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April 25, 1956.

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We, the undersigned members of the Committee appointed by you to examine the thesis submitted by Mr. Gordon James Mogenson in partial fulfillment of the requirements for the degree of Master of Arts, beg to report that we consider the thesis satisfactory both in form and content.

Subject of Thesis: The Effect of Psychological Stress Procedures on the Coagulation System in the Albino Rat.

We also report that Mr. Gordon James Mogenson has successfully passed an oral examination in the general field of the subject of the thesis.

GAM/mh

THE EFFECT OF PSYCHOLOGICAL STRESS PROCEDURES
ON THE COAGULATION SYSTEM IN THE ALBINO RAT

A Thesis

Submitted to The Faculty of Graduate Studies
in Partial Fulfilment of the Requirements

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In the Department of Psychology

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by

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Saskatoon, Saskatchewan

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INTRODUCTION

Research dealing with changes in the blood coagulation system as a result of psychological stress procedures appears to be rather limited. Apart from the work of Cannon about forty years ago, and more recent clinical observations, the accumulated experimental findings in this area are few. However, each of the separate topics, blood coagulation and stress, has aroused great interest, and hence each is the subject of many articles.

This introduction will first give a systematic account in chronological sequence of research that bears quite directly on the changes in blood coagulation produced by psychological stress. Then, will follow accounts of the present views on stress and blood coagulation. In doing this it will be much easier to see the nature and position of the present problem.

William Hewson (26) in 1771 noted that hypercoagulability of the blood followed hemorrhage and stress. Vosburgh and Richards (84) in 1903, while studying changes in blood sugar levels of dogs after giving adrenalin, observed that more rapid coagulation accompanied an increase in blood sugar. Since the adrenalin had been applied to the pancreas, they concluded that adrenalin acted in some way on the pancreas to alter coagulation. In 1909 Wiggers (5-P133), also using dogs, and doing work on the effects of adrenalin on internal hemorrhage, did not obtain the same results as Vosburgh and Richards and found instead that adrenalin injected or added to the blood did not hasten coagulation. Two years later von den Velden (5-P133) found that adrenalin decreased the blood coagulation time in man. This he attributed to tissue juices entering the

blood as a result of vasoconstriction of blood vessels. This is reminiscent of the work of Milain (28) in 1901 who found that blood obtained by pressure on the finger or ear (capillary puncture) showed a progressive reduction in clotting time. This he believed was the result of an influx of tissue juices. Dale and Laidlaw (5-Pl33) were not able to reproduce von den Velden's results and found no change in coagulation time when adrenalin was given orally.

Aware of these inconsistent observations, and seeing the relevance of changes in coagulability from adrenalin to his work on bodily changes during pain and emotional excitement, Cannon did further work along these lines. Using decerebrated cats, he gave subcutaneous and intravenous injections of adrenalin (9) and stimulated the splanchnic and sciatic nerves (9, 8). Clotting times were measured by the graphic method devised by Mendenhall and Cannon (7). Subcutaneous injections of adrenalin, after a delay of as long as twenty minutes, produced a very considerable reduction in clotting time lasting as long as one hour. Most of Cannon's work was done using intravenous injections of adrenalin since the rate and amount entering the vascular system could be more accurately controlled. With this procedure, the response to small doses occurred after a briefer delay and lasted about 20-30 minutes. Cannon found that larger doses of adrenalin usually produced an increase in blood coagulation time followed sometimes by a reduction (6). Howell (30), working with dogs, obtained similar results with larger doses of adrenalin. Since adrenalin may both shorten and lengthen clotting time, Cannon postulated that two factors were operating, one hastening and the other retarding coagulation, and

that the equilibrium between them was upset by adrenalin.

Cannon believed that his results confirmed earlier findings that a reduction of blood coagulation time occurred when adrenalin was given in proper dosage. However, he presented evidence against von den Velden's conclusion that this was due to tissue juices entering the blood from vasoconstriction. Work with the 'anterior animal' (circulation confined to the anterior part of the body) revealed that some organ, probably the liver, was involved as well as adrenalin (6). Cannon reasoned that possibly the liver furnished a factor for the clotting process and that adrenalin stimulated the liver to discharge this factor. Grabfield (29) in 1916 suggested that minimal doses of adrenalin increase the amount of prothrombin in the blood and hence the coagulation time is reduced. Further support for this view that adrenalin in suitable amounts shortens coagulation time came during the 1920's from work by Takasaki, Hirayama, La Barre, Hartman, Mills, Necheler, and Chu (5).

Since the injected adrenalin altered blood coagulation, Cannon was interested in seeing if increased adrenal activity would also do this (8). It had previously been shown that the splanchnic nerve provided the sole innervation of the adrenal and that adrenalin was secreted when the splanchnic was stimulated (5-Pl66). Cannon also claimed that during pain and emotion the adrenals were reflexly stimulated. Hence artificial stimulation of the splanchnic in experiments was duplicating what happened in the organism during pain and emotions. Cannon found that splanchnic stimulation was followed immediately, or after a short delay, by a shortening of the coagulation time. By means of adrenalectomy he was able to show that this effect was produced through the adrenals. It was the

influence of adrenalin on the liver that produced this change.

As a controlled experimental procedure to simulate pain Cannon artificially stimulated the sciatic nerve. This stimulation, or the pain of surgical operation, resulted in faster clotting. It was noted that some cats reacted violently and vigorously to being bound. This was used as a means of studying the effects of emotional excitement. Again there was faster coagulation which Cannon attributed to the release of adrenalin. Thus, shortening of the blood coagulation time occurred when adrenalin was either injected or released endogenously during pain or emotional excitement. Cannon regarded this as one of the fundamental bodily changes contributing to the individual's preservation and increased efficiency in physical struggle.

Cole (16) in 1942 published a preliminary study which showed a marked decrease in blood clotting time of rats following exposure to sound five days a week for a period of several weeks started immediately after weaning. However, his results are based on a small number of determinations. In 1944 Ungar (83) working with guinea pigs obtained results similar to Cannon's work on pain. The difference was that Cannon stimulated the sciatic nerve whereas Ungar traumatized the animal (pain plus tissue damage). At six hours and, again, at three days after ~~traumatization~~ traumatization there was a marked shortening of bleeding time. After eight days it had returned to normal. Moreau (51, 52) in 1948 found electrical injury to the brain or legs of frogs resulted in liberation of some substance into the blood having thromboplastic properties. Injury was followed by a slight decrease in coagulation time and then an increase.

Thus, early experimental work showed that adrenalin, injected or endogeneous, in proper doses resulted in hypercoagulability of the blood. It was suggested that adrenalin acted by stimulating the production of prothrombin in the liver. More recently, rapid clotting time has been observed following audiogenic seizures and trauma.

Reports from the clinical field also reveal an alteration of the blood coagulation system due to the stress of surgery and the emotions of fear and anxiety. Since Dustin's (71) original emphasis on post-operative disease, a great deal of attention has been given to thrombosis and thrombophlebitis. Sandrock (66) has suggested that increased prothrombin activity on the second or third post-operative day is indicative of impending venous thrombosis. Takats (82) has been especially interested in post-operative changes in the blood clotting mechanisms. He has suggested that these changes are a manifestation of Selye's General Adaptation Syndrome and has tried adrenalin pre-operatively to minimize the post-operative tendencies to thrombosis. Recently Schneider (69) has emphasized the significant role of emotional stress in the pathogenesis of thrombophlebitis. He found that patients prone to thrombophlebitis exhibited a shortened clotting time during anxiety, fear, hostility, and while discussing stressful topics.

Schneider (70) has also reported alterations in clotting time and relative viscosity as a result of stress procedures with people free from thromboembolic symptoms. Using normotensive and hypertensive subjects, he found in all people tested a marked shortening of clotting time and prothrombin time following exercise. However, in the cold pressor test only subjects showing a significant elevation of blood pressure dis-

played shortening of the clotting time. Blood clotting changes following a stress interview seemed also to go with an elevation of the blood pressure. The same general results are reported by Macht (47) in studying healthy persons in the blood bank of a hospital. Normal subjects had an average clotting time of 8.3 minutes, those judged as apprehensive 3.4 minutes, and the highly nervous and hysterical 2.2 minutes. It was observed that the apprehensive and nervous groups also showed elevated blood pressure while at the blood bank. Schneider's conclusion "that shortened clotting time and increased blood viscosity are biologically useful in stress where bleeding may follow as in mortal combat" (68-P831) sounds much like Cannon's view regarding the adaptive nature of this reaction. However, the influence of newer concepts of stress is seen when Schneider continues "this pattern of response is made use of in the hypertensive subject perhaps to his detriment in that these changes may predispose to coronary and cerebral thromboses" (68-P.831).

Kast and co-workers (36) have recently published a study on psychotic patients receiving Electroshock Therapy. The subjects were divided into three groups. The first group received EST at appointed times. The second group received identical treatment except that no electric current was applied. The third group suddenly received EST without anticipating it. They found that both groups one and two showed a marked shortening in blood clotting time, while group three remained normal. They concluded that the anxious anticipation of Electroshock was essential to produce changes in blood clotting time and not the electric current itself. Hence it would appear that adrenocortical stimulation following EST does not result from the electric current acting directly on the

hypothalamus and pituitary gland. Rather, Kast claimed, adrenocortical stimulation follows ACTH production as a result of pituitary activation by the adrenalin released during the emotional response. Thus Electro-shock is a non-specific stimulus which can be replaced by any noxious stimulus capable of arousing anxiety and excitement.

