0°C, under $N_2$, or in presence of metabolic inhibitors eliminated "active" transport and eliminated strontium-calcium discrimination. (5) Ca-Sr discrimination under given conditions was dependent upon a metabolically active membrane.

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## Effect of Guanylic Acid on Plasma NEFA Response to Stress.* (25739)

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A physiological role for the guanine nucleotides in the stress response is suggested by experiments in which pretreatment with these agents significantly extended the swimming time of heavily weighted normal rats(1). We have investigated the effect of guanylic acid on plasma NEFA response to stresses of fasting and tourniquet application in normal and adrenalectomized rats, since this is one metabolic parameter of stress response.

*Methods.* Male albino rats of the Sprague Dawley strain, weighing 210-230 g were used in most experiments. Male mongrel dogs were used in a few experiments. The rats were maintained on Purina rat chow and water *ad libitum* with the exception of the adrenalectomized animals which were given 0.9% saline in place of tap water. Bilateral adrenalectomy was performed under ether anaesthesia and the animals were not used until at least 7 days postoperative. The fasting period used was 16 hrs. Each experimental and control group contained 8 animals. In the first experiment, normal and adrenalectomized animals were pretreated for 7 days with a daily subc. injection of either 0.3 mg/kg or 1 mg/kg hydrocortisone and then fasted. In the second experiment, normal and adrenalectomized animals were pretreated with only one subc. injection of 1 mg/kg hydrocortisone immediately preceding fasting period. In the third experimental group, normal and adrenalectomized animals were pretreated with 10 mg/kg guanylic acid (racemic sodium guanylate) subc. immediately preceding fasting period. Plasma NEFA levels were determined at end of fasting period. In series 4, tourniquets were applied across both hind limbs just below the inguinal ligament, using a firmly fixed wire clamp. Normal and adrenalectomized animals were used. In these experiments, one group of rats was pretreated with guanylic acid (10 mg/kg) daily, subc., for 7 or 14 days prior to application of tourniquet. A second group received only one injection of the drug on day of experiment. In all cases, the tourniquet was kept in place for 5 hr and then removed. No food was available during this period nor for 10 hr after removal of tourniquet. At the end of this time blood was drawn for analysis and plasma NEFA levels determined. In all experiments blood was taken from the abdominal aorta via a plastic cannula, under ether anaesthesia. NEFA levels were determined by the method of Dole(2). Survival time of the adrenalectomized animals subjected to tourniquet stress was measured after removal of tourniquet. Blood sugar levels were measured in normal dogs at hourly intervals after 10 mg/kg guanylic acid was administered. Simultaneous blood pressure and E.C.G. measurements were made.

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TABLE I. Effect of Hydrocortisone or Guanylic Acid on 16 Hr Fasting Plasma NEFA Levels in 8 Rats/Series.

| | meq/l ± S.D. |
|---|---|
| Intact, untreated | 1.113 ± .128 |
| Adrenx, " | 1.550 ± .149 |
| Hydrocortisone treated | |
| 1 mg/kg, 1 day | .997 ± .082 |
| " , 7 days | .980 ± .107 |
| Adrenx + .3 mg/kg, 7 days | 1.739 ± .137 |
| " + 1.0 " , " | 1.006 ± .128 |
| " + 1.0 " , 1 day | .964 ± .085 |
| Guanylic acid treated 10 mg/kg, 1 day | .883 ± .149 |
| Adrenx + 10 mg/kg, 1 day | .794 ± .091 |

*Results.* Pretreatment with a physiologic dose of hydrocortisone (1 mg/kg) for either 7 days prior to the 16 hr fast or by single injection immediately preceding the fasting period, restored to normal the NEFA response of the adrenalectomized rats ($P = < .01$, Table I). The smaller dose of hydrocortisone (0.3 mg/kg) was ineffective. In the normal animal, hydrocortisone had an insignificant effect on the characteristic NEFA elevation due to fasting (Table I). Pretreatment of normal and adrenalectomized rats with guanylic acid abolished the usual rise in NEFA levels following fasting ($P = < .01$. Table I). When tourniquets were applied to normal rats, they all survived the stress but plasma NEFA levels remained elevated 10 hours after removal of the tourniquet. Either chronic or acute pretreatment with guanylic acid prevented this rise in plasma NEFA resulting from the tourniquet stress (Table II). All adrenalectomized rats ultimately succumbed to tourniquet shock but 14 days pretreatment with guanylic acid significantly extended survival time of these animals (Fig. 1). Irritability induced by the tourniquet was markedly diminished in those animals which had been treated with guanylic acid.

