error	64	0.15	63	0.212	

* or ** Statistically significant at $P < 0.05$ or 0.01

APPENDIX F

Formula of vitamin mixture, mineral mixture and basal diets for Experiment 4

Components of vitamin mixture			
Vitamin		percent	
Thiamine		0.01	
Riboflavin		0.02	
Pantothenic acid		0.10	
Niacin		0.10	
Biotin		0.002	
Pyridoxine		0.01	
Vitamin B12(1 percent)		0.05	
Folacin		0.005	
Inositol		0.004	
Choline		8.00	
Vitamin A (325,000 IU/g)		0.03	
Vitamin D ₃ (200,000/g)		0.013	
Vitamin E (125,000/lb)		1.0	
Vitamin K (menodione)		0.4	
Corn starch		90.5	

Composition of Basal diet		Elements of mineral pre-mixture	
Barley	34.40	NaCl	4.45
Sucrose	15.00	CaHPO ₄	76.42
Lard (stabilized)	10.00	KHCO ₃	14.46
Cellulose (BW200)	5.00	Mg	2.00
Starch	26.10	MnSO ₄ , H ₂ O	0.33
Vitamin pre-mix	5.00	FeSO ₄ , 2H ₂ O	0.33
Mineral pre-mix	4.5	CuSO ₄ , 5H ₂ O	0.11
		ZnO	0.11

APPENDIX G

Variance analysis and co-variance of oxygen uptake by mice fed different rapeseed meals. (Experiment 4)

Variance analysis			Co-variance		
Source of variance	df	MS	df	MS	
Meals	11	0.607**	11	0.308**	
Myrosinase	1	1.592**	1	0.452**	
Meals x Myrosinase	11	0.106**	11	0.060*	
Error	216	0.034	95	0.028	

Variance analysis and co-variance of feed intake of mice fed different rapeseed meals. (Experiment 4)

Variance analysis			Co-variance		
Source of variance	df	MS	df	MS	
Meals	11	0.675**	11	0.637**	
Myrosinase	1	5.125**	1	3.362**	
Meals x Myrosinase	11	0.562**	11	0.575**	
Error	96	0.200	95	0.200	

* or ** Statistically significant at $P < 0.05$ or 0.01

APPENDIX H

Analyses of variance of gains, liver, kidney weights and feed intake of mice fed rapeseed meal with and without myrosinase supplementation and prepared with buffer solution with various pH's. (Experiment5)

Source of Variance	df	gain	Variance				
			liver (abs.wt.)	liver (P. wt.)	kidney (abs.wt.)	kidney (P.wt.)	feed intake
pH	3	30.17**	0.168*	43.06**	10.30	4.23	27.96
Myrosinase	1	113.43**	0.022	286.82**	0.07	22.29**	383.96**
pH x myro.	3	7.87*	0.012	8.95	0.039	1.5	93.85*
Error	32	2.19	0.047	8.06	0.34	0.63	23.49

* or ** Statistically significant at $P < 0.05$ or 0.01
P = Percent

APPENDIX I

Analyses of variance of gains, liver, kidney weights and feed intake of mice fed rapeseed meal with and without myrosinase supplementation and prepared with buffer solutions of various pH's. (Experiment 5)

Source of Variance	df	gain	Variance				
			liver (abs.wt.)	liver (P.wt.)	kidney (abs.wt.)	kidney (P.wt.)	feed intake
pH	3	10.74**	0.027	239.82**	0.0013	19.44**	104.53**
Myrosinase	1	164.67**	0.344**	2572.48**	0.0265**	161.10**	805.24**
pH x myro.	3	1.29	0.068*	222.04	0.0016	11.53*	50.96
Error	32	1.359	0.022	31.21	0.0016	2.77	26.02

* or ** Statistically significant at $P < 0.05$ or 0.01

APPENDIX J

Ration formulation of Experiment 7

Ration No.	Ingredient	Percent
1	Casein	13.26
	Basal	86.77
2	Bronowski meal	31.55
	Basal	86.45
	Rapeseed meal (<u>Brassica napus</u>)	28.67

APPENDIX K

The amino acid contents of Casein, Bronowski and Commercial rapeseed meal¹

Amino acid	Casein percent	Bronowski percent	C. meal ² percent
Alanine	-	-	4.44
Arginine	3.69	6.22	5.71
Aspartic acid	7.24	5.75	6.87
Cystine	-	2.16(3)	2.16
Glutamic acid	29.97	16.51	18.11
Glycine	-	4.61	4.95
Histidine	3.02	2.80	2.69
Isoleucine	4.57	3.51	3.80
Leucine	9.37	6.42	6.78
Lysine	8.18	5.65	6.00
Methionine	2.96	1.85	1.86
Phenylalanine	5.20	3.79	3.88
Proline	-	5.07	6.21
Serine	6.67	4.08	4.49
Threonine	3.10	4.08	4.39
Tryptophan	1.04(3)	-	1.15
Tyrosine	5.97	2.58	2.22
Valine	5.78	4.34	4.89

¹g amino acid/100g

²Obtained from The University of Alberta, Edmonton, Alberta.