Another line of evidence for adrenocortical involvement in the etiology of altered blood coagulation comes from clinical reports of ACTH and cortisone therapy. Cosgriff (17) (18), Smith (78), and Garrett (26) have reported hypercoagulability of the blood and thromboembolic disease following the administration of these hormones. Cosgriff's (17) case for this being due to a pre-formed thrombin molecule is highly questionable. It is probable that the clotting of blood in a tube despite normally adequate oxalate is more related to the taking of the blood sample than to a pre-formed thrombin. McGraw (30) suggests that ACTH tends to delay coagulation and that patients tend to develop thrombosis when the therapy is stopped. Reconciliation of these opposing views as to the effect of ACTH on blood coagulation is probably found in Smith's statement, "The alterations following these hormonal agents appear to be, in part, a function of both the initial level of adrenal cortical activity and the integrity of the coagulation mechanism itself, which existed prior to the hormonal administration". (78-P. 296).

STRESS

Stress is an organismic response which may be initiated by a variety of stress agents or stressors. Selye (72) (73) has listed these in a classification that covers a wide variety of physical, physiological, and psychological agents. Not only are the initiating agents varied, but the response also involves diffuse body changes. Important among these are the enlargement of the adrenal cortex together with a discharge of its lipid granules and, possibly, involution of thymus, lymph nodes, and spleen. Often there is general tissue breakdown manifested by gastro-intestinal erosions, inflammation of arteries and heart, hypertension, and behavioral disorders. Selye (73, 74) in his annual reports has reviewed the literature describing pathological changes in various organs and systems of the body occurring in response to different kinds of stress agents.

Present knowledge points to the importance of the adrenal cortex in mediating the stress response. Many experiments have shown that when the adrenal cortex is removed the stress response is inadequate or absent, and that under conditions of stress the activity of the adrenal cortex is increased (15, 25, 50, 60). Hence methods of determining adrenal cortical activity such as (1) ascorbic acid depletion, (2) eosinopenia, and (3) adrenal steroid levels in the blood and urine have been of great importance (23).

The adrenal cortex is itself activated by ACTH. This fact has directed attention to the mechanisms activating the anterior pituitary. Three possibilities have been noted: (1) a feedback mechanism,

suggested by Sayers (69) in which the level of adrenal corticoids circulating in the blood regulates ACTH secretion in an inverse manner.

During stress, the cells utilize more corticoids, thus lowering their circulating level, and thereby stimulating the anterior pituitary;

(2) a hormonal control mechanism in which some factor (Long (44) suggests adrenalin) directly stimulates the anterior pituitary; (3) a hypothalamic mechanism in which a neurohormone, secreted by the hypothalamus, is transported through the hypophyseal portal veins to the anterior pituitary, where it has a direct stimulating action (31). In support of this McCann (49) has found that lesions in the hypothalamus interfere with ACTH secretion.

This pituitary-adrenocortical mechanism active in stress may be aroused experimentally by a number of physical and chemical agents. Such agents as x-irradiation, frostbite, and injections of sodium chloride or formaldehyde, result in cellular changes or alterations of the cell environment. The stress response follows such damage. However, the hormonal and hypothalamic control mechanisms suggest that this activation may not require direct alteration of cells, but might be initiated by emotional and cognitive responses which arise as a result of interoceptive and exteroceptive stimuli. Thus in situations of anxiety, excitement, or other strong emotions adrenalin is poured into the blood in large quantities. This adrenalin then excites the anterior pituitary. Similarly, higher cognitive factors involving perception of conflict and failure with accompanying emotion can result in ACTH secretion via their influence on the hypothalamus.

Stress due to direct cellular damage as a result of physical and chemical agents is hereafter called physiological stress. Stress due to the action of exteroceptive and interoceptive stimuli mediated by cognitive and emotional processes is hereafter called psychological stress. Aside from the initiating agents, physiological and psychological stress appear to be very similar. In each case the hyperactivity of the pituitary-adrenocortical axis is involved, by which the organism may achieve adequate adjustment, or some of the pathologic changes previously cited may occur. The response to physiological stress agents is affected by the previous history of stress and the present state of endocrine balance. The response in psychological stress depends even more on individual differences, constitutional and cultural factors, early conditioning, and previous history (1, 4, 10, 11, 12, 54, 55, 61, 86). These factors determine whether perceptual information is interpreted as threatening or not (67) and whether the stress response will be adaptive or damaging.

BLOOD COAGULATION

Reference has been made to the diffuse body response to stress. This study has been concerned primarily with the effects on the blood coagulation system. Therefore it is important to examine what happens when blood clots and where, according to the measures used in these experiments, this system is altered during stress.

The classical theory of blood coagulation had four factors: prothrombin, thromboplastin, calcium, and fibrinogen (2). In the presence of thromboplastin and calcium prothrombin is changed to thrombin which then converts fibrinogen to fibrin. The change of prothrombin to thrombin has been called the conversion phase, and the change of fibrinogen to fibrin the clotting phase.

It has been noted that Cannon suggested that the liver discharges a substance which hastens blood clotting and that Grabfield (27) believed that this was prothrombin. For twenty years it has been known that prothrombin production by the liver depends on the availability of vitamin K. According to the classical theory it was believed that vitamin K deficiency led to a reduced formation of prothrombin only and that since dicumarol was antagonistic to vitamin K it thus reduced the available prothrombin circulating in the blood.

In the past decade other coagulation factors have been discovered. This has necessitated a revision of theory. Figure 1 shows the coagulation theory adapted for this treatment. The conversion phase is no longer regarded as thromboplastin acting on prothrombin in the presence of calcium. Rather it is assumed that thromboplastin activates proconvertin (Factor VII) which it changes to convertin (20, 21).

Convertin then transfers prothrombin to thrombin aided by accelerin (Factor V). The precursor of accelerin (pro-accelerin) is probably influenced by thrombin as it forms. According to this revised position, deficiency in vitamin K would influence not only prothrombin production but also proconvertin. The use of dicumarol then alters the level of both prothrombin and proconvertin, probably affecting the latter to a greater extent.

It is necessary to give these details since the one-stage prothrombin time was used as a measure of coagulability in these experiments. This method measures the time for the conversion and clotting phases to occur at a constant temperature when thromboplastin and calcium are supplied in excess. The one-stage method does not measure the amount of prothrombin present, but the rate of thrombin formation (33). Variations in prothrombin time depend on changes in the amounts of prothrombin, proconvertin, pro-accelerin, and fibrinogen present in the blood. Fibrinogen is seldom a limiting factor. Reduced amounts of prothrombin can increase the prothrombin time, but the change is slight until the reduction has reached ten per cent of normal (56). Pro-accelerin has been described by Owren (57) as the limiting factor in a rare hemorrhagic state called parahaemophilia. However, in most cases the one-stage prothrombin time is a measure of proconvertin (56). This conclusion is justified when the addition of proconvertin, using normal serum, corrects the change (2). Dicumarol probably reduces both prothrombin and proconvertin (33). A prolonged prothrombin time following stress may result from reduction of both prothrombin and proconvertin. However, as mentioned above, prothrombin time by the one-stage method is probably more closely related to the amount of proconvertin.

THE PRESENT PROBLEM

In 1951 it was reported that the anticoagulant drugs phenylindanedione (PID) and dicumarol delayed the onset of gangrene following experimental frostbite in rabbits (45). Further work in this laboratory showed that frostbite produced an increase in the prothrombin time of rats, and in rabbits increased the effects of the anticoagulant drugs. Rabbits maintained on PID or dicumarol and exposed to frostbite showed a greater increase in prothrombin time and a large number of deaths from hemorrhage (46). It was suggested that the effects of frostbite on coagulation were not specific, but the result of a stress response.

The effects of other stress agents (NaCl, insulin, formaldehyde) on the coagulation system were then investigated (13). All three stressors produced an increase in the prothrombin time in rats, but not with rabbits. In the case of rabbits thrombopenic drugs were required to demonstrate that the coagulation factors had been affected. Changes in the prothrombin time in the rat occurred as early as six hours after stress and lasted for over forty-eight hours. Adrenalectomy eliminated these effects, showing that following stress the adrenal gland is necessary for changes to occur in the coagulation system.

It is then clear that the changes in blood coagulation originally noted following frostbite are not specific for frostbite, but result from other physiological stress agents as well. The experiments reported here extend the work on the effects of physiological stress agents on blood coagulation to include psychological stress procedures. They are also directed towards finding out more about hemorrhagic death following stress.

PROCEDURES

1. Forced Jumping - The Lashley Jumping Stand was used for training rats in jumping and discrimination. The apparatus consisted of a box with two doors, and a stand wired so that electric shocks could be delivered to the feet of rats that did not want to jump. The stand could be set at varying distances from the box. One group was trained to jump to the door with a white circle. This was done by consistently blocking the door with a black circle. When they jumped to the door with the white circle they got in and received food. The second group was made to learn a place habit which consisted of always jumping to the door on the right. The test proper was done after the rats had learned the discrimination or place habit and had had sufficient practice to establish the habit. On the test day the rats were given three or four successful jumps and allowed to eat. Then both doors were locked and the animals forced to jump for a ten minute period during which they usually jumped thirty to forty times. They were shocked with the electric current if necessary. The control animals were given a regular ten minute practice session.
2. Electrified Water Source - Rats were placed in individual cages wired in such a way that they received shocks when attempting to drink. One electrode was attached to the cage, and the other was introduced into the water through the rubber stopper of the water bottles. Sodium chloride was used as an electrolyte. The two electrodes were connected to the regular A C supply through a variable transformer which was set at thirty-five volts. For two hours each morning the experimental animals, as well as control animals were allowed to drink and were fed in ordinary individual cages. The lights in the room were on during this time.

Except for this two hour period, whenever the experimental animals attempted to drink they received a shock. During this time the room was dark except for a small light over the cages.

3. Sound Induced Seizures - (a) Rats were placed in a 12" x 10" x 10" aluminum box which could be closed with a lucite lid. A Hartmann whistle, connected to a source of compressed air, was introduced at one end of the box. The whistle was adjusted to give a sound stimulus of 13,000 c.p.s. The whistle was on for from two to five minutes depending on how quickly seizures occurred. (b) For some experiments the above apparatus was not used. Instead the whistle was turned on while the rats were in their cages. Large rats in individual cages, or weanling rats in group cages were brought into the room. The whistle was placed on top of the cage and turned on.

