and solid elements of the blood(2). It is suggested that the particular immunochemistry of an animal, insofar as it determines adsorption forces of the surfaces of cells and macromolecules, may be a factor in causing these differences. An *in vitro* and *in vivo* study of these phenomena with radioactive oxytetracycline and tetracycline is presently underway.

Comparisons have been made of the ratios of radioactive assay to microbiological assay. As might be expected, the radioactive assay has given uniformly higher calculated values in serum for oxytetracycline than the microbiological assay has indicated. The differences appear to be accounted for if one postulates inactive complexes of oxytetracycline with blood constituents (*e.g.*, amino acids, ions, proteins and protein fragments and cells) and/or rapid metabolic conversion to inactive forms by the liver or other tissues. Data in C-57 mice(2) and in Swiss mice with H^3-tetracycline(4) have indicated that high liver concentrations of oxytetracycline are achieved early; it is most probable that the antibiotic undergoes metabolic changes, including both degradation and conjugation to inactive forms. The products of these changes then return to the blood stream without significant bioactivity.

With respect to the observed advantage in serum blood levels produced by glucosamine (Tables I and II), the following mechanisms are suggested as possibilities: 1. Increased absorption. 2. Decreased elimination by bile, urine, or feces. 3. Alteration in the binding equilibria between cells, proteins, and aqueous phases of the blood. 4. Alteration in cellular permeability, in favor of extracellular spaces. 5. Alteration in liver metabolism, whereby less of the absorbed antibiotic is sequestered by this organ.

Summary. 1. A new technic for studying serum levels of oxytetracycline is presented, involving estimation of the C^{14}-oxytetracycline and derivatives thereof in serum solids by liquid scintillation counting. 2. The technic is illustrated by data from studies on the effect of glucosamine HCl on serum levels of beagle dogs and Strain A mice. 3. Enhanced serum levels were found with glucosamine HCl between 0 and 24 hours in dogs and mice. Various hypotheses, which are currently being tested, were advanced to explain this phenomenon.

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Effect of Hydrocortisone on Activation and Activity of Anaphylatoxin *in vitro.* (23970)

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The physiological importance of anaphylatoxin, a naturally occurring substance in blood of rat, guinea pig, dog, cat, horse, cow and man, has not yet been established(1). Rocha e Silva and Aronson have shown that anaphylatoxin becomes a potent histamine releaser when activated by incubation with polysaccharides such as inulin, dextran, and agar(2). The activation takes place rapidly when plasma is incubated with agar at 37°C; time and temperature curves have been determined by Rothschild and Rocha e Silva (3). Anaphylatoxin preparations produce contractions of isolated guinea pig ileum, attributable to histamine release because they are blocked by antihistaminic drugs(3). Fur-

TABLE I. Effect of Hydrocortisone on Activation of Anaphylatoxin *In Vitro.*

Conc. hydrocortisone	Inhibition
2.5 mg/ml	11/11
1.25	18/24
.25	0/2

thermore, as isolated ileum is treated repeatedly with anaphylatoxin, tachyphylaxis soon takes place believed to result from exhaustion of the store of bound histamine in muscle, since sensitivity of muscle to histamine remains unimpaired.

Mucopolysaccharides of the ground substance and synovial fluid have for some time been implicated (by association) with the development of inflammatory reactions. Activation of anaphylatoxin by polysaccharides *in vitro* has led Rocha e Silva to suspect mucopolysaccharides as potential activators *in vivo* of anaphylatoxin(1). To approach the problem of inflammatory reactions, it was of interest to study the effects of hydrocortisone on activation and activity of anaphylatoxin *in vitro.*