³Determined by microbiological assay

APPENDIX L

Analyses of variance of gains, liver, kidney weights and feed intake of mice

Source of Variance	df	Variance					
		Gain (g)	Liver wt.(g)	Liver wt.percent	Kidney wt.(g)	Kidney percent intake (g)	
Cystine	4	3.035	22.483	17.624**	0.802	1.169*	15.977
Protein source	2	194.491**	26.222	148.531**	0.157	3.817**	256.331**
Filtration	1	6.823	32.025	0.015	0.864	2.013	71.183
Cyst. x Proteins	8	24.193**	24.084	18.275**	0.206	1.313**	59.118
Cyst. x Filt.	4	12.053	26.051	3.425	0.773	0.910	32.976
Proteins x Filt.	2	4.943	23.881	4.862	0.194	0.291	80.306
Cyst.x Proteins x Filt.	8	9.952**	24.259	9.187*	0.223	0.981*	56.228
Error	118 ¹	3.068	24.288	4.549	0.320	10.407	48.101

¹ missing data = 2

* or ** Statistically significant at $P < 0.05$ or 0.01

APPENDIX M

Experimental design for Experiment 8

FeSO ₄ percent in meal	Myrosinase percent in meal	<u>0.0% cystine</u> pH5	Untreated	<u>0.13% cystine</u> pH5	Untreated	<u>0.26% cystine</u> pH5	Untreated
0.0	0.0						
	0.3						
0.35	0.0						
	0.3						
0.70	0.0						
	0.7						
8.01	0.0						
	0.3						

APPENDIX N

Analyses of variance of gain, liver, kidney and feed intake of mice fed diets containing treated rapeseed meal with cystine, kidney and feed intake of mice fed diets containing treated rapeseed meal with cystine, ferrous sulfate and myrosinase at various pH's

Source of Variance	df	Gain (g)	Liver wt. (g)	Liver percent	Kidney wt. (g)	Kidney percent	Feed In-take (g)
Cystine	2	44.41**	0.538**	10335.85**	0.0175**	775.17**	180.77**
pH	1	111.16**	0.215**	5934.48**	0.0016**	837.53*	291.74**
Myrosinase	1	533.28**	1.468**	22141.62**	0.1325**	1392.10**	2046.40**
FeSO ₄ 7H ₂ O	3	33.92**	0.289**	12584.52**	0.0203**	874.14**	216.18**
Cyst. x pH	2	57.40**	0.184**	66216.25**	0.0113**	6676.66**	183.19**
Cyst. x Myro.	2	10.87**	0.062**	5585.26**	0.0178**	777.91**	84.48**
Cyst. x FeSO ₄	6	21.54**	0.155**	11820.06**	0.0170**	854.69**	16.58**
pH x Myro.	1	221.49**	0.307**	107963.10**	0.075**	10996.36**	1197.64**
pH x FeSO ₄	3	62.03**	0.185**	65264.96**	0.0142**	6724.67**	440.22**
Myro. x FeSO ₄	3	6.66**	0.018**	2741.24**	0.0109**	487.78**	17.28
Cyst. x pH x Myro.	2	6.02**	0.250**	24765.85**	0.0079**	3378.39**	6.54
Cyst. x pH x Fe	6	11.74**	0.111**	55758.07**	0.0117**	5903.57**	47.07
pH x Myro. x Fe	6	14.47**	0.115**	2278.06**	0.0132**	412.64**	60.22*
Cyst. x Myro. x FeSO ₄	3	1.88**	0.175**	23858.81**	0.0158**	3115.38**	99.43*
Cyst. x pH x Myro. x Fe	6	71.56**	0.161**	12903.762	0.0498**	1432.718	89.76
Error	192	0.49	0.025	20.068	0.0017	2.882	26.96

* or ** Statistically significant at P < 0.05 or 0.01

APPENDIX O

Average growth responses obtained for the interaction effects of cystine levels, FeSO₄ level, myrosinase levels and pH treatments (g) *

FeSO ₄ % in meal	Myrosinase % in meal	0% Cystine		0.13% Cystine		0.26% Cystine	
		pH 5	Untreated	pH 5	Untreated	pH 5	Untreated
0.0	0.0	5.50	7.32	8.43	7.45	7.70	8.45
	0.3	1.71	5.62	0.58	5.58	5.25	7.85
0.35	0.0	5.68	3.40	7.22	5.07	8.42	8.37
	0.3	0.79	8.56	1.98	7.63	3.26	2.33
0.70	0.0	6.10	8.88	6.25	10.31	7.99	6.68
	0.3	1.12	7.40	2.57	4.92	2.13	8.14
8.00	0.0	5.27	8.72	8.64	0.87	11.34	4.97
	0.3	1.68	5.26	0.18	-0.37	5.59	2.60

* LSR values for minimum and maximum: FeSO₄ x myrosinase x cystine x pH 1.14 and 1.40g.