4. Electroshock - An adjustable alternating current stimulator connected to the 110 volt line was used. Microhm jelly was applied to the rat's ears and small alligator clips were then attached. The current was put on by means of a press key. As a means of confinement the animals were placed in a 12" x 12" x 5" plastic box during the electroshock procedure.

5. Obtaining the Samples of Blood - The effect of these psychological stress procedures was measured by doing a one-stage Prothrombin Time test on blood samples after stress. The manner of taking samples and measuring the prothrombin time were as follows:

(a) Cardiac Puncture - While the rat was under light ether anesthesia a two ml. sample of blood was taken from the heart using a 22 gauge needle in a siliconed syringe containing 0.2 ml. of sodium citrate.

(b) Tail Vein Puncture - The rat was put under light ether anesthesia. The tail was placed in a beaker of warm water (45°C - 50°C) for about one minute to dilate the veins. Using a 0.5 ml. siliconed syringe and a 25 gauge needle, about 0.1 ml. of blood was drawn from the lateral vein of the tail.

6. Determinations of Prothrombin Time -

(a) Modified Quick Procedure - Blood obtained by cardiac puncture was centrifuged and the plasma removed. One tenth ml. of plasma was placed in a test tube in a water bath set at 37°C . To this was added 0.1 ml. of thromboplastin. After about fifteen seconds, 0.1 ml. of 0.02 molar calcium chloride solution was quickly blown into the mixture, and the stop-watch started. At the first appearance of a clot or fibrin threads the stop-watch was stopped. The time recorded was taken as the prothrombin time.

(b) Bed-side Prothrombin Time Determination - A modification of the Schwager - Jaques method was used (34). One drop of thromboplastin was placed on a one and one-half inch watch glass with a one-quarter ml. syringe. To this was added one drop of blood from the tail vein sample. The timer was then started and the blood stirred with the needle until the clot formed. The timer was immediately stopped and the prothrombin time recorded in seconds.

7. Administration of Dicumarol - Several experiments were done in which stress and dicumarol were combined. Dicumarol made the effects of stress more apparent both in changes of prothrombin time and mortality. Dicumarol was administered orally in the feed. This feed was the same as the standard colony diet, except that it was in powdered form instead

of as pellets. The mixture was prepared in lots of 600 gm with enough dicumarol added to give a dosage of 10 mg./kg. or 20 mg./kg. of body weight. The amount of this prepared feed was given according to weight so that a two hundred gram rat received 5 gm. of feed daily. The animals were fed daily at 9.00 a.m. in deep-walled dishes to avoid spilling. Any prepared diet not consumed by the afternoon was weighed and additional pellets of the standard colony variety added so that each rat received a normal quantity of food.

8. Adrenalectomy - Rats were adrenalectomized to study the effects of stress and of anticoagulant in such animals. The operation was performed after the manner described by D'Amour and Blood (21).

9. Drugs Used -

(a) Thromboplastin - 0.15 gm. of dehydrated rabbit brain thromboplastin obtained from Cappel Laboratories was suspended in 4.0 ml. of 0.1 molar sodium oxalate. This suspension was placed in a water bath at 45°C - 50°C for fifteen minutes and then centrifuged. The supernatant liquid was poured off and used in prothrombin time determinations.

(b) 0.9% sodium chloride - This was prepared by dissolving 9 gm. of NaCl in 1000 ml. of distilled water, and kept as stock solution. Adrenalectomized rats were given this to drink ad libitum.

(c) Calcium chloride solution - a 0.02 molar solution of CaCl_2 was made by dissolving 0.222 gm. of anhydrous calcium chloride in 100 ml. of distilled water.

(d) Sodium citrate - a 3.8 per cent solution of sodium citrate was made by dissolving 3.8 gm. of sodium citrate in 100 ml. of distilled water.

RESULTS AND DISCUSSION

The procedures used produced changes in the blood coagulation system of rats. The most obvious change is the increased variability of prothrombin time, with some animals showing a marked increase over control levels, and others, a decrease. Consequently, the mean often changes very little and results in the acceptance of the null hypothesis when the t test is used. Yet the size of the standard deviation of experimental groups is often much larger than that of control groups. The significance of this change in variance may be tested by a procedure outlined by Edwards (22). This test for the heterogeneity of variance using the F ratio has been extensively used in evaluating the data.

Forced Jumping did not produce wild running and seizures as some investigators have reported (53). However, following such stress, the animals were usually hyperactive. Rats varied in their reaction to bumping into the door of the box and falling into the net below. Some animals jumped more forcibly and with a shorter latency when the door was blocked. Others did not want to jump, and were forced to move by the administration of mild electric shocks to the feet.

Table I shows the effect of Forced Jumping on prothrombin time. Twenty minutes after a single exposure to such treatment the prothrombin time was significantly increased over the prothrombin time of control animals. When these same animals were stressed for six days by Forced Jumping, the increase in prothrombin time taken twenty minutes after stress was even more marked. Both t test and F test showed significance. Ninety minutes after Forced Jumping the prothrombin time was not significantly changed. The group of animals in which the blood samples were

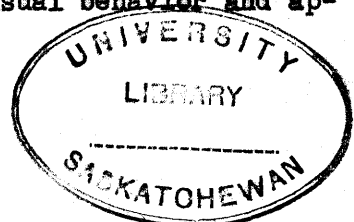
taken by cardiac puncture three hours following stress showed changes in the variance of prothrombin times that were very significant.

Thus it appears that Forced Jumping in the Lashley jumping apparatus produced changes in prothrombin time that are detectable as long as three hours following stress. In most animals there is a longer prothrombin time, but in some it is shorter.

Most of the animals in the Electrified Water Source Apparatus became hyper-sensitive and over-active. When anyone came into the little room where they were kept they ran about wildly in their cages, often clinging to the walls and ceiling. Care had to be taken when the door was opened in order to place food trays in the cage during the daily feeding period. On at least one occasion rats jumped from the cage when the door was opened and ran wildly about on the floor. Rats normally approach the food as they are being fed or remain timidly at the back of the cage, but do not jump out of their cages.

The results of experiments with animals in the Electrified Water Source Apparatus are shown in Table II. Blood samples were taken by cardiac puncture on the fifteenth day after the animals were placed in the apparatus. The mean prothrombin time was not significantly changed. However, since one-half of the experimental animals had prothrombin times either much higher or lower than controls the F test for heterogeneity of variances was very significant.

The same experiment with five experimental and five control animals in the apparatus for twenty-one days showed no changes in the prothrombin time. These animals displayed no unusual behavior and ap-



peared no more excited than the controls. These rats were removed routinely every other day to be weighed. It could be that the handling involved in this had something to do with their lack of response. There was also some indication that the rats were not receiving shocks when drinking, due to short circuiting of the current or the rusting of wires.

Table III shows the results when samples were taken from rats which had just arrived after transportation for 1800 miles by rail. These animals had food provided in the crates and were watered enroute by ice being placed on the screen which covered the top of the crates. Yet, in spite of these precautions, nine of 50 rats shipped at this time had died before arrival. It has been reported that the transportation of animals is an emotional as well as a physical stress (10). However, no one has previously reported that the blood coagulation was altered by the stress of transportation. Table III shows that by the F test there was a significant change in variance.

Rats vary in incidence of sound induced seizures. There was noticeable variation in susceptibility of the strains of animals used in these experiments. One strain, for instance, showed 10 per cent susceptibility and another strain 20 per cent susceptibility. The response is also affected by the degree to which the animals are permitted extraneous or manipulative behavior during the sound stimulation (29). Thus rats that began to scratch or lick themselves in a somewhat unusual manner never had seizures. Rats put into new surroundings often tended to explore the situation, and were less prone to react violently to sound. Presumably manipulatory or substitute behavior reduces tension, permits channeling of the aroused state into some sort of response, and this

facilitates handling of the situation (32).

The excitability of the nervous system seems to be important in the occurrence of sound seizures (79). Thus excitant drugs such as metrazol, coramine, caffeine and sodium benzoate, which increase the irritability of the nervous system, increase the susceptibility to seizures (35, 65, 77). Metrazol used in these experiments in sub-convulsive doses (2.5 to 3.0 ml. per 100 g. body weight) made it possible to convulse nearly all rats exposed to sound.

The sound induced seizures were very much like those so completely described by Finger (24). There was a startle response when the sound was turned on, often even though no seizure occurred. When seizures occurred they usually started with a sudden burst of violent running. This was followed by the tonic, clonic phases. A comatose period then occurred lasting as long as one-half hour, during which the animal was very passive and limp and could be molded into any shape. Some animals did not go into a passive phase following the convulsions proper, but hopped stiffly and violently about the side of the box or cage on hind legs. It was noted that once the seizure began the intensity of the sound necessary to continue the phenomenon was considerably less than that required for its initiation.

In the case of drug-facilitated sound seizures the running was more violent and much shorter. This has been reported before (35). The convulsion was probably more severe also, since a number of deaths occurred during or immediately after the seizure. The convulsion proper of the metrazol-facilitated sound seizure began when in the course of wild

running the rat flopped on its side with its four legs drawn toward the front of the body in a tonic fashion. The front legs were brought down tight and stiff against the chest. Gradually the hind legs moved towards the tail and then began to vibrate and quiver in the clonic phase. All this time the eyes were closed tightly and the shape of the head distorted by the continuous tension of the muscles. Following this the sequence took one of two forms similar to the behavior described for sound induced seizures. Either the animal remained very passive and limp with its body obviously relaxed, or it began hopping stiffly about on its hind legs. In each case the animals generally breathed deeply and rapidly for 15-30 minutes after the seizure, and seemed to have difficulty in getting enough oxygen.

