The Effect of Psychological Stress Procedures on the Prothrombin Time of Rats

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VARIOUS WORKERS have reported experimental and clinical observations which suggested that psychological stress might affect the blood coagulation system. As early as 1771 William Hewson noted hypercoagulability of the blood followed hemorrhage. In 1903 Vosburgh and Richards[29] observed more rapid coagulation accompanied an increase in the blood sugar of dogs receiving adrenalin. In view of the relevance of changes in coagulability from adrenalin to Cannon's work on bodily changes during pain and emotional excitement, the latter conducted several studies and found that in decerebrate cats adrenalin in suitable doses considerably reduced the clotting time, while larger doses usually produced an increase in the blood coagulation time followed in some cases by a reduction. Pain evoked by stimulation of the splanchnic nerve, and emotional reactions were also followed by a shortening of the blood coagulation time.[1-3] More recent reports by Cole[5] on rats exposed to sound, by Ungar[28] on guinea pigs traumatized in a drum, and by Moreau[17] on frogs receiving electrical injury showed decrease in the coagulation time of blood. Clinical reports have revealed an alteration of the blood coagulation system due to the stress of surgery and the emotions of fear and anxiety. A shortened clotting time during anxiety, fear, hostility, and while discussing stressful topics was found by Schneider[20, 21] and Macht.[16] Kast[15], studying psychotic patients receiving electroshock therapy (ECT), found that subjects receiving ECT at appointed times and subjects anticipating ECT showed a marked shortening in blood clotting time, while subjects receiving ECT without previous warning showed no change. Clinical reports on the use of ACTH and cortisone suggest adrenocortical involvement in the etiology of altered blood coagulation. Cosgriff[6, 7], Smith[23], and Garrett[9] have reported hypercoagulability of the blood and thromboembolic disease following the administration of these hormones. McGraw 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".[24]

The most general finding in the experimental and clinical reports has been shortening of blood coagulation time after a variety of psychological stresses. In contrast to this, results obtained in this laboratory with certain severe physical and chemical stress agents (frostbite, 10 per cent sodium chloride intraperi-

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toneally, 4 per cent formaldehyde subcutaneously and insulin shock[13]) indicated a marked increase in the prothrombin time of rats. This paper reports the extension of this work in rats to include psychological stress procedures. As found in the previous investigations, the simultaneous administration of Dicumarol made the effects of stress more apparent both in the change in prothrombin time and in the incidence of mortality.

Methods

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 so wired so that electric shocks could be delivered to the feet of rats that did not want to jump. The rats were made to learn a place habit which consisted of always jumping to the door on the left. On the test days 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. The control animals were given a regular ten minute practice session. Forced jumping did not produce wild running and seizures as some investigators have reported.[18] However, following such stress, the animals were usually hyperactive.

Electrified Water Source—Rats were placed in individual cages wired in such a way that they received a shock of 35 volts 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, with sodium chloride as an electrolyte. For two hours each morning the experimental animals as well as control animals were allowed to drink and were fed in ordinary individual cages. Most of the animals kept in the Electrified Water Source Apparatus became hypersensitive and overactive.

Transportation—Blood samples were taken immediately after a rail trip of 1500 miles.

Sound Induced Seizures—A Hartmann whistle, connected to a source of compressed air was adjusted to give a sound stimulus of 13,000 c.p.s. and the whistle turned on for two to five minutes depending on how quickly seizures occurred. The rats were either confined in a small aluminum box or kept in their cages while exposed to the sound. Rats vary in incidence of sound-induced seizures. For the strains of animals used in these experiments, one strain showed 10 per cent susceptibility and another strain 30 per cent susceptibility. The occurrence of seizures is affected by the degree to which the animals are permitted extraneous or manipulative behavior during the sound stimulation[11] and by the excitability of the nervous system as excitant drugs increase susceptibility.[14,19] Metrazol was used in some experiments in subconvulsive doses (0.25 to 0.3 ml. per 100 Gm. body weight) and this made it possible to convulse nearly all rats exposed to sound. The sound-induced seizures were very much like those described by Finger.[8]

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. As a means of confinement the animals were placed in a 12″ x 12″ x 5″ plastic box during the electroshock procedure. Electrically induced seizures are very similar to sound-induced seizures.[27] The strength and duration of the electric current required to convulse rats was found to be fairly constant, 130 to 140 volts for 0.4–0.7 seconds.