Methods. Blood was collected from Sprague-Dawley rats under light ether anesthesia by cardiac puncture using heparin, 0.1 mg/ml of blood, as anti-coagulant. Plasma anaphylatoxin was assayed *in vitro* on guinea pig terminal ileum. The muscle was suspended in 4 ml oxygenated Tyrode's solution at 30°C, and muscle contractions recorded on a kymograph. One minute elapsed after chamber was thoroughly washed with Tyrode's solution, before any new substance was introduced for test. *Exp. 1.* Separate aliquots of rat plasma were treated as follows: 1) diluted 1:1 with saline as control; 2) activated by mixing 1:1 with 0.1% agar solution; 3) activated with agar in presence of hydrocortisone-hemisuccinate or equivalent amount of neutralized succinic acid; 4) mixed with hydrocortisone-hemisuccinate or neutralized succinic acid, without agar, as another control. The mixtures were incubated 10 minutes at 37°C, then 0.01-0.1 ml were transferred to tissue bath and tested on isolated ileum. Samples to be compared were tested in the order A,B,C,D, A to ascertain whether a similar muscular response could be obtained with A after exposure to the other substances. Allowing for any decrease in responsiveness of muscle, it was determined whether A,B,C, and D were comparable in activity. *Exp. 2.* Anaphylatoxin was activated with agar and assayed *in vitro* on guinea pig ileum as in Exp. 1. Hydrocortisone-hemisuccinate or equivalent neutralized succinic acid was introduced directly into tissue bath immediately before the activated plasma, to determine effects on activity of anaphylatoxin *in vitro.* Trials in a series were rotated as in Exp. 1 to allow for tachyphylaxis of the isolated ileum.

Results. Exp. 1, effect of hydrocortisone on activation of anaphylatoxin in vitro. Anaphylatoxin activated by agar in the presence of 2.5 mg/ml hydrocortisone (as the hemisuccinate) was only half as active as anaphylatoxin activated with agar alone. In 18 of 24 trials with 1.25 mg hydrocortisone/ml, activation of anaphylatoxin was inhibited (Table I). Activity of hydrocortisone-hemisuccinate was not attributable to the succinate since equivalent concentrations of succinate alone did not produce inhibition in Exp. 1 or 2.

Exp. 2. Effect of hydrocortisone on activity of anaphylatoxin in vitro. At 1.25 mg/ml in the tissue bath, hydrocortisone clearly inhibited response of ileum to activated anaphylatoxin (Table II). Responses of isolated ileum to exogenous histamine, acetylcholine, and bradykinin were also inhibited by 1.25 mg hydrocortisone/ml in the tissue bath (Table III). Inhibition of muscle response was only temporary since the muscle responded to these agents as soon as hydrocortisone was washed out of bath.

In another experiment blood levels of anaphylatoxin were studied. Pretreatment of rats with 10 mg hydrocortisone acetate 24 and 4 hours prior to bleeding did not decrease anaphylatoxin activity in 6 of 8 trials. Ana-

TABLE II. Effect of Hydrocortisone in Tissue Bath on Activity of Anaphylatoxin *In Vitro.*

Conc. hydrocortisone	Inhibition
1.25 mg/ml	7/7
.625	3/7
.25	0/3
.125	0/2

TABLE III. Effect of Hydrocortisone in Tissue Bath on Activity of Exogenous Histamine, Acetylcholine, and Bradykinin *In Vitro*.

Substance	Conc. hydrocortisone	Inhibition
Histamine	1.25 mg/ml	13/13
	.625	4/6
	.25	4/4
	.125	1/5
Acetylcholine	1.25	9/12
	.625	6/8
	.125	0/4
Bradykinin	1.25	7/7
	.625	7/7
	.125	0/6

phylatoxin levels in rats bearing pneumodermal pouch inflammations(4) for 7 to 10 days were the same as in normal animals, in 6 of 7 comparisons.

Discussion. Haining found that sodium salicylate did not inhibit activation of anaphylatoxin *in vitro*(5). Exp. 1 demonstrated, however, that hydrocortisone in the incubation medium inhibited activation of anaphylatoxin. The pH of Tyrode's solution was not changed by the hydrocortisone, and calculations showed that the ionic strength was increased less than 5%, which would not explain the inhibition, since Rothschild and Rocha e Silva have reported that increases in ionic strength of the order of 100% are required to inhibit activation of anaphylatoxin (3).

In the second experiment the mechanism by which hydrocortisone inhibited the response of the isolated muscle to anaphylatoxin was not established unequivocally. As hydrocortisone did not inhibit the tachyphylaxis produced by anaphylatoxin alone, histamine release was not blocked; therefore hydrocortisone must have inhibited the muscle response to liberated histamine. Since acetylcholine, bradykinin, and histamine were all inhibited by hydrocortisone, the most likely mechanism for hydrocortisone would appear to be a non-specific desensitization of the muscle rather than by blockade of histamine receptor sites. Lefcoe has reported that comparable concentrations of hydrocortisone inhibit the responses of guinea pig tracheal muscle to histamine and cat tracheal muscle to acetylcholine(6).