There was no uniform shift of prothrombin time after a single exposure to sound. However, at $1\frac{1}{2}$, 3, and 6 hours after such stress some rats had a prothrombin time that was longer than normal and others shorter. It is shown in Table IV that at these times the variances were significantly different. Of the twenty-four experimental animals having samples taken 3 and 6 hours after exposure to sound, twelve were more than two standard deviations from the mean of the controls. It is pertinent that about equal numbers were longer and shorter. After 12 hours the prothrombin time was normal.

The results of a series of experiments in which single drug-facilitated sound seizures were used are given in Table V. Twenty-four hours after such a stressor the F test was significant. These results are similar to those obtained with a series of drug-facilitated sound

seizures. However, there was no change at six hours and seventy-two hours. Because a number of rats died during or immediately following seizures, samples were taken from a few animals about fifteen minutes after being convulsed. The prothrombin time was significantly longer.

It seems unusual that the prothrombin time was significantly changed fifteen minutes and twenty-four hours after such stress, but not after six hours. This probably has something to do with the phasic nature of the changes following stress. In the language of Selye (72). it could be that at the transition from the Alarm Reaction to the Stage of Resistance there is a period when changes from the physiological normal are minimal. Thus a rat that at first had a lengthened prothrombin time may later have a shortened prothrombin time, and vice versa. It follows then that a period when no prothrombin time change is evident could be preceded and followed by significant changes.

Nearly 20 per cent mortality was reported in animals having single drug-facilitated sound seizures. On the basis of a limited number of cases it appears that the mortality is higher when a series of such seizures ~~is~~ given.

The pathological reports on rats dying after drug-facilitated sound seizures indicated generalized congestion of the lungs, liver, and kidneys. Examination of other animals following death revealed enlarged livers as well. It is probable that congestion and/or hemorrhage occurred as a result of most drug facilitated sound seizures, as there was often laboured breathing and traces of blood from the nostrils. In view of the damage to the liver it seems quite logical that the prothrombin time

should be longer immediately following seizures, since the liver is the probable site of prothrombin and proconvertin production. Later, perhaps at twenty-four hours, the prothrombin time of some rats is shorter due to increased resistance on the part of the animal. These results regarding the effects of sound seizures on blood coagulation are not in complete agreement with another report in which it was found that the clotting time was reduced from 114 sec. to 35 sec. following sound seizures (16).

Electrically induced seizures are very similar to sound induced seizures (80). For this reason and since the strains of rats used were not very susceptible to sound induced seizures, it was decided to use Electroschock (ES) extensively. It is also a relatively simple procedure which can be done under fairly constant conditions.

The strength and duration of the electric current required to convulse rats was found to be fairly constant. The instrument was set at 130 to 140 volts and the current put on for 0.4 - 0.7 seconds. When the current was put on the rats made a violent start, often jumping them into the air. Sometimes there were rapid movements of the legs. When the convulsion began the animal rolled on its side with its hind legs forward, front legs back along the chest, eyes tightly closed, back arched, and sometimes with the mouth widely open. Frequently there was erection, ejaculation, and usually urination and defecation occurred. After about ten seconds the back straightened somewhat and the hind legs were brought down and stretched out along the tail, the front legs remaining along the chest. This lasted for about five to ten seconds dur-

ing which the muscles were in tonic contraction and the back, legs, and tail quivered. Then gradually the body relaxed and the eyes opened. The animals remained on their side for a little while or lay quietly in the cage when returned. Within two or three minutes they assumed a semi-crouch posture which made them appear defensive and defiant. They were hypersensitive for some time. The breathing was rapid and the eyes protruded. After about thirty minutes appearance again seemed normal.

The nervous system is of prime importance in the stress response to ES. Thus a number of reports indicate that rats under anesthesia do not display seizures from ES (59, 77), and no changes in the size of the adrenal gland result (63). In these experiments, rats which were given ES while under ether anesthesia showed some movement of the upper part of the body while the current was on, but no seizure occurred. It appears that the activation of the pituitary adrenal-cortical axis following ES (15, 25, 50, 60) is mediated via the nervous system by means of the hypothalamic mechanism; and that anesthesia by depressing activity in the CNS blocks this mechanism.

The literature contains evidence that the adrenals of feral rats are much larger than those of domesticated animals (62). Since fewer feral rats display grand mal seizures as compared to tame rats (79), it has been suggested that the size of the adrenal is directly related to the resistance to seizures. If this is so, adrenalectomized animals should be convulsed easier or with a weaker current than rats with adrenals intact. However, this does not seem to be the case since adrenalectomized rats convulsed with ES in our experiments did not differ from others in susceptibility or response.

The effect of a single ES on the prothrombin time of rats is shown in Table VI. This experiment was done using male rats weighing 225 - 300 gm. The stress of a single ES showed a significant change in the prothrombin time when samples were taken after one and one-half hours. Again there was no uniform shift, some rats had a longer prothrombin time, and others a shorter prothrombin time. A single ES administered to smaller animals weighing 130 to 160 grams did produce a significant difference in prothrombin time means. These results are reported in Table VII. Since the animals were small, it seems likely that ES constituted a more severe stress for them and that the hypocoagulability reflected damage to liver and kidney.

The prothrombin time changes at various times after ES have not been worked out very well. However, on the basis of preliminary experiments it was found that at one and one-half hours the change was maximal.

Attention during the course of this research has been directed to the occurrence of hemorrhage and mortality in rats exposed to stress. Hemorrhage or hemorrhagic death is a good index of an altered state of the coagulation system. Of 52 weanling rats exposed to sound for two to three minutes daily for three weeks, thirteen died. Post mortem examination revealed congested lungs, haemothorax, and gastro-intestinal congestion. Such stress had another interesting effect on these young rats. Four groups of these animals were given the following treatment: Group A - gentling (64, 85, 86) ten minutes daily for three weeks, Group B - control, Group C - gentling and exposure to sound daily for three weeks, Group D - exposure to sound daily for three weeks. All

groups were matched for weight, the pre-treatment means of each group being 48 grams. Six weeks after weaning the mean weights were: Group A - 154 gm., Group B - 132 gm., Group C - 136 gm., Group D - 137 gm. The rats of Group A were significantly heavier than the controls ($t = 5.8$, $p = 0.01$). This confirms results reported by Weininger (89) on the effects of gentling on weight gain. Sound alone did not produce a reduction in weight, but it did nullify the beneficial effects of gentling.

Drug-facilitated sound seizures produced a number of deaths which occurred during or immediately following convulsions. These animals showed hemorrhagic symptoms. The pathological reports given in the appendix, on two of these rats indicate that the lungs, livers, and kidneys were especially affected.

Rats occasionally died of hemorrhage when blood samples were taken by cardiac puncture following stress. After repeated administration of ES rats showed a lengthened prothrombin time and many deaths from cardiac puncture. The mortality in rats from cardiac puncture following a single ES is shown in Table VII. The mortality is significantly higher than that of control animals receiving only cardiac puncture. Mortality from ES and cardiac puncture was markedly reduced when rats had received stress and cardiac puncture two or three weeks previous. In fact the number of deaths was less than that from cardiac puncture alone in control animals. It was found that it was the previous cardiac puncture and not the previous stress that produced this reduced mortality. This finding has a very practical application in that it shows that it is not expedient to use rats in stress experiments if they have had blood taken by cardiac puncture.

In order to make these procedures more sensitive to changes in the coagulation system and more effective in producing hemorrhage and mortality the animals were fed small doses of dicumarol. Such doses ordinarily do not produce hemorrhage in rats. However, stress along with the anticoagulant can produce appreciable hemorrhage. It is suggested that the degree of stress and the dosage of anticoagulant probably bear an inverse relationship as shown in Figure 2. Thus as the intensity of the stressor is increased the dose of dicumarol required to produce hemorrhagic death is less.

A large number of rats were fed 10 mg./kg. of dicumarol daily in their feed for five days. ES was administered to some of the animals on the fourth day and blood samples taken one and one-half hours later by cardiac puncture. Table VIII shows that with new rats the mortality was significantly higher in those receiving dicumarol and ES than in those getting only dicumarol. The prothrombin time of the stressed group was also very significantly longer. However, when animals receiving previous cardiac puncture were used, stress did not increase the prothrombin time or mortality.

Work is continuing at the present time on the effects of stress in rats fed different doses of dicumarol. The tail vein method of obtaining blood samples has made it possible to follow the prothrombin time in rats over a period of time without encountering the effects of cardiac puncture on the stress response. An experiment has been completed on about one hundred animals. At present another group of about the same size is being studied.

This research is designed to examine the effects on blood coagulation, especially on hemorrhage and mortality, of different dosages of anticoagulant combined with different forms of stress. First 102 rats were standardized on dicumarol at a dosage of 10 mg./kg. of body weight. This consisted of feeding them dicumarol in their feed daily for eight days, and taking samples of blood by tail vein puncture on days four, six, and eight. Prothrombin time determinations by the Schwager-Jaques method (34) were made on these samples. The rats were then judged as reactors or non-reactors, depending on the level of prothrombin time recorded for the three days. A reactor was considered to be any animal that had an increase of at least one hundred per cent in the prothrombin time on days four, six, and eight. It was found that 21 of 102 animals were reactors. Of these 21 reactors, six died of hemorrhage during the standardization. The 15 reactors on 10 mg./kg. of dicumarol, along with 15 non-reactors, were divided into the various groups for stress procedures during which they were fed 10 mg./kg. The remaining 66 non-reactors were then standardized on dicumarol at 20 mg./kg. On this dosage five died, 38 were judged as reactors, and 23 as non-reactors. These rats were again fed 20 mg./kg. of dicumarol and divided into groups for stress procedures. The various experimental groups were: (1) dicumarol, (2) dicumarol and ES, (3) dicumarol and sound seizures, (4) dicumarol and ES while under ether anesthesia, (5) adrenalectomy and ES, (6) adrenalectomy, dicumarol, and ES, (7) adrenalectomy and dicumarol, (8) ES, (9) sound seizures.