Determination of Prothrombin Time—The rat was placed under light ether anesthesia and using a siliconed syringe a two ml. sample of blood was taken by cardiac puncture, through a 22 gauge needle or about 0.1 ml. of blood was drawn from the lateral vein of the tail with a 25 gauge needle. The Modified Quick Procedure was performed on citrated plasma with commercial rabbit brain thromboplastin and 0.02 molar calcium chloride solution, using the first appearance of a clot or fibrin thread as the end-point. A modification of the Schwager-Jaques method for the Bed-side Prothrombin Time was also used.[22] One drop of thromboplastin was placed on a one and one-half inch watch glass with a one-quarter ml. syringe, one drop of blood from the tail vein sample added, the timer started and the blood stirred with the needle until the clot formed.

Dicumarol (Abbott) was added to the standard colony diet, 40 mg. 100 Gm. of feed and fed 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. and after this was consumed, additional food without Dicumarol given in the afternoon.

Statistical Treatment of Data—While the original experiments with chemical and physical stress agents gave a consistent increase in prothrombin time, more recently it has been found that this is not the case with many groups of rats, due to variations in genetic constitution and past experience. Rather, the effect of stress agents on prothrombin time is to cause a marked increase in the prothrombin time for only some in a group, and even a shortening of prothrombin time for some rats. This means that the mean values obtained for groups of animals are not useful nor a valid measure of the effect observed. Rather the increased variability is the significant change. The significance of a difference in variance may be tested by a procedure developed by Snedecor.[25] It is the ratio of variance and has been called the F ratio. $F = S_1^2/S_2^2$. This test for the heterogeneity of variance using the F ratio has been extensively used in evaluating the data.

Results

The effect of certain psychological stress procedures on the prothrombin time of rats is shown in table 1. Twenty minutes after a single exposure to Forced Jumping, the prothrombin time was significantly different from the prothrombin time of control animals ($F = 24.8$, $p < 0.01$). When animals were stressed for six days by Forced Jumping, the increase in prothrombin time taken twenty minutes after stress was even more marked, with both t test and F test indicating a highly significant change in prothrombin time. The group of animals in which the blood samples were taken by cardiac puncture and measured by the Quick procedure showed similar changes in the variance of prothrombin times that were very significant. This was three hours following Forced Jumping. Thus it appears that Forced Jumping in the Lashley jumping apparatus produced changes in prothrombin time detectable as long as three hours following stress. In individual

TABLE 1.—*Effect of Psychological Stress Procedures on Prothrombin Time in Rats*

Procedure	Time intervening between stress and taking blood	No. of animals per group	Prothrombin Time*		Tests of significance
			Control	Exptl.	
Forced jumping	20 min.	12	26.4 ± 1.05	29.5 ± 5.2	F = 24.8, p < .01
	20 min.‡	5	24.5 ± 0.84	32.6 ± 4.8	F = 32.8, p < .05
	3 hr.	5	16.6 ± 0.7†	20.6 ± 7.2†	F = 100, p < .01
Electrified water source	After 15 days of stress	20	17.7 ± 0.87†	18.9 ± 2.70†	F = 10.1, p < .01
Transportation		46	28.4 ± 1.52	27.3 ± 3.58	F = 5.53, p < .01
Electroshock	1½ hours	17	28.8 ± 1.5	27.9 ± 4.1	F = 7.4, p < .01
	1½ hours	22	14.1 ± 0.7†	16.3 ± 2.5†	F = 20.5, p < .01

* Mean ± standard deviation. Blood samples by tail vein puncture († by cardiac puncture.)

‡ Stress of Forced Jumping for six consecutive days.