TABLE II. Effect of Guanylic Acid on NEFA Response of Intact Rats to Tourniquet Stress. 8 rats/series.

| Exp. and dose | NEFA, meq/l ± S.D. |
|---|---|
| Control, untreated | 1.119 ± .144 |
| Tourniquet, untreated | 1.616 ± .076 |
| " , 10 mg/kg, 7 days | .923 ± .044 |
| " , " , 1 day | 1.013 ± .091 |

In nonfasting dogs treated with guanylic acid, depression of the NEFA levels from 0.689 meq/l to 0.488 was not accompanied by a significant change in blood sugar levels. The drug had no significant effect on blood pressure or E.C.G. of the dog.

*Discussion.* The mechanism whereby stress elicits an elevation in plasma NEFA levels is not clear. Release of catechol amines as well as cortical steroids may contribute to this response. Glucose utilization, rather than blood glucose levels *per se,* seems to correlate with release of the nonesterified fatty acids. However, hypoglycemia is usually accompanied by an elevation in plasma NEFA(3). In the adrenalectomized animal, medullary catechol amines as well as cortical steroids are

EFFECT OF GUANYLIC ACID ON SURVIVAL TIME OF ADRENALECTOMIZED ANIMALS

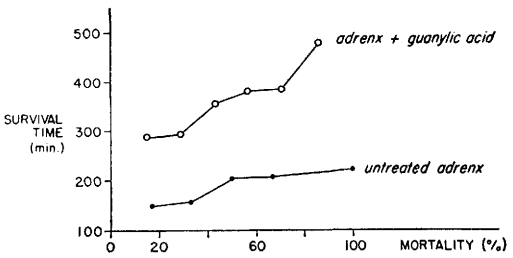


FIG. 1. Chart showing effect of guanylic acid on survival time of adrenalectomized animals subjected to tourniquet stress.

absent. Despite this, the elevation in plasma NEFA levels in stressed adrenalectomized animal is greater than in normal controls. The contribution of the sympathetic nervous system to this response has not been clarified. The hypoglycemia of the stressed adrenalectomized animal is probably a major factor. Treatment with hydrocortisone restores to normal the NEFA response to fasting in the adrenalectomized rat but has little or no effect on the intact animal when administered in physiologic amounts. The same amount of hydrocortisone will also maintain adequate blood sugar levels in the adrenalectomized preparation but has little or no effect on blood sugar of normal animals(4). Guanylic acid seems to affect plasma NEFA levels in the normal animal without a concomitant effect on blood glucose levels. Blood pressure and

E.C.G. measurements in the dog did not significantly change during the period in which the NEFA levels were falling.

Pretreatment with guanylic acid abolished the NEFA elevation seen in either fasting or tourniquet stress in the intact animal. It was more effective than hydrocortisone in preventing the NEFA rise seen in fasting adrenalectomized animals. Our preliminary data also suggest that pretreatment with guanylic acid significantly extends survival time of traumatized adrenalectomized animals. We are repeating these experiments with addition of guanylic acid injected during the stress period and the period following removal of the tourniquet since availability of this agent was presumably diminishing in the experiments reported.

The mechanism of the effects of guanine derivatives is unknown. The guanine nucleotides are known to participate in formation of the nucleic acids(5). Recent evidence indicates that they may also act as coenzymes in the system that catalyses the phosphorylation of ADP coupled to breakdown of succinyl CoA(6). In addition, guanosine triphosphate has been shown to activate the enzyme systems for fatty acid oxidation, independently of ATP. Of special interest is the finding that lymphatic tissue contains unusually high concentrations of guanine compounds. One of the invariable consequences of acute stress is release of adrenal steroids followed by involution of lymphatic tissues. It is possible that this mechanism releases nucleotides into the circulation and makes them available for protective effects on other organ systems. Once maximal involution of lymphatic tissue has occurred, additional exogenous steroids would presumably be ineffective in this regard. Additional guanine derivatives, however, might continue to contribute to increased resistance to stress. This would explain the results obtained by Kokas, *et al.*(1) in which cortical steroids had no effect on swimming time of weighted intact rats but treatment with guanine compounds increased swimming time 5-fold. We are investigating the response to other stresses under these conditions.

*Summary.* Pretreatment with hydrocortisone restores plasma NEFA response of adrenalectomized animals to normal but is without effect on intact rats. Pretreatment with guanylic acid, a purine nucleotide, abolishes NEFA response to fasting and tourniquet stress in the normal animal. It prevents elevation in plasma NEFA in the fasting adrenalectomized rats. Survival time of adrenalectomized animals subjected to tourniquet stress is significantly prolonged by pretreatment with guanylic acid. A physiologic mechanism is suggested which operates via lymphatic release of nucleotides resulting from cortical steroid activity.

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## Accumulation of Cholesterol in Inositol Deficiency. (25740)

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Inositol, an essential cellular component occurring in almost all types of cells, is an essential nutrient to various organisms such as strains of yeast, fungi, a mutant *Neurospora crassa,* mice, rats, cotton rats, hamster

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