A complete statistical analysis of the results of this experiment will not be made until the second section of the work is completed.

However, a number of interesting trends appear when the results from the first hundred animals are examined. In Figure 3 it is clear that for the four types of rats, reactors and non-reactors at 10 mg./kg. and reactors and non-reactors at 20 mg./kg., the prothrombin time after adrenalectomy is very much higher. Thus in the 20 mg./kg. reactors the prothrombin time on dicumarol after adrenalectomy was, on days six and eight, 350% of the initial standardization. Animals not receiving dicumarol had normal prothrombin times. All of the sixteen adrenalectomized animals fed dicumarol eventually died, most of them between day five and day eight. It did not seem to matter whether animals received stress or were reactors. The important thing was being fed dicumarol. Post mortem examinations showed hemorrhagic symptoms.

This marked rise in prothrombin time of adrenalectomized rats fed dicumarol has two possible explanations. One is that the adrenal cortex is necessary, indirectly, in the synthesis of proteins, and that since the adrenals are removed the level of proteins necessary in coagulation is reduced. The second explanation is related to the work of Kramar and others (39) in which it was found that capillary resistance is permanently lowered after adrenalectomy. For example in the rat the resistance drops from a normal of 60-70 mm.Hg. to a value of 5 mm. Hg. In hemostasis there are three lines of defence: (1) the coagulation of the blood, (2) agglutination of platelets and blood cells, (3) constriction of blood vessels and decrease in permeability. If one of these defenses is defective adjustment can still be made. However, if two of these are impaired it is unlikely that hemorrhage can be prevented. The adrenalectomized rat on dicumarol has two of these defenses affected since both the coagul-

ability of the blood and capillary resistance are lowered. This accounts for the hemorrhage and death. But why is the prothrombin time higher during the period when dicumarol is fed to adrenalectomized rats than it was on the initial standardization? This is probably due to the tendency for blood to escape through the vessels having reduced capillary resistance (41). Prothrombin, pro-convertin, and other factors of blood coagulation are utilized in checking the escape of blood. A reduction of these factors is reflected in a longer prothrombin time.

The second interesting observation from Figure 3 is that reactors at 10 mg./kg. have a prothrombin time on the second feeding of dicumarol which is only 50 per cent of the first, and the non-reactors at 20 mg./kg. have a prothrombin time on the second feeding about 175 per cent of the first. In other words, hyper-reactors at 10 mg./kg. have become reactor and non-reactors at 20 mg./kg. have become reactors. A total of 12 reactors fed 10 mg./kg. dicumarol and 15 non-reactors fed 20 mg./kg. dicumarol show this trend. As yet there is no explanation for this. Any elucidation of these changes must await further understanding of what is involved in a rat being a reactor or a non-reactor.

The results show that the stress of ES or sound seizures has increased the prothrombin time on the sixth day, when the samples were taken one and one-half hours after exposure to stress. This is in agreement with the results given in Table VIII, where it was shown that new rats (without previous cardiac puncture) on dicumarol and receiving ES had a significantly longer prothrombin time than those on dicumarol alone, when samples were taken one and one-half hours following ES.

Figure 3 shows the interesting fact that in the case of reactors at both

dosage levels of dicumarol the prothrombin time is even longer on day eight, 48 hours after stress, than it was on day six. Thus, in reactors, stress increased prothrombin time with the change lasting for at least 48 hours. In non-reactors, prothrombin time following stress was shorter on day eight than on day six. The mortality data in Table IX provide further evidence that the effects of stress were more severe for the reactors than for the non-reactors. This is not so apparent in animals fed 10 mg./kg. dicumarol in which 1/12 reactors and 0/12 non-reactors died. However, when dicumarol was fed 20 mg./kg., 10/21 reactors died following stress while only 2/11 non-reactors died ($t = 2.46$, $p < 0.02$). When ES was given while the animals were under ether anesthesia the effects of stress measured by prothrombin time change and mortality were reduced.

This research shows that following stress the prothrombin time may be longer or shorter depending on such things as the severity of stress, time after stress, and individual differences. Severe stress, such as drug-facilitated sound seizures and stress over a period of time such as daily exposures of young rats to sound, produced hemorrhagic death. Stress administered while rats were fed thrombopenic drugs resulted in increased prothrombin time and mortality. It is not clear how stress acts in producing these changes. Cannon's claim that adrenalin stimulated the production of prothrombin in the stressed animals is not an adequate explanation. The hormones of the adrenal cortex are probably more involved in the stress response than adrenalin. Recent work has shown that capillary resistance exhibits phasic changes in the stress response. At certain times the capillary resistance falls to a low level and remains low for some time (37, 38, 39, 40, 41, 42). This has been

called the capillary crisis. The part played by the adrenal cortex in this regard is not clear. However, since the capillary resistance falls to a low level after adrenalectomy the adrenal cortex must play a vital part in regulating capillary permeability. During stress two things happen which are in some way related to the adrenal cortex. There is an increase in prothrombin time and a reduction of capillary resistance at certain times. This reduction in capillary resistance has been attributed to a hypo-secretion of cortisone or to the antagonism of Somatotrophic hormone on cortisone (38). If these changes are brought about in rats whose coagulation system is already altered by thrombopenic drugs, hemorrhagic symptoms result. Adrenalectomy apparently reduces the capillary resistance to such a low level that stress makes no difference in this regard. Consequently, adrenalectomized rats fed dicumarol inevitably die of hemorrhage whether stress is given or not.

SUMMARY AND CONCLUSIONS

Procedures including Forced Jumping, Electrified Water Source, Transportation, Sound Induced Seizures, and Electroshock were used in studying the effects of psychological stress procedures on the blood coagulation system in the albino rat. Both prothrombin time and mortality were used as indices of an altered coagulability. The thrombopenic drug, dicumarol was used in a number of experiments because it made the effects of stress on both prothrombin time and mortality more pronounced. The use of the tail vein puncture together with the Schwager-Jaques Method of prothrombin time determination made it possible to follow the prothrombin time for a considerable time and opened up many possibilities for future research.

Prothrombin time was increased when blood samples were taken a short time after exposure to severe types of stress. Significant increases in mean prothrombin time were also obtained when stress was given with an anti-coagulant. Usually, however, the altered coagulability was reflected in a greater heterogeneity of variance due to some animals having a longer prothrombin time and others shorter.

Hemorrhagic death was observed in connection with stress. Mortality occurred following drug-facilitated sound seizures, exposure of young rats to sound for a number of days, after ES and cardiac puncture, after stress in rats receiving prothrombopenic drugs, and in adrenalectomized rats fed anticoagulant.

Changes in the prothrombin time and hemorrhagic deaths are related in some way to the adrenal cortex. It is suggested that since

both stress and adrenalectomy reduce the capillary resistance, stressed and adrenalectomized rats fed prothrombopenic drugs have two means of hemostasis impaired and hence are not able to maintain hemostasis.

BIBLIOGRAPHY

1. Berkeley, A.W. Level of aspiration in relation to adrenal cortical activity and the concept of stress. J. Comp. physiol. Psychol., 1952, 45, 443.
2. Biggs, R., & Macfarlane, R.G. Human blood coagulation and its disorders. Springfield, Illinois: Thomas, 1953.
3. Bitterman, M.E. A reply to Dr. Finger. J. Comp. physiol. Psychol., 1946, 53, 116.
4. Bowlby, J. Maternal care and mental health. World Health Organization, 1952.
5. Cannon, W.B. Bodily changes in pain, hunger, fear, and rage. Boston: Chas. T. Branford Co., 1953.
6. Cannon, W.B., & Gray, H. The hastening or retarding of coagulation by adrenalin injections. Amer. J. Physiol., 1914, 34, 232.
7. Cannon, W.B., & Mendenhall, W.L. Factors affecting the coagulation time of blood. 1. The graphic method of recording coagulation used in these experiments. Amer. J. Physiol., 1914, 34, 225.
8. Cannon, W.B., & Mendenhall, W.L. The hastening of coagulation by stimulating the splanchnic nerves. Amer. J. Physiol., 1914, 34, 243.
9. Cannon, W.B., & Mendenhall, W.L. The hastening of coagulation in pain and emotional excitement. Amer. J. Physiol., 1914, 34, 251.
10. Carworth Farms Quarterly Letter, No. 38, New City, New York, July 1, 1955.
11. Carworth Farms Quarterly Letter, No. 39, New City, New York, October 1, 1955.

12. Christie, R. Experimental naivete and experiential naivete.
Psychol. Bull., 1951, 48, 329.
13. Chubaty, W. Anticoagulant in frostbite. M.A. Thesis, University of Saskatchewan, 1954.
14. Chubaty, W., & Jaques, L.B. An effect of stress agents on prothrombin time. Rev. Hemat., 1954, 9, 523.
15. Cleghorn, R.A., Goodman, A.J., Graham, B.F., Jones, M.H., & Rublee, N.R. Activation of the adrenal cortex in human subjects following electro-convulsive therapy and psychomotor stress.
J. clin. Endocrin., 1948, 8, 608.
16. Cole, W.H., Yeakel, E.H., & Farris, E.J. A preliminary study of changes in blood of gray Norway rats following audiogenic seizures.
Anat. Rec., 1942, 84, 524.
17. Cosgriff, S.W. Thromboembolic complications associated with ACTH and cortisone therapy. J. Amer. Med. Ass., 1951, 147, 924.
18. Cosgriff, S.W. Hypercoagulability of the blood associated with ACTH and cortisone therapy. Amer. J. Med., 1950, 9, 752.
19. Dam, H. Recent studies on vitamin K. Proc. Nutr. Soc., 1953, 12, 114.
20. Dam, H. Vitamins and antivitamins in blood coagulation.
Ric. sci., 1953, 23, Suppl. 4, P3.
21. D'Amour, F.E., & Blood, F.R. Manual for laboratory work in mammalian physiology. Chicago: The University of Chicago Press, 1948.
22. Edwards, A.L. Experimental design in psychological research. New York: Rinehart & Co. Inc., 1950.