TABLE 2.—*Effect of Sound on Prothrombin Time in Rats*

Procedure	Time between stress and taking blood samples	No. of animals per group	Prothrombin Time*		Tests of Significance
			Control	Exptl.	
Exposure to sound	1½ hr.	6	25.4 ± 1.70†	26.1 ± 5.00	F = 8.6, p < .05
	3 hr.	14	14.3 ± 0.95	14.3 ± 2.49	F = 6.9, p < .01
	6 hr.	10	13.6 ± 0.44	14.2 ± 1.82	F = 17.4, p < .01
	12 hr.	5	13.3 ± 0.62	13.1 ± 0.63	F = 1, p > .10
Metrazol-facilitated sound seizures	15 min.	5	16.3 ± 0.4	23.5 ± 2.6	F = 42, p < .01
	6 hr.	7	16.3 ± 0.7	15.6 ± 1.0	F = 2, p > .10
	24 hr.	8	14.6 ± 0.65	14.2 ± 1.66	F = 7.3, p < .05
	72 hr.	4	16.8 ± 0.83	16.3 ± 1.6	F = 3.7, p > .10

* Mean ± standard deviation. Blood samples by cardiac puncture. († by tail vein puncture.)

animals, some showed a larger prothrombin time, some, a shorter time, while some showed no change.

The results of experiments with animals in the Electrified Water Source Apparatus are also shown in table 1. 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 variance was very significant.

Blood samples were taken from rats which had just arrived after transportation for 1500 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. In spite of these precautions, nearly 50 per cent of the rats died enroute or shortly after arrival, indicating the severity of the stress suffered. It has been reported that the transportation of animals is an emotional as well as a physical stress.[4] However, no one has previously reported that the blood coagulation was altered by the stress of transportation. The prothrombin time of some of these transported animals was affected, since as shown in table 1, by the F test there was a significant change in variance.

The effect of a single electroshock on the prothrombin time of rats is also shown in table 1. This experiment was done using male rats weighing 225–300 Gm. In preliminary experiments it appeared the change was maximal at one and one-half hours after electroshock. As shown the stress of a single electroshock gave 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 electroshock administered to smaller animals weighing 130 to 160 grams did produce a significant increase in the mean prothrombin time.

The results of exposure to high frequency sound are shown in table 2. There was no uniform shift of prothrombin time after a single exposure to sound. 1½, 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 2 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, the values for twelve were more than two standard deviations from the mean of the controls. It is pertinent that the number of longer and shorter values were the same. After 21 hours the variation of the prothrombin time was normal. The results of experiments in which drug-facilitated sound seizures were used are also presented in table 2. As shown 24 hours following this stressor the F test was significant and immediately following seizures the variation in prothrombin times was even greater, and the mean prothrombin time was significantly greater than the control. This was not the case for animals 6 and 72 hours after seizures. Similar results were obtained when drug-facilitated sound seizures were repeated daily for 10 days.

Rats occasionally died of hemorrhage when blood samples were taken by cardiac puncture following stress. After repeated administration of electroshock, rats showed a lengthened prothrombin time and many deaths from cardiac puncture. The mortality in rats from cardiac puncture following a single electroshock is shown in table 3. The mortality is significantly higher than that of control animals receiving only cardiac puncture. However, mortality from cardiac puncture with electroshock was markedly reduced when rats had received stress and cardiac puncture two or three weeks previously. 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. Further, previous cardiac puncture likewise suppressed the increased variability in prothrombin time due to electroshock. This finding has a very practical application in that it shows 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, we have found that stress along with the anticoagulant can produce appreciable hemorrhage. A large number of rats were fed 10 mg/Kg. of Dicumarol daily in their feed for five days. Electroshock was administered to some of the animals on the fourth day and blood samples taken one and

TABLE 3.—*Prothrombin Time and Mortality from Cardiac Puncture in Rats Receiving Electroshock*

Treatment	Prothrombin Time*		Mortality from Cardiac Puncture†	
	—	‡	—	‡
Control	(1) 14.3 ± 0.56	(2) 14.3 ± 0.2	(1) 4/16	(2) 0/4
Electroshock	(3) 16.3 ± 2.50	(4) 15.3 ± 0.8	(3) 14/22	(4) 2/11
Dicumarol	(a) 22.8 ± 8.7	(b) 23.5 ± 9.3	(a) 11/29	(b) 11/47
Dicumarol and Electroshock	(c) 41.5 ± 27.6	(d) 23.1 ± 10.7	(c) 32/50	(d) 12/37

* Differences between (1) and (3), (2) and (3), (a) and (c) significant at 1% level. Other differences not significant.

† Differences between (1) and (3), (a) and (c), significant at 2% level.

‡ Subjected to cardiac puncture 10 days–3 weeks before experiment.

