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Received September 20, 1965. P.S.E.B.M., 1966, v121.

Effect of Stress on Blood Levels of Guanine Nucleotides.* (30709)

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Previous work from this laboratory showed that pretreatment of stressed intact rats with guanine nucleotides abolished some of the characteristic metabolic alterations of stress (1,2). In addition, the survival time of adrenalectomized rats subjected to tourniquet application was significantly prolonged by guanylic acid pretreatment(2). These experiments suggested a physiological role for the guanine nucleotides in the stress response. The present study was undertaken to determine whether an applied stress alters the metabolic handling of the guanine nucleotides themselves in normal and adrenalectomized rats. As a first approach to this problem we measured the rate of disappearance of C^{14} labelled guanylic acid from the blood of tourniquet stressed and unstressed intact and adrenalectomized animals.

Materials and methods. Male albino rats of the Sprague-Dawley strain, weighing 200 g ± 10 g were used in these studies. The rats were maintained on Purina rat chow and

* Supported in part by U.S.P.H.S. Grant AM-04714.

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TABLE I. Disappearance Rate of GMP-8-C^{14} from Blood of Stressed and Non-Stressed Intact Rats.

GMP-8-C^{14} cpm/ml.

Time (secs)	Intact	Intact + stress	P (value)
10	6521 ± 596	8343 ± 1475	<.05
30	3918 ± 596	5441 ± 422	<.001
60	2482 ± 513	4117 ± 1115	<.02
120	2298 ± 359	2639 ± 209	N.S.
300	1991 ± 233	2828 ± 742	<.05

water *ad libitum* with the exception of the adrenalectomized animals given 1.0% saline in place of tap water. Bilateral adrenalectomy was performed under ether anaesthesia and the animals were not used until the seventh post-operative day. Tourniquet stress was produced by tight ligation of the hind limbs at the inguinal level with a double rubber band. The ligature was kept in place for 4 hours.

Intact and adrenalectomized animals in both the stressed and non-stressed state were given 0.3 ml of guanosine monophosphate-8-C^{14} containing 7.5×10^{-4} μmoles (4.9×10^5 cpm) into the femoral vein. Stressed animals were similarly treated 5 minutes after removal of the tourniquet. Groups of animals (5 to 7 animals/group) were decapitated at intervals of 10, 30, 60, 120 and 300 seconds and blood samples were collected by drainage from the severed neck vessels into heparinized tubes. One ml aliquots of mixed blood were deproteinized with $HClO_4$ and neutralized with 5 N KOH. Aliquots of this solution were removed and placed in vials containing 15 ml of counting solution which contained 1% 2,5-diphenyloxazole, 0.05% p-bis (2-(5-phenyloxazolyl)-benzene), 5% naphthalene in p-dioxane and ethyleneglycolmonethyl ether.

Results. In these studies our initial injection of the radioactive material was of the order of 490,000 cpm into a 200 g rat with a blood volume of approximately 14 ml. The highest possible blood concentration after complete mixing would thus be about 35,000 cpm/ml blood. If this value is taken as the theoretical base line, it can be seen from Table I that the disappearance of the labelled nucleotide from the blood is rapid within the first 10 seconds in all animals but the stressed intact animal shows a slower rate of disappearance than the non-stressed animal. The differences in disappearance rates as between stressed and non-stressed intact rats were significant at all time intervals except the 120-second sampling.

Data from comparable studies with adrenalectomized animals are shown in Table II. These data indicate similarly that in a stressful situation the rate of disappearance of the labelled nucleotides is slower than in the resting state. Significant differences were observed at all time intervals used.