23. Engel, F.L. General concepts of adrenocortical functions in relation to the response to stress. Psychosom. Med., 1953, 15, 565.
24. Finger, F.W. Convulsive behavior in the rat. Psychol. Bull., 1947, 44, 210.
25. Frost, J.W., Dryer, R.L., & Kohlstaedt, K.G., Stress studies on auto race drivers. J. Lab. clin. Med., 1951, 38, 523.
26. Garrett, J.V. Heparin and the adrenal cortex. J. clin. Path., 1953, 6, 294.
27. Grabfield, G.P. The effect of adrenin on the factors of coagulation. Amer. J. Physiol., 1916, 42, 46.
28. Gray, H. & Lunt, L.K. The effects of hemorrhage before and after exclusion of abdominal circulation adrenals or intestine. Amer. J. Physiol., 1914, 34, 332.
29. Griffiths, W.J. The influence of behavioral factors on the incidence of audiogenic seizures in rats. J. Comp. physiol. Psychol., 1953, 46, 150.
30. Howell, W.H. The condition of the blood in hemophilia, thrombosis and purpura. Amer. J. Physiol., 1914, 34, 321.
31. Hume, D.A. The role of the hypothalamus in the pituitary-adrenal cortical response to stress. J. clin. Invest., 1949, 28, 790.
32. Hurder, W. P., & Sanders, A.F. Audiogenic seizure and the adrenal cortex. Science, 1953, 117, 324.
33. Jaques, L.B. Blood clotting and hemostasis. Annu. Rev. Physiol., 1954, 16, 175.

34. Jaques, L.B. Estimation of the blood prothrombin time by the bedside method. In L.M. Tocantins. The coagulation of blood, methods of study. New York: Grune and Stratton, 1955.
35. Karn, H.W., Ladowski, C.H., & Patton, R.A. The effect of metrazol on the susceptibility of rats to sound induced seizures. J. comp. Psychol., 1941, 32, 563.
36. Kast, E., & Zweibel, A. Changes in blood clotting time and blood sugar levels in relation to electroshock therapy. Psychosom. Med., 1954, 16, 334.
37. Kramar, J. Stress and capillary resistance. Amer. J. Physiol., 1953, 69, 175.
38. Kramar, J. Endocrine regulation of the capillary resistance. Science, 1954, 119, 790.
39. Kramar, J., & Kramar, M.S. The effect of adrenalectomy, surgical trauma, and ether anesthesia upon the capillary resistance of the albino rat. Endocrinology, 1953, 52, 453.
40. Kramar, J. Stress of fasting and realimentation as reflected in the capillary resistance and eosinophils. Amer. J. Physiol., 1954, 178, 486.
41. Kramar, J., Meyers, W., & Peetz, D.J. Correlation between capillary resistance and circulating eosinophils. J. Lab. clin. Med., 1954, 43, 395.
42. Kramar, J., Peetz, D.J., & McCarthy, H.H. Capillary response in stress. Psychosom. Med., 1954, 16, 393.
43. Long, C.N.H. Secretions of adrenal cortex. Bull. N.Y. Acad. Med., 1947, 23, 260.

44. Long, C.N.H. The conditions associated with the secretion of the adrenal cortex. Fed. Proc., 1947, 6, 461.
45. Lepp, E., Ogilvie, M., & Jaques, L.B. Effect of danilone and dicumarol on experimental frostbite. Proc. Canad. Physiol. Soc., XV Annual Meeting, 1951, P. 27.
46. Lepp, E., Chubaty, W., & Jaques, L.B. Effect of phenylindanedione and dicumarol in experimental frostbite. Canad. J. med. Sc., 31, 173.
47. Macht, D.I. Influence of acute emotions on blood clotting. Fed. Proc., 1951, 10, 88.
48. Macht, D.A. Effects of drugs and of emotions on blood coagulation. J. Amer. med. Assoc., 1952, 148, 265.
49. McCann, S.M. Effects of hypothalamic lesions in adrenal cortical response to stress. Amer. J. Physiol., 1952, 171, 746.
50. Michael, S.G., & Brown, W.T. Blood lymphocytes and adrenal functions in ECT of psychoses. J. Nerv. ment. Dis., 1951, 113, 538.
51. Moreau, L. Studies of shock in frogs. 7. Shock due to electrical injury. Fed. Proc., 1952, 7, 82.
52. Moreau, L. Shock due to electrical injury in frogs. Amer. J. Physiol., 1948, 154, 38.
53. Munn, N.L. Handbook of psychological research on the rat. Boston: Houghton Mifflin Co., 1950.
54. Noble, R.L. The development of resistance by rats and guinea pigs to amounts of trauma usually fatal. Amer. J. Physiol., 1943, 138, 346.
55. Orlansky, H. Infant care and personality. Psychol. Bull., 1949, 46, 1.

56. Owen, C., & Bollman, J.L. Analysis of the one-stage test for prothrombin time. J. Appl. Physiol., 1953, 5, 515.
57. Owren, P.A. Parahaemophilia: Haemorrhagic diathesis due to absence of a previously unknown clotting factor. Lancet, 1947, 1, 446.
58. Page, J.D. Electrically induced convulsions. J. comp. Psychol., 1941, 31, 181.
59. Porter, F.B., & Stone, C.P. AGS in rats under ether anesthesia. J. Comp. physiol. Psych., 1947, 40, 441.
60. Raskin, N., Howard, G., & Singer, S. Lymphocytic and eosinophilic reaction to electric shock treatment. Amer. J. Psychiat., 1951, 107, 758.
61. Richter, C.P. Psychotic behavior produced in wild Norway and Alexandrine rats apparently by the fear of food poisoning. In Reymert, M.L. Feelings and emotions, the Mooseheart symposium. New York: McGraw Hill, 1950, pp 189 - 202.
62. Rogers, P.V., & Richter, C.P. Anatomical comparison between the adrenal glands of the wild Norway, wild Alexandrine, and domestic Norway rats. Endocrinology, 1948, 42, 46.
63. Rosvold, H.E., Keyslan, S.J., & Stevenson, J.A. Effect of electroconvulsive shock on adrenal cortex of the rat. Proc. Soc. exp. Biol., 1952, 80, 60.
64. Ruegamer, W.R., Bernstein, L., & Benjamin, J.D. Growth, food utilization, and thyroid activity in the albino rat as a function of early handling. Science, 1954, 120, 184.

65. Sachs, J., & Glaser, N.M. Changes in susceptibility to the convulsant action of metrazol. J. Pharmacol., 1941, 73, 289.
66. Sandrock, R.S., & Mahoney, E.B. Prothrombin activity: a diagnostic test for early post-operative venous thrombosis. Ann. Surg., 1948, 128, 521.
67. Sayers, G. The adrenal cortex and homeostasis. Physiol. Rev., 1950, 30, 241.
68. Schneider, R.A. The relation of stress to clotting time, relative viscosity and certain other biophysical alterations of the blood in the normo-tensive and hyper-tensive subject. Res. Publ. Ass. nerv. ment. Dis., 1950, 29, 818.
69. Schneider, R.A. Recurrent thrombophlebitis: a study of life situations, emotions and the clotting time and relative viscosity of the blood. Bull. N.Y. Acad. Med., 1951, 27, 389.
70. Schneider, R.A. & Zangari, V.M. Variations in clotting time, relative viscosity and other physicochemical properties of the blood accompanying physical and emotional stress in the normotensive and hypertensive subject. Psychosom. Med., 1951, 13, 289.
71. Selye, H. Stress and the general adaptation syndrome. Brit. med. J., 1950, 1383.
72. Selye, H. The physiology and pathology of exposure to stress, Montreal: ACTA Inc., 1950.
73. Selye, H. First annual report on stress. Montreal: ACTA Inc., 1951.
74. Selye, H. Second annual report on stress. Montreal: ACTA Inc., 1952.

75. Selye, H. Fourth annual report on stress. Montreal: ACTA Inc., 1954.
76. Selye, H. Stress and disease. Science, 1955, 122, 625.
77. Siegel, P.S., McGinnies, E.M., & Box, J.C. The runway performance of rats subjected to electro-convulsive shock following nembutal anesthesia. J. Comp. physiol. Psychol., 1949, 42, 417.
78. Smith, R.W., Margulis, R.R., Brennan, M.J., & Monto, R.W. The influence of ACTH and cortisone on certain factors of blood coagulation. Science, 1950, 112, 295.
79. Snee, T.J., Terrence, C.F., & Crowley, M.E. Drug facilitation of audiogenic seizures. J. Psychol., 1942, 13, 223.
80. Stainbrook, E.J. A note on induced convulsions in the rat. J. Psychol. 1941, 31, 337.
81. Stone, C.P. Effects of electro-convulsive shocks on wildness and savageness in feral rats. J. Comp. physiol. Psychol., 1953, 46, 373.
82. Takats, G., & Marshal, M.H. The response of the clotting equilibrium to post-operative stress. Surgery, 31, 13, 1952.
83. Ungar, G. The effect of trauma on bleeding time and capillary resistance. J. Physiol., 1944, 103, 18.
84. Vosburgh, C.H., & Richards, A.N. An experimental study of the sugar content and extra-vascular coagulation of the blood after the administration of adrenalin. Amer. J. Physiol., 1903, 9, 35.
85. Weininger, O., & McClelland, A. Gentling and weight gain in the albino rat. Canad. J. Psychol., 1954, 8, 147.

86. Weininger, O. Physiological damage under emotional stress as a function of early experience. Science, 1954, 119, 285.
87. Wolff, H.G. Stress and disease. Springfield, Illinois: Thomas, 1953.