Blood Samples by Cardiac Puncture.

one-half hours later by cardiac puncture. It is shown in Table 3 that with new rats the mortality was significantly higher in those receiving Dicumarol and electroshock (64 per cent) than in those getting only Dicumarol (mortality of 38 per cent). The prothrombin time of the stressed group was also very significantly longer. However, when animals which had been subjected to cardiac puncture sometime previously were used, electroshock did not increase either the prothrombin time or the incidence of mortality.

Discussion

The psychological stress procedures used in rats produced in each case a similar type of alteration of the blood coagulation system. This was an increased variability of prothrombin time, for some animals had a prothrombin time longer than normal and others a shorter time. It should be noted that some animals had no change in prothrombin time. The increased variability is similar to the findings of Cannon with cats, in which suitable doses of adrenalin reduced coagulation time. Cannon believed two factors were operating, one hastening and the other retarding coagulation and that adrenalin upset the equilibrium between them. Grabfield[10] suggested that adrenalin stimulated the liver to produce prothrombin. Today we know that prothrombin is seldom a limiting factor in blood coagulation and that an increase in prothrombin in this way would probably not affect these tests. It is becoming clear that a stress response is involved and that altered blood coagulation is one manifestation of the stress response. Adrenalin may be important in evoking this response, as in the case of Cannon's work. However, other mediators of the stress response are also possible. The previous report[13] that the change in prothrombin time could not be obtained in the adrenalectomized rat suggests the pituitary-adrenalcortical axis plays a role.

The way in which the stress response can lead to changes in coagulability of the blood is not known. Rats which died after drug-facilitated sound seizures were examined and generalized congestion of the lungs, liver and kidneys found. 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 other experiments in which weanling rats were exposed to sound for two minutes daily for three weeks, 13 of 52 died. Deaths occurred only in rats that had a number of convulsions from sound. Post mortem examination in these young rats revealed congested liver, lungs, hemothorax, and gastro-intestinal congestion. Pathologic changes were also observed in the liver, lungs and sometimes the heart of rats which died after electroshock and cardiac puncture while being fed small doses of Dicumarol. In view of the damage to the liver and the relation of the liver to prothrombin and proconvertin it seems logical that the prothrombin time should be longer, although the fact that the effect was observed 15 minutes after seizures suggests a more direct effect on the coagulation system. More difficult to explain is the finding of a prothrombin time shorter than normal with some rats.

It seems odd that the prothrombin time was significantly changed 15 minutes and 24 hours after sound 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,[23] it could be that at the transition from the Alarm Reaction to the Stage

of Resistance there is a period when prothrombin values show a minimal deviation from the physiologic normal. 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 change in prothrombin time is evident, could be preceded and followed by significant changes. The response to stress depends on many factors, which may be summarized under state of the organism, stressor used, length of time since stress.

Summary

Forced jumping in the Lashley jumping apparatus, maintenance in cages with an electrified water source, transportation, sound-induced seizures, and electroconvulsive treatment were used in studying the effects of psychological stress on the prothrombin time of the albino rat. An increased prothrombin time occurred at certain times following forced jumping, sound-induced seizures, and electroshock. Frequently the altered coagulability was reflected in a greater heterogeneity of variance due to some animals having a longer prothrombin time and others shorter. The changes in the prothrombin time following stress were more pronounced when the prothrombopenic drug, Dicumarol, was administered. This was evident from two indices, prothrombin time and mortality.

Summario in Interlingua

Pro studiar le effecto de stress psychologic super le tempore de prothrombina in rattos albin, saltation fortiate in le apparato saltatori de Lashley, mantenentia in cavias con electrificate fontes de aqua, transportation, convulsiones inducite per medio de sonos, e tractamento electroconvulsive esseva empleate. Un prolongate tempore de prothrombina occurreva a certe vices post saltation fortiate, convulsiones inducite per sonos, e electrochoc. Frequentemente le alterate coagulabilitate esseva reflectite in un plus grande heterogeneitate de variantia statistic, proque alicun animales habeva un plus longe tempore de prothrombina, alteres un plus curte. Le cambiamentos in le tempore de prothrombina sequente stress esseva plus pronunciate post le administration del droga prothrombopenic, Dicumarol. Isto es evidente in duo indices, le tempore de prothrombina e le mortalitate.

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