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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and chick. However, virtually nothing is known about the exact role of inositol except that it is an important constituent of lipid (1-3). Gavin and McHenry(4) noted that inositol prevented accumulation of fat and cholesterol in liver of rats, and were confirmed by subsequent workers(5-9). The lipotropic effect of this vitamin in human beings was studied by Abel and co-workers(10-12), who showed that gastrointestinal cancers invariably led to infiltration of fat in the liver. Administration of inositol post-operatively lowered the fat content to normal levels. Though there appears to be general agreement that inositol is a lipotropic factor, there is no agreement as to the mechanism involved and limitation of lipotropic potency. This report deals with the role of inositol particularly in relation to cholesterol metabolism. The γ-isomer of hexachlorocyclohexane (gammexane) is an antimetabolite of inositol. Kirkwood and Phillips(13) showed growth inhibiting effect of γ-isomer and its reversal by inositol. Sarma(14) first observed that gammexane increased cholesterol content of tissues of rice moth larvae (*Corcyra cephalonica* strain). It was of interest to find out whether free or esterified fraction of total cholesterol was increased during gammexane feeding. The effect of gammexane feeding has therefore been studied in both *Corcyra cephalonica* and albino rats.

Material and methods. 1. *Effect of gammexane and inositol on cholesterol content of larvae, Corcyra cephalonica.* Gammexane was obtained from I.C.I., Madras. Experimental procedure was that of Sarma(14) except that gammexane was added at higher levels. Rice moth larvae were fed on 4 types of diets. Diet I was the basal diet, 2 parts wheat + 1 part potato starch. Diet II, basal diet + 4 mg % of gammexane. Diet III, basal diet + 4 mg % of gammexane + 100 mg % of inositol. Diet IV, basal diet + 100 mg % of inositol. Results are presented in Table I.

TABLE I. Effect of Gammexane and Inositol on Cholesterol Content of Rice Moth Larvae (*Corcyra cephalonica*).

mg/100 g of tissue.

Diet	Wt of 10 larvae on 25th day (mg)	Cholesterol content Free	Ester	Ratio E/F
I	250 ± 2.4	32 ± 1.1	48 ± 1.9	1.5
II	80 ± 1.2	141 ± 1.7	39 ± 1.3	.28
III	200 ± 2.3	52 ± 1.4	68 ± 1.4	1.31
IV	265 ± 3.1	34 ± .2	54 ± 1.3	1.60

Avg of 8 batches.

TABLE II. Effect of Gammexane and Inositol on Cholesterol Content of Serum of Albino Rats.

mg/100 ml serum.

Group	Cholesterol content Free	Ester	Ratio E/F
I	40.5 ± .46	76 ± 4.0	1.9
II	142.3 ± 5.4	58.6 ± 5.1	.41
III	70.0 ± 5.0	135.8 ± 9.9	1.94
IV	46.3 ± 3.3	80.2 ± 5.4	1.73

Avg of 8 values.

2. *Effect of gammexane and inositol on cholesterol content of serum of albino rats.* Rats weighing 60-70 g were divided into 4 groups of 8 rats each. They were housed in individual cages and given the following diets: Group I, casein 12%, groundnut oil 8%, salt mixture A 2%, salt mixture B 2%, Vit. B complex 1%, fat soluble vitamin 2%,‡ sucrose 72.6%, and choline 0.4%; Group II, basal diet + 1 mg "gammexane"/g diet; Group III, basal diet + 1 mg gammexane/g of diet + 4 mg inositol/g diet; Group IV, basal diet + 4 mg inositol/g of diet. Animals were fed for 4 weeks, and all received the same amount of diet. At end of fourth week the animals were killed; blood was taken by heart puncture. Serum was separated by centrifugation and analysed(15) as before for free, total, and ester cholesterol contents. Results are given in Table II.

Results. Rats which received diet containing gammexane (Group II), exhibited hyperirritability, hyperactivity, retardation of growth and mild alopecia. Some animals died and others showed no gain in weight. Addition of inositol seemed to combat slightly the growth depressant effect of γ-isomer. There is a marked increase of cholesterol in both larval tissues and serum of rats. The increase is found to be mainly in free cholesterol and the ester fraction is low, thereby bringing a marked change in ratio between ester (E) and free cholesterol (F). This effect of gammexane is almost completely reversed by inositol in the levels used. Thus inositol appears to

‡ Vitamin mixtures and salt mixture(16).

have greater influence on esterification of cholesterol.

Whether gammexane brings about inhibition of cholesterase, the enzyme system responsible for esterification of cholesterol in liver(17) and pancreas(18), in a manner reversible by excess inositol is under investigation.

Lowering of total cholesterol and increase of ester fraction has been observed by Avigan and Steinberg(19) by feeding rats with unsaturated fatty acids. Klein(20) demonstrated that serum cholesterol is preferentially esterified with poly-unsaturated fatty acids. Therefore the possible role of inositol directly or indirectly influencing the liver to bring about unsaturation of fatty acid deserves further study.

Summary. Tissues of *Corcyra cephalonica* and serum of albino rats fed a diet containing gammexane accumulated more free cholesterol with concomitant decrease in ester fraction. Inositol almost completely counteracted the effect of gammexane. Inositol thus appears to have great influence on esterification of cholesterol.

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Metabolism of Chlorpromazine: Organic-Extractable Fraction From Human Urine.* (25741)

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Despite its widespread clinical use, no detailed data are available on the metabolism of chlorpromazine in humans. Experiments with dogs have led to isolation of chlorpromazine sulfoxide from urine(1) and to renewed emphasis on sulfoxidation as a major metabolic pathway common to the phenothiazines(1,2, 3). More recently, Walkenstein and Seifter (3) have demonstrated that the side chain of promazine (and chlorpromazine) undergoes N-monodemethylation in animals. This finding would probably account for the release of $C^{14}O_2$ following administration of chlorpromazine-(N-methyl)-C^{14} to rats(4).

The present work was undertaken preparatory to a more general study on biochemical and therapeutic significance of variations in

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