APPENDIX

TABLE I EFFECT OF FORCED JUMPING ON PROTHROMBIN TIME IN RATS

Time interven- ing between stress and tak- ing blood	No. of Animals		Control		Expt'l		Tests of Significance	
	Control	Expt'l	Prothrombin Time Mean	S.D.	Prothrombin Time Mean	S.D.	Mean	Variance
20 min.	3	5	24.7	0.4	28.4	2.47	t = 2.5 p < .05	F = 39 p < .10
90 min.	3	5	28.9	2.1	29.0	4.1	t = .01 p > .10	F = 4 p > .10
3 hrs. ^x	4	5	16.6	0.7	20.6	7.2	t = .74 p > .10	F = 100 p < .01
20 min. ^{x x}	3	5	24.5	.84	32.6	4.8	t = 2.6 p < .05	F = 32.8 p < .05
^x Blood samples by cardiac puncture. Others by tail vein puncture. ^{xx} Animals stressed for 6 days, other groups single stress.								

TABLE II EFFECT OF ELECTRIFIED WATER SOURCE PROCEDURE
ON PROTHROMBIN TIME IN RATS

Treatment	No. of Animals	Prothrombin Time in Seconds	
		Mean	S.D.
Control	10	17.7	1.2
Expt'l	10	18.9	3.5
Tests of		$t = 1.03$	$F = 8.5$
Significance		$p > 0.10$	$p < 0.01$

TABLE III PROTHROMBIN TIME IN RATS AFTER STRESS DUE
TO TRANSPORTATION

Treatment	No. of Animals	Prothrombin Time in Seconds	
		Mean	S.D.
Control	12	29.1	1.2
Expt'l	12	28.7	3.0
Tests of		t = .43	F = 6.13
Significance		p > .10	p < .02
Samples by tail vein puncture.			

TABLE IV EFFECT OF SINGLE EXPOSURES TO SOUND ON PROTHROMBIN TIME IN RATS

Time [■]			Control		Expt'l		Tests of significance	
	No. of Animals		Prothrombin Time in Seconds		Prothrombin Time in Seconds			
	Control	Expt'l	Mean	S.D.	Mean	S.D.	Mean	Variance
1½ hr. [■]	6	6	25.4	1.70	26.1	5.00	t = .47 p > .10	F = 8.6 p < .05
3 hr.	15	14	14.3	0.95	14.3	2.49	t = 0	F = 6.9 p < .01
6 hr.	8	10	13.6	0.44	14.2	1.82	t = .91 p > .10	F = 17.4 p < 0.01
12 hr.	5	5	13.3	0.62	13.1	0.63	t = .23 p > .10	F = 1 p > .10
<p>■ Time intervening between stress and taking of samples of blood.</p> <p>■ Blood samples by tail vein punctures.</p>								

TABLE V EFFECT OF SINGLE METRAZOL-FACILITATED SOUND SEIZURES^{xx} ON PROTHROMBIN
TIME IN RATS

Time ^{xxx}	No. of Animals		Prothrombin Time in Seconds				Tests of significance	
	Control	Expt'l	Control		Expt'l		Mean	Variance
			Mean	S.D.	Mean	S.D.		
15 min.	5	5	16.3	0.4	23.5	2.6	t = 6 p < 0.01	F = 42 p < .01
6 hr.	9	7	16.3	0.7	15.6	1.0	t = 1.49 p > 0.10	F = 2 p > .10
24 hr.	8	8	14.6	0.65	14.2	1.66	t = .3 p > .10	F = 7.3 p < .05
72 hr.	5	4	16.8	0.83	16.3	1.6	t = .3 p > .10	F = 3.7 p > .10

^x Rats sensitized with metrazol (0.3 cc/100 gm. body weight) so that seizures would occur to sound.

^{xxx} Time intervening between seizure and taking of blood sample.

TABLE VI EFFECT OF A SINGLE ELECTROSHOCK ON THE
PROTHROMBIN TIME IN RATS[⌘]

Treatment	No. of Animals	Prothrombin Time in Seconds	
		Mean	S.D.
Control	8	29.1	1.4
Electroshock	12	28.3	4.3
Tests of		t = 0.51	F = 9.7
Significance		p > 0.10	p < 0.01
⌘ Samples by tail vein puncture. 1½ hours after stress.			

TABLE VII. EFFECT OF ELECTROSHOCK ON PROTHROMBIN TIME AND MORTALITY IN
RATS HAVING PREVIOUS CARDIAC PUNCTURE AND NO PREVIOUS CARDIAC PUNCTURE

Treatment	Prothrombin Time		Tests of Significance		Mortality	Significance
	Mean (sec.)	S.D.	Mean	Variance		
(1) Control - c.p.	14.2	0.56			4/16	
(2) New rats given ES and c.p.	16.3	2.52	(1) and (2) $t = 3.18$ $F = 20.5$ $p < .01$ $p < .01$		14/22	(1) and (2) $\lambda = 4.61$ $p < .05$
(3) Rats receiving stress + c.p. 2 weeks before, given ES and c.p.	15.3	0.8	(1) and (3) $t = 10.9$ $F = 2.1$ $p < .01$ $p > .10$ (2) and (3) $t = 1.18$ $F = 9.9$ $p > .10$ $p < .01$		2/11	(1) and (3) $\lambda = .142$ $p > .10$ (2) and (3) $\lambda = 5.9$ $p < .02$

TABLE VIII PROTHROMBIN TIME AND MORTALITY FROM CARDIAC PUNCTURE IN RATS RECEIVING
DICUMAROL AND ELECTROSHOCK

	Prothrombin Time in Seconds				Mortality	
	Dicumarol		Dicumarol + ES		Dicumarol	Dicumarol + ES
	Mean	S.D.	Mean	S.D.		
New Rats	(a) 22.8	8.7	(b) 41.5	27.56	(a) 11/29 = .38	(b) 32/50 = .64
Rats having previous c.p.	(c) 23.5	9.25	(d) 23.1	10.7	(c) 11/47 = .30	(d) 12/37 = .32
<div> <p>Comparing (a) and (b) $t = 4.2$, $p < 0.01$ $F = 10$, $p < .01$,</p> <p>For (a) and (b), difference between two percentages. $t = 2.36$; $p < .02$.</p> </div>						

TABLE IX MORTALITY FOLLOWING STRESS IN RATS FED DICUMAROL

Treatment	Dicumarol 10 mg./Kg.		Dicumarol 20 mg./Kg.	
	Reactors	Non-reactors	Reactors	Non-reactors
Dicumarol	0/3	0/3	1/9	0/4
Dicumarol + ES	1/3	0/3	5/10	1/6
Dicumarol + Sound Seizures	0/3	0/3	2/2	0/0
Dicumarol + ES While Under Ether	0/3	0/3	3/9	1/5
Adrenalectomy				
(1) ES			0/3	0/3
(2) Dicumarol	1/1	2/2	2/2	3/3
(3) Dicumarol + ES	2/2	1/1	3/3	2/2

PATHOLOGICAL REPORT ON RATS DYING OF SOUND SEIZURES

Rat 1. (a) Microscopic:

Lungs - There was very marked vascular congestion and oedema with small patches of perivascular and intra-alveolar haemorrhage.

Liver - mild central vein area congestion.

Kidneys - were the site of mild chronic pyelonephritis.

(b) Diagnosis

1. Acute pulmonary oedema.
2. Acute passive congestion of
 - (a) lungs - marked
 - (b) liver - slight
3. Petechial hemorrhage of lungs
4. Chronic pyelonephritis.

Rat 2. (a) Microscopic:

Lungs - were markedly congested and there were several intra- and sub-pleural recent petechial.

Liver - moderately severe central vein area congestion and marked fatty degeneration of the portal zones.

Kidney - moderate vascular congestion, also present was a considerable degree of acute pyelonephritis.