APPENDIX P

Average liver weights obtained for the interaction effects of cystine levels, FeSO_4 levels, myrosinase levels and pH treatments (g)*

FeSO_4 % in meal	Myrosinase % in meal	0% Cystine		0.13% Cystine		0.26% Cystine	
		pH 5	Untreated	pH 5	Untreated	pH 5	Untreated
0.0	0.0	0.94	1.17	1.28	1.18	1.12	1.36
	0.3	0.86	1.00	0.79	1.05	1.20	1.38
0.35	0.0	1.14	0.80	1.13	1.02	1.27	1.24
	0.3	0.67	1.22	0.74	1.12	1.00	0.80
0.70	0.0	0.98	1.00	1.06	1.21	1.21	1.19
	0.3	0.76	1.01	0.83	1.01	0.80	1.03
8.00	0.0	0.97	1.20	1.12	0.67	1.06	1.03
	0.3	0.80	1.04	0.71	0.60	1.31	0.78

* LSR values for minimum and maximum: FeSO_4 x myrosinase x cystine x pH 0.26 and 0.31g.

APPENDIX Q

Average adjusted liver weights obtained for the interaction effects of cystine levels, FeSO_4 levels, myrosinase levels and pH treatments (g)*

FeSO ₄ % in meal	Myrosinase % in meal	0% Cystine		0.13% Cystine		0.26% Cystine	
		pH 5	Untreated	pH 5	Untreated	pH 5	Untreated
0.0	0.0	17.09	15.98	15.18	15.83	14.54	16.09
	0.3	50.29	17.79	136.20	18.81	22.85	17.57
0.35	0.0	20.07	23.52	15.65	20.11	15.08	14.81
	0.3	84.81	14.25	37.37	14.67	13.67	34.33
0.70	0.0	6.06	11.26	16.96	11.73	15.14	17.81
	0.3	67.85	13.64	32.29	20.52	37.55	12.65
8.00	0.0	18.40	13.67	12.96	77.01	9.34	20.72
	0.3	47.61	19.77	443.75	6.00	24.30	30.00

* LSR values for minimum and maximum: FeSO_4 x myrosinase x cystine x pH 7.29 and 8.83g.

APPENDIX R

Average kidney weights obtained for the interaction effects of cystine levels, FeSO₄ levels, myrosinase levels and pH treatments (g)*

FeSO ₄ % in meal	Myrosinase % in meal	0% Cystine		0.13% Cystine		0.26% Cystine	
		pH 5	Untreated	pH 5	Untreated	pH 5	Untreated
0.0	0.0	0.27	0.28	0.39	0.29	0.31	0.37
	0.3	0.19	0.26	0.34	0.24	0.27	0.35
0.35	0.0	0.31	0.22	0.32	0.24	0.33	0.26
	0.3	0.21	0.35	0.21	0.30	0.23	0.23
0.70	0.0	0.28	0.29	0.29	0.38	0.30	0.28
	0.3	0.19	0.27	0.21	0.28	0.21	0.28
8.00	0.0	0.26	0.29	0.31	0.19	0.26	0.23
	0.3	0.21	0.24	0.18	0.17	0.38	0.24

* LSR values for minimum and maximum: FeSO₄ x myrosinase x cystine x pH 0.02 and 0.08g.

APPENDIX S

Average adjusted kidney weights obtained for the interaction effects of cystine levels, FeSO₄ levels, myrosinase levels and pH treatments (g)*

FeSO ₄ % in meal	Myrosinase % in meal	0% Cystine		0.13% Cystine		0.26% Cystine	
		pH 5	Untreated	pH 5	Untreated	pH 5	Untreated
0.0	0.0	4.90	3.82	4.62	3.89	4.02	4.37
	0.3	11.11	4.62	41.37	4.30	5.14	4.45
0.35	0.0	5.45	7.94	4.43	4.73	3.91	3.10
	0.3	26.58	4.08	10.60	3.93	7.04	9.87
0.70	0.0	4.59	3.26	4.64	3.68	3.75	4.19
	0.3	16.96	3.64	12.06	5.69	9.85	3.43
8.00	0.0	4.93	3.32	3.58	21.83	2.29	4.62
	0.3	12.50	4.56	112.50	1.70	7.05	9.23

* LSR values for minimum and maximum: FeSO₄ x myrosinase x cystine x pH 2.76 and 3.35g.

