normal as well as in hyper- and hypothyroid rats. Higher percentage of liver glycogen and lower blood sugar was found in animals fed with hydrolyzed GCA, as compared to their appropriate controls.

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Urinary Excretion of Acid Mucopolysaccharides by Rabbits Injected with Chelating Agents and with Chondroitin Sulfate.*† (25596)

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Of recent years there has been interest in treatment of scleroderma by chelation(1-4). The therapeutic efficacy of this approach is yet to be established. However the possibility of connective tissue being changed by chelators is intriguing. The present pilot study was undertaken in rabbits prior to studying humans to see if a change in excretion of acid mucopolysaccharides in urine could be detected following administration of chelating agents. Bryant, Leder and Stetten(5) have shown quantitation of urinary acid mucopolysaccharides to be a potentially rewarding approach to reflection of experimental alteration of connective tissue in rabbits. as demonstrated by their data on urinary levels following injection of papain intravenously. Their observations convey the implicit assumption that proteolytic enzyme as administered could affect connective tissue extravascularly and that chondroitin sulfate presumably released from protein binding *in situ* could then pass intravascularly to the kidneys where it could be excreted at least in part in the urine. In the present study it is shown that chondroitin sulfate† injected in large amounts intravenously into rabbits resulted in excretion of a small proportion but nonetheless a large amount of chondroitin sulfate in urine. When chelators‡ were administered extravascularly to rabbits, a transient and frequently mild rise in urinary excretion of acid mucopolysaccharides was noted; time of onset and degree and

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† Commercial chondroitin sulfate (General Biochemicals) of cartilaginous source, containing 15 to 20% protein.

‡ Disodium EDTA, with and without calcium furnished kindly as Endrate by Abbott Research Labs., North Chicago, Ill.

duration of rise was highly variable from animal to animal.

Method. Healthy male albino rabbits were used. Weights varied from 1.9 to 2.7 kg at time of injection, except the 2 animals receiving repeated injections of chondroitin sulfate, which weighed 4 kg. Baseline 24 hour urine collections were made under toluene on 7-11 days during 10-14 days preceding injection. I. Four rabbits received a single intravenous injection of 1000 mg of chondroitin sulfate in 5 ml saline. 24-hour urine collections continued thereafter. At 48 and 72 hours after injection, 2 rabbits received repeated intravenous injections of chondroitin sulfate, 1000 mg. II. Three rabbits§ received a series of 3 subcutaneous injections of Disodium Endrate, Abbott, 500 mg in 5 ml‖ daily on successive days, with production of an intense and hemorrhagic local reaction sometimes with necrosis. III. Five rabbits§ (Group III A) received 3-10 successive daily subcutaneous injections of Disodium Calcium Endrate, 500 mg in 5 ml,‖ without apparent local reaction. Two rabbits (Group III B) received similar injections daily for 10 days and simultaneously daily intramuscular injections of cortisone (Cortone Acetate, Sharp & Dohme), 12.5 mg in 0.5 ml for 10 days, for suppression of any inapparent inflammatory reaction. In all instances 24 hour urine collections were continued for about 15 days following completion of injections. Urine samples were held under toluene at 5°C until used for analysis. Acid mucopolysaccharides were recovered from duplicate 3-5 ml aliquots of urine by modification of the method of DiFerrante(6). After overnight dialysis against running tap water and precipitation with cetyltrimethylammonium bromide at pH 5.0, the centrifuged acid mucopolysaccharide complex was resuspended in a small portion of the supernate and dissociated from the quaternary amine with an equal volume of salt-saturated ethanol. The acid mucopolysaccharides were then reprecipitated with 4 volumes of ethanol and dissolved in demineralized water for measurement of hexuronic acid content by the carbazole method of Dische (7). Standard curves and criteria for acceptance were as defined previously(8). Repeated recoveries of 76-89% of 200 μg of chondroitin sulfate added to the original urine aliquot were demonstrated.

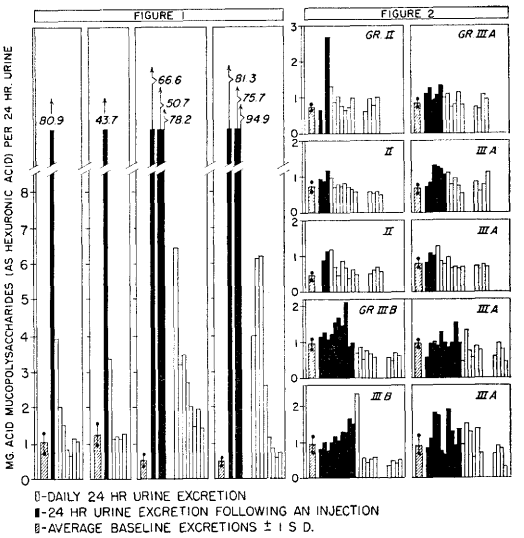

FIG. 1. 24-hr urinary excretions of acid mucopolysaccharides in rabbits (Group I) inj. intrav. with chondroitin sulfate.

FIG. 2. 24-hr urinary excretions of acid mucopolysaccharides in rabbits inj. subcut. (Group II) with Disodium Endrate (Abbott), (Group III A) with Disodium Calcium Endrate (Abbott), and (Group III B) with Disodium Calcium Endrate (Abbott) and Cortone Acetate (Sharp & Dohme).

Results. The acid mucopolysaccharides recovered were entirely similar in metachromatic and chromatographic characteristics to those described earlier(8) in human urine; extensive qualitative characterization has been deferred.