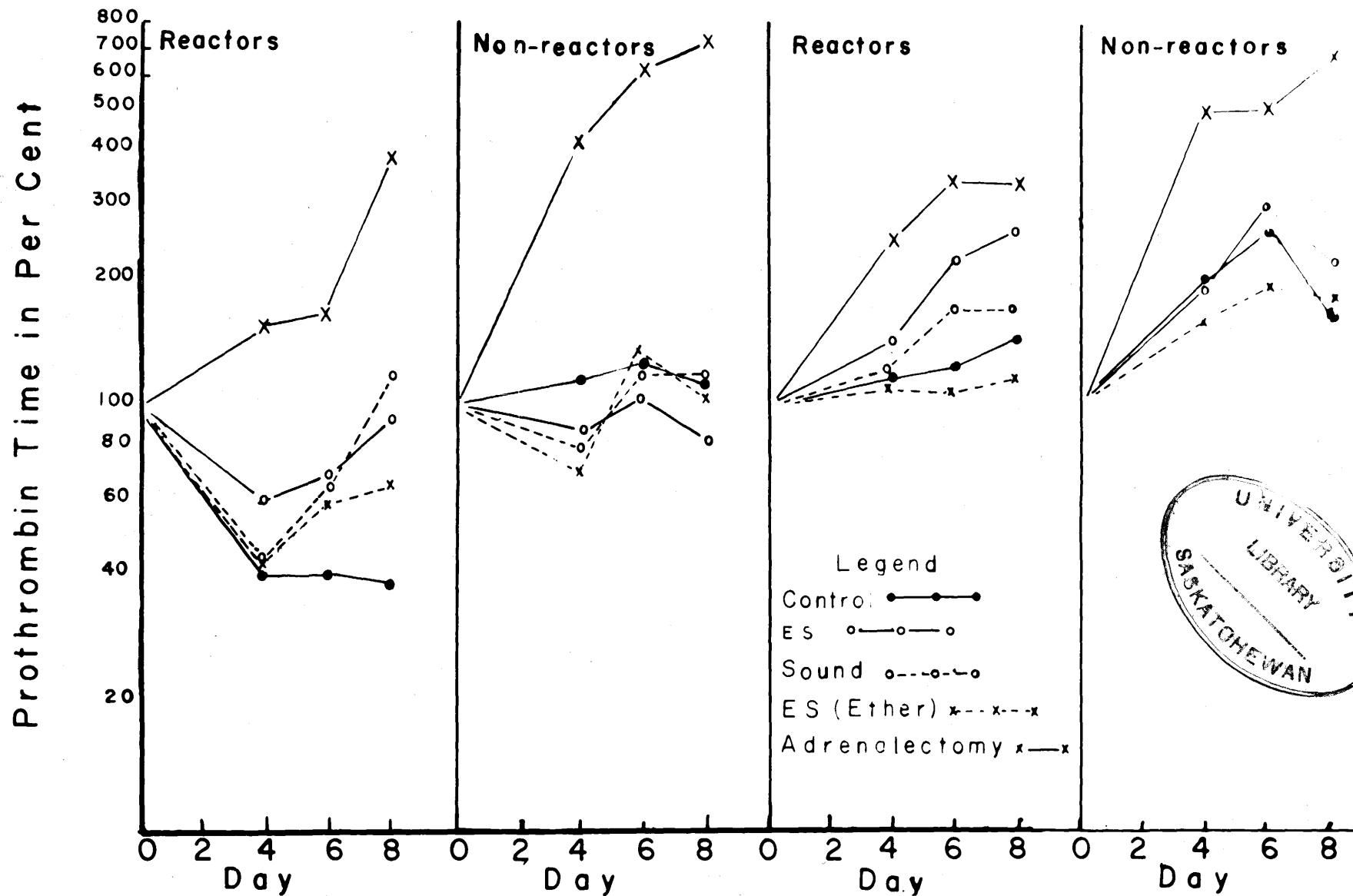
(b) Diagnosis

1. Acute passive congestion of viscera
2. Petechial hemorrhage of lungs.
3. Fatty degeneration of liver - marked portal
4. Acute Pyelonephritis.

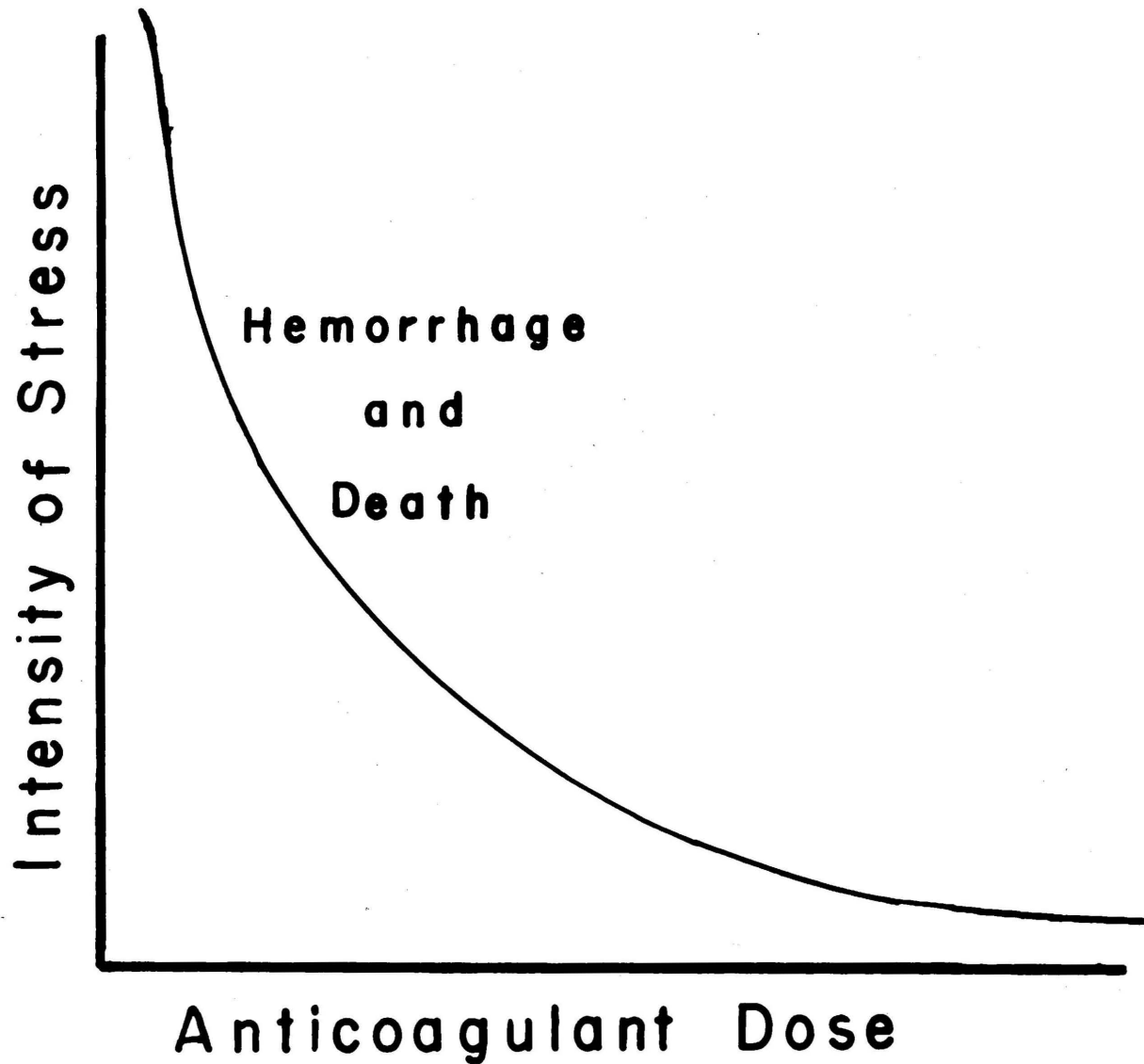
Prothrombin Time After Anticoagulant and Stress

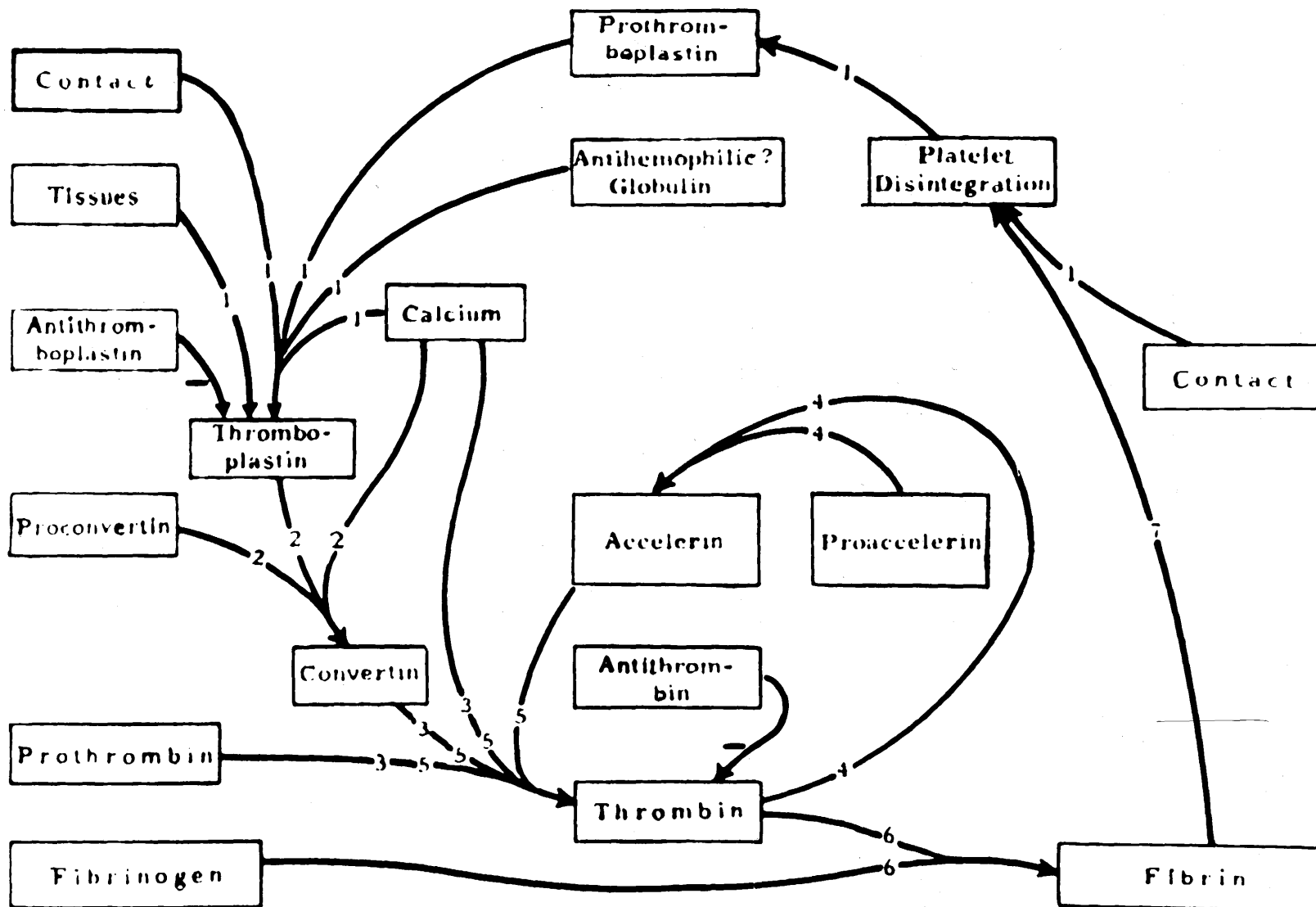
Dicumarol-10 mg/kg.

Dicumarol-20mg/kg.



The Relation of Stress and Anticoagulant in Hemorrhage





Prothrombin Time After Anticoagulant and Stress

Dicumarol-10 mg/kg.

Dicumarol-20mg/kg.

Prothrombin Time in Per Cent

