Radioactive Oxytetracycline (Terramycin*). III. Effect of Glucosamine HCl on Serum Concentrations.† (23969)

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Biological labelling of oxytetracycline by 2-C^{14} acetic acid has been reported(1). The need for such material to determine physiological handling and distribution had been felt for some time. Preliminary studies on its distribution in mice have been reported(2). One of the important questions that such a material might help resolve was the question of antibiotic blood levels—a problem difficult to attack with certainty by methods depending solely upon microbiological assay. The multiplicity of factors which might interfere with bioassay was realized, but no satisfactory method for eliminating or neutralizing them has been devised. Such factors include subtle interferences with the test due to antagonistic effects, chelation, serum protein binding, and adsorption by cellular elements of the blood. Extraction technics are always open to some doubt, especially with regard to completeness of extraction and dangers of biological inactivation of the antibiotic during the extraction process. Furthermore, quantitative extraction of blood can offer difficulties at the level of blood concentrations shown by most antibiotics. Hence, the importance of an independent assay method, such as isotopically labelled antibiotics afford, was not overlooked. Use of radioactive antibiotics in estimation of blood level can offer data which resolves many problems encountered in biological assay. Its precision and freedom from interferences of the kind that plague bioassay are great advantages. However, there are problems, too, especially those of relation between bioactivity and radioactivity at various time periods. Some of these will be discussed below.

Materials and procedures. Radioactive C^{14} oxytetracycline. The free base used contained 263 d/m/μg. For administration to mice and dogs, it was dissolved in 0.01 N HCl. Sufficient 0.1 N HCl was added to produce complete solution. *Additives.* Glucosamine HCl tested for effects on serum level was dissolved with the antibiotic prior to administration. *Dosage.* Dogs were administered 1-2 ml of the radioactive solution by stomach tube at a dosage level of 10 mg/kg. Dosage in mice (administered by blunt hypodermic needle to the stomach) was 250 mg/kg. Male beagles weighing 8 to 13 kg each were used. The mice were inbred (brother-sister matings) Strain A produced in our laboratory from stock obtained from Jackson Memorial Laboratory. Male animals weighing 18-20 g were used. *Procedure.* 0.5 ml or more of whole blood was centrifuged; a measured volume of serum was removed into weighed 10 ml ampoules, and freeze-dried overnight at 200 μ. The ampoule was weighed, and the mg/ml solids were calculated by difference. Five to 10 mg of the solids were then weighed and evenly suspended in vials containing 20 ml of a homogeneous suspension of Thixcin (2.5%) in a toluene solution of 0.25% 2,5-diphenyloxazole(5), the background of which had been previously determined by liquid scintillation counting in a Tri-Carb Scintillation Spectrometer. After 4-6 ten minute counts had been taken on each sample, 200 μl of a toluene solution of standard benzoic acid containing 2674 d/m were added to each vial, and the counting efficiency of the vial and contents determined. The net counts on the sample were then evaluated by subtracting background; the net count was corrected to net d/m by application of the individual efficiency factor. This value was converted to d/m/mg by dividing d/m by the weight of sample counted, and converted to d/m/ml by multiplying by number of mg/cc of serum. Dividing this value by specific activity of the radioactive oxytetracycline produced a calculated value for the μg/ml shown in the Tables.

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TABLE I. Effect of Glucosamine HCl on Serum Levels of C^{14}-Oxytetracycline.

Formulation	Dog	Time (hr) 0	.5	1	24
		μg/ml			
C^{14}-oxytetracycline HCl	1	.0	.381	.780	.378
	2	.0	.309	.701	.427
	3	.0	.568	.353	.231
	4	.0	.618	4.48	.225
	Avg	.00	.469	1.58	.315
Idem + glucosamine HCl (1:1)	6	.0	1.80	.978	.478
	8	.0	.611	4.03	.755
	9	.0	1.06	8.32	.734
	10	.0	.878	1.13	1.05
	Avg	.00	1.087	3.61	.754
Ratio 2/1 (avg)		—	2.32	2.24	2.39

Results. Table I presents results from an experiment designed to determine the effect of glucosamine HCl on rate of serum level increase and time of retention of radioactivity. It is apparent from the ratios of the averages that glucosamine HCl produced higher serum levels within one hour, and that these higher levels persisted at 24 hours (oxytetracycline or degradation products).

Table II indicates the data of a serum level study performed in inbred Strain A mice. These mice were chosen because of their excellent uniformity in other tests, *e.g.*, phagocytosis(6). These data indicate a serum level in favor of the glucosamine-oxytetracycline combination at every period of time assayed between 5 minutes and 24 hours following oral administration. These data also illustrate a trend which we believe to be significant in mice, *i.e.*, a decrease in serum levels between 15 minutes and 4 hours. This phenomenon is presently under detailed study.

Discussion. The radioactive oxytetracycline used in these studies is an example of a compound which is labelled non-uniformly during its production from acetate precursor by *S. rimosus*(1). The preparation used here contained about 200 d/m/μg. By increasing the size of sample that could be counted (from as little as fractional milligrams up to several hundred milligrams) with good efficiency (30-46%) through liquid scintillation counting, as little as 0.05 μg in as much as 5 cc of serum could be determined with good precision (± 10%) within reasonable lengths of time. The excellent stability characteristics of the Tri-Carb Liquid Scintillation Spectrometer could be extended even further than was attempted here.

TABLE II. Effect of Glucosamine HCl on C^{14}-Oxytetracycline Serum Level in Strain A Mice.*

Time	C^{14}-oxytetracycline d/m/ml	μg/ml	C^{14}-oxytetracycline HCl + glucosamine HCl (1:1) d/m/ml	μg/ml
5 min.	972	3.70	1475	5.60
15	933	3.55	2050	7.80
30	1195	4.55	1432	5.44
60	641	2.44	703	2.67
120	954	3.63	1005	3.82
240	885	3.36	1135	4.31
24 hr	421	1.56	563	2.14

* Each figure is avg of 2 mice.

One of the advantages of the non-uniformly labelled C^{14}-oxytetracycline over 7-tritio tetracycline, for example, is that one stands a better chance of picking up more biologically inactive metabolites of the antibiotic(3). Since the compound is labelled in the nucleus with carbon, it is not subject to losing its label by peripheral attack, such as a simple dehydrogenation of a tritium-labelled compound might accomplish.

One of the striking results of this study was to emphasize once again the tremendous individual differences with regard to serum levels shown by different individual animals (Table I). Since the radiochemical test is subject to greater precision than the microbiological test, one can now say more definitely that there are differences of this magnitude in the sera. However, the magnitude of the differences may arise not only from differences in absorption rate, but also by differences in binding equilibria between serum

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-