Discussion. In these experiments we studied the disappearance rate of a labelled guanine nucleotide from the blood of stressed and unstressed intact and adrenalectomized rats. The design of the experiment was suggested by previous work which showed that exogenous guanine nucleotides could significantly alter metabolic responses to stress and could extend the survival time of stressed adrenalectomized animals(1,2). Since nucleotides are found in high concentration in lymphatic tissue which involutes during a stressful situation, we hypothesized that this corticoid mediated involution made available endogenous nucleotides which then acted in the homeostatic response. As a corollary, nucleotides might be used in greater amounts by peripheral tissues during the stress response. Our measurements of the rate of disappearance of GMP-8-C^{14} from the blood do not support this concept, however. Tourniquet stress appeared actually to slow the clearance rate of the label in both intact and adrenalectomized animals.

These results resemble the clearance data obtained with labelled corticoids in similar

TABLE II. Disappearance Rate of GMP-8-C^{14} from Blood of Stressed and Non-Stressed Adrenalectomized Rats.

GMP-8-C^{14} cpm/ml.

Time (secs)	Adrenalectomized	Adrenalectomized + stress	P (value)
10	7449 ± 1550	10961 ± 933	<.01
30	3678 ± 634	6152 ± 641	<.001
60	2409 ± 384	4559 ± 577	<.001
120	2294 ± 411	3177 ± 664	<.02
300	1913 ± 244	2907 ± 191	<.001

experiments(3-7). When the critical role of the glucocorticoids in the stress response became apparent it was suggested that stress created an increased demand for cortical hormones which accelerated their removal from the blood(3). Attempts to confirm this hypothesis were not signally successful. In fact, the bulk of the evidence indicated that stress slowed the disappearance of glucocorticoids from the blood(4,5,6). Thus, Firschein *et al* found that the clearance of injected cortisol-4-C^{14} was slower in tourniquet stressed rats than in normal animals(6). When they raised blood corticoid levels in non-stressed animals by administration of large amounts of unlabelled corticosterone they found a similarly delayed clearance. In both instances increased steroids evoked a decreased turnover rate in plasma, liver and muscle. Since the liver is known to be one of the principal sites of steroid metabolism, a stress or steroid induced depression of liver turnover could lead to a slower clearance of steroids not only from the liver itself but also from the plasma.

Alteration in circulatory dynamics could account for the slower removal of the label following a severe stress. But the work of Ulrich and Long(5) would tend to minimize the role of circulatory factors. They found that exercise, histamine injection, nephrectomy or evisceration differed very little in their effect on the disappearance rate of C^{14} labelled hydrocortisone or corticosterone from the serum of normal or adrenalectomized rats. Since these procedures produce very different changes in circulatory dynamics, it would appear that this does not represent the common denominator in this particular stress response.

Our data may reflect a change in liver function during the homeostatic responses to stress. Feigelson's work suggests that an increase in circulating corticoids may lead to increased purine synthesis in the liver(8). This is further increased by release of nucleotides from lympathic tissue. At the same time, there may be a decreased clearance of plasma nucleotides. Under stressful circumstances, therefore, the larger nucleotide pool could be reflected in a slower disappearance of the labelled nucleotide from the blood. In the case of the adrenalectomized animal no corticoids are available to set in train all these changes but there is evidence from our laboratory that the liver clearance of nucleotides is probably slowed during a stress and some nucleotides are released from damaged tissues. This, in turn, might lead to a decreased disappearance rate of the label from the blood.

In all these experiments we measured only the labelled carbon and not the nucleotide moieties as such. It is possible that a rapid metabolism of the labelled compounds with recirculation of the metabolites might also have contributed to the apparent slowing of plasma clearance of the nucleotides themselves.

These data show only that stress alters in some way the handling of the labelled nucleotide by the whole animal.

Summary. Studies using C^{14}-labelled guanylic acid demonstrated a rapid disappearance of these substances from the blood of intact and adrenalectomized rats. The application of tourniquets across the hind limbs of these animals produced a significantly decreased clearance of the label from the blood after the tourniquet was removed. It is suggested that changes in liver function together with increased release of intracellular nucleotides resulting from lymphatic involution or cellular damage may act to increase the blood nucleotide pool and cause an apparent slowing of the disappearance of the C^{14} from the blood.

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Received September 21, 1965. P.S.E.B.M., 1966, v121.