APPENDIX T

Average feed intakes obtained for the interaction effects of cystine levels, FeSO₄ levels, myrosinase levels and pH treatments (g)*

FeSO ₄ % in meal	Myrosinase % in meal	0% Cystine		0.13% Cystine		0.26% Cystine	
		pH 5	Untreated	pH 5	Untreated	pH 5	Untreated
0.0	0.0	37.07	38.18	43.82	41.53	42.05	42.72
	0.3	32.48	38.44	27.82	40.44	35.80	41.47
0.35	0.0	36.28	33.20	38.43	42.04	49.48	39.83
	0.3	21.82	44.25	27.58	38.58	32.77	41.17
0.70	0.0	41.98	42.10	41.48	48.93	39.33	39.44
	0.3	28.40	38.97	31.78	38.17	32.76	41.39
8.00	0.0	42.28	41.34	41.49	26.69	43.74	32.32
	0.3	32.95	32.58	26.84	27.74	36.68	28.76

* LSR values for minimum and maximum: FeSO₄ x myrosinase x cystine x pH8.91 ans 10.79g.

APPENDIX U

Variance analyses of growth, liver weight, kidney weight, and feed intake of weanling mice fed rapeseed meal treated with FeSO₄, pH5, cystine, wet heat and filtration (Experiment 9)

Source of Variance	df	Variance					
		Gain (gm)	Liver (Abs.wt.)	Liver percent	Kidney (Abs.wt.) (gm)	Kidney percent	Feed Intake (g)
Temperature	2	9.06**	0.079*	3.116	0.005	0.330	74.58*
Filtration	1	291.59**	1.419**	199.575**	0.053**	2.999**	1548.67**
Ferrous sulfate	3	8.13*	0.043	13.988	0.001	3.484	60.95*
Temp. x Filt.	2	1.72	0.029	1.851	0.007*	0.650	13.52
Temp. x FeSO ₄	6	3.50	0.0320	6.204	0.001	1.236	14.41
Filt. x FeSO ₄	3	4.24	0.067	8.961	0.005	2.005	46.47
Temp. x Filt. x FeSO ₄	6	2.05	0.007	5.623	0.003	0.274	40.48
Error	96	2.16	0.028	9.437	0.002	0.852	18.90

* or ** Statistically significant at P < 0.05 or 0.01

APPENDIX V

Variance analyses of growth, liver, kidney weight and feed intake of weanling mice fed treated rapeseed meal with cold and hot water and cystine

Source of Variance	df	Gain	Variance					
			Liver Abs.wt.	Liver Percent	Kidney Abs.wt.	Kidney Percent	Feed Intake	
Temperature	2	21.46**	0.024	41.130*	0.002	2.852	26.99	
Filtration	1	0.181	0.003	19.305	0.001	2.414	10.09	
Cystine	2	6.83	0.039	4.600	0.001	1.210	52.72	
Temp. x Filt.	2	31.28**	0.247**	35.504*	0.018	1.427	123.91*	
Temp. x Cystine	4	7.88	0.079	8.823	0.002	1.613	73.06	
Filt. x Cystine	2	2.78	0.017	11.593	0.006	1.273	1.23	
Temp. x Filt. x Cyst.	4	5.76	0.009	24.085	0.003	1.017	46.22	
Error	72	3.79	0.027	9.148	0.003	0.9577	30.45	

* or ** Statistically significant at $P < 0.05$ or 0.01

APPENDIX W

Variance analyses of growth, liver, kidney weight and feed intake of weanling mice fed treated rapeseed meal with cold and hot water, ferrous sulfate and filtration treatments

Source of Variance	df	Gain (g)	Liver wt.(g)	Variance			Kidney percent Intake(g)	Feed Intake(g)
				Liver percent	Kidney wt.(g)	Kidney wt.percent		
Temperature	2	36.56**	0.27**	71.99**	0.013**	8.05**	35.81**	
Filtration	1	70.72**	0.18**	153.05**	0.051**	2.31*	89.47**	
FeSO ₄	3	35.72**	0.24**	87.78**	0.016**	4.18**	12.24*	
Temp. x Filt.	2	25.01**	0.08*	100.87**	0.011**	5.49**	13.50*	
Temp. x FeSO ₄	6	13.80*	0.03	21.92	0.003	1.36*	5.57	
Filt. x FeSO ₄	3	17.48**	0.13**	30.51	0.010**	1.02	11.50*	
Temp. x Filt. x FeSO ₄	6	5.82	0.01	30.31	0.001	1.50*	3.01	
Error	96	2.77	0.020	12.73	0.002	0.55	3.36	

* or ** Statistically significant at $P < 0.05$ or 0.01