Haining demonstrated that 4 mg sodium salicylate/ml inhibited the action of anaphylatoxin on isolated guinea pig ileum(5). Sodium salicylate prevented histamine release, however, since repeated anaphylatoxin treatments in the presence of salicylate did not produce tachyphylaxis.

In our experiments, hydrocortisone inhibited both activation of anaphylatoxin *in vitro* and response of isolated muscle to it. Hydrocortisone probably does not influence the inflammatory process by affecting activation or activity of anaphylatoxin *in vivo* since concentrations of hydrocortisone that were active *in vitro* were at least 1000 times greater than the levels of 11-oxygenated steroids usually quoted for peripheral blood(7,8). Furthermore, neither inflammation in the pneumodermal pouch nor administration of hydrocortisone to normal rats changed circulating levels of anaphylatoxin. These considerations suggest that anaphylatoxin does not play an important role in inflammation. Local alterations in the mucopolysaccharides at the site of inflammation, which could not be simulated in these experiments, still might regulate the process of inflammation *via* anaphylatoxin; but the role of anaphylatoxin in the inflammatory process remains to be demonstrated.

Summary. Effects of hydrocortisone on activation of and response to anaphylatoxin were studied using isolated guinea pig ileum. Hydrocortisone at 1.25 mg/ml inhibited activation of anaphylatoxin *in vitro*. At the same concentration in the tissue bath it also inhibited effects of activated anaphylatoxin on isolated guinea pig ileum. This inhibition was attributed to a non-specific desensitization of the muscle rather than to direct inhibition of anaphylatoxin or histamine release.

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Effect of Low Sodium Diet on Sodium Content and Radiosodium Exchange in Rat Bone.* (23971)

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Skeletal tissues are rich in sodium, a part of which readily exchanges with radiosodium. In recent years a number of workers have been interested in studying the degree of lability of this skeletal component under a variety of experimental situations. Among these is the effect of a low sodium diet.

Orent-Keiles, Robinson and McCollum(1) noted that the bones of rats reared on a low Na diet were soft in consistency. Bergstrom and Wallace(2) found that the bones of young rats kept on a low Na diet for 2 weeks contained only half as much Na as the controls. More recently Munro, Satoskar and Wilson (3) in a study of adult rats observed a decrease of 10% in bone Na content during a 14 week period of low Na feeding. The latter workers also determined radiosodium exchangeability in the bones of their animals, and finding no change concluded that the Na lost from bone came from the non-exchangeable fraction.

The present study was undertaken to investigate this problem.

Experimental. Twelve 20-day-old male albino rats of the Rochester strain were fed Big Red dog pellets and tap water for 10 days, at which time they were divided into 2 equal groups according to a table of random numbers. The flip of a coin then decided which group was to receive the low Na diet. The diet was constructed by blending Crisco 300 g, Lonalac 600 g, rolled oats 2070 g, brewer's yeast 30 g, $CaCl_2$ 12 g, $CaHPO_4 \cdot 2H_2O$ 11.4 g, and polyvisol 1.8 cc. On analysis it was found to contain 0.011% Na, 0.7% Cl, 0.42% K and 0.44% Ca. For the control group 60 g of $NaHCO_3$ was added, bringing the Na concentration to 0.55%. Distilled water was provided for drinking.

Since preliminary trials had indicated that food consumption was poor on the low Na diet, it was decided to pair-feed the 2 groups. Records of food consumption were kept, and the control group offered only as much food as the average consumed by the low Na group.

The experimental period lasted 4 weeks. The animals were then given radiosodium^{22} (2.5 μc/100 g) by intraperitoneal injection, and 2½ to 4 hours later killed by decapitation. The long bones were dissected free of adherent muscle and periosteum, the ends clipped off, and the marrow removed. Bone water was determined by drying *in vacuo* at 70°C. The dried samples were then ashed at 525°C and the ash taken up in a small amount of 2 N HCl. Sodium content was determined in this ash solution by means of a Baird internal standard flame photometer equipped with a Na III-Li II filter and a manufactured gas burner, with natural gas as a fuel. Using this apparatus we found that interfering substances present in bone produce a small, and constant, error in sodium readings over a concentration range of 0.1-0.4 meq/l. Serum sodium was determined by flame photometry, and chloride by Volhard titration. Radiosodium was determined in a scintillation counter.

In view of the uncertainty regarding the position of Cl in bone, the total Na con-

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