Fig. I records excretion of acid mucopolysaccharides in urine following single and repeated injections of 1000 mg of chondroitin sulfate intravenously. Total excretions of acid mucopolysaccharides during the 2-4 days required to return to baseline levels following a single injection of chondroitin sulfate indicated excretion of the order of magnitude of 5-10% of the injected amount. The sharp

§ Some animals had received Disodium Endrate and/or cortisone in a limited series of observations about 3 weeks prior to present studies. Baseline collections recorded here were made in 10 days immediately preceding present injections.

‖ Without further 200 X dilution recommended by Abbott Research Labs. for intravenous administration to humans.

drop in excretion during 24-48 hour period following first injection in the 2 rabbits receiving 3 repeated injections indicated the probability of a similar pattern of excretion in these animals. On completion of the 3 injections in these rabbits, however, increased excretion was maintained in some degree during entire 12-day period of continued observation.

Fig. II summarizes data in animals receiving Disodium Endrate and Disodium Calcium Endrate subcutaneously. A subsequent increase in excretion of acid mucopolysaccharides was noted at some time in all 10 rabbits thereafter, but onset and degree and duration of apparent response varied greatly from animal to animal. There was no significant difference in this response in the various groups of rabbits despite the fact that Group II (Disodium Endrate) exhibited intense local tissue injury, Group III A (Disodium Calcium Endrate) showed no apparent local reaction, and Group III B additionally received cortisone for suppression of any inapparent inflammatory reaction. All animals were handled equally on all injection and weighing days.

Daily volumes and specific gravity of urines were the only other parameters measured. These did not correlate in any way with levels of acid mucopolysaccharide excretion.

Discussion. The sharp rise in urinary excretion of acid mucopolysaccharides following intravenous injection of chondroitin sulfate into rabbits confirms the assumption of Bryant, Leder and Stetten(5) that a similar rise noted by them following intravenous injection of papain could have been caused by elevated level of chondroitin sulfate in the circulating blood of their rabbits. A comparison of quantitative data in the 2 studies further strengthens this assumption. In the present study, the general order of magnitude of excreted chondroitin sulfate in urine was 5-10% of total amount injected. In the previous study(5), serum levels rose by about 700-1100 μg/ml serum in rabbits weighing 800-1000 g and hence with circulating serum volume of perhaps 30-40 ml, the total rise in acid mucopolysaccharides therefore reflecting appearance in total circulating blood of an amount in the order of magnitude of 20-45 mg. Average increase in urinary excretion noted thereafter by the authors was 3.3 mg. Thus the percent excretion in their studies would appear to have been of the same order of magnitude as noted in the present studies where chondroitin sulfate was introduced directly into the blood stream. The fate of the remaining 90% was not determined. Following 3 successive injections of chondroitin sulfate, urinary excretion no longer returned promptly to the baseline in 3-5 days as after a single injection but was still somewhat elevated when urine collections were discontinued 12 days after the third injection. Levels were still 5-fold greater than baseline levels one week after the third injection.¶

The observation that about 5 to 10% of chondroitin sulfate introduced into the circulating blood of rabbits appeared in the urine does not warrant the assumption that elevated urine levels necessarily reflect an increased blood level of acid mucopolysaccharides. Indeed in humans nothing is yet reported regarding the fate of acid mucopolysaccharides introduced into the blood stream, other than as is apparent that urinary excretions rise sharply in patients receiving heparin. Jaques, Napke and Levy(10) present the protocol on one human subject given heparin intravenously. This individual showed total metachromatic activity in urine equivalent to 5% of amount injected.

Introduction of chelating agents subcutaneously in rabbits was followed by a transient and frequently mild increase in urinary excretion of acid mucopolysaccharides. Although levels of acid mucopolysaccharides in human plasma have been shown to rise nonspecifically in the presence of inflammation (11), the increased urinary excretion observed here in rabbits did not correlate grossly with degree of apparent local tissue injury

¶ One of each pair of animals was sacrificed at 3 and 7 weeks, respectively, following either the last or only injection of chondroitin sulfate. Histologic examination of kidneys and other tissues, using the acridine orange fluorescent technic of Hicks and Matthaei(9), failed to reveal any unusual accumulations of fluorescent material. Cartilage from the same animals fluoresced brilliantly, as described for acid mucopolysaccharides in(9).

which ranged from obvious intense injury with necrosis to no apparent inflammatory reaction and suppression of any inapparent inflammation by adrenocorticosteroids. Thus the possibility must be entertained that increased excretion was related to introduction of chelating agents. The observation warrants extension of studies to humans where use of chelators may offer an additional experimental approach to a study of factors influencing urinary levels of acid mucopolysaccharides in humans. The mechanism by which chelators may have induced an increased level of excretion in rabbits is not known. Whether the same observation of increased excretions holds in humans will be investigated.

Summary. (1) Introduction of chondroitin sulfate into the blood stream of rabbits was followed by elevated excretion of acid mucopolysaccharides in urine totalling 5-10% of injected amount over 2-4 days thereafter. Repeated injections were followed by excretions which were still 5-fold greater than baseline levels one week after third and last injection. (2) Repeated subcutaneous injection of disodium EDTA and disodium calcium EDTA into rabbits was followed by a brief and frequently mild rise in urinary excretion of acid mucopolysaccharides; onset, degree and duration of rise varied widely from animal to animal.

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Radiation Avoidance in the Mouse. (25597)

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It is commonly stated that animals are incapable of detecting high energy radiation with the unaided senses but there is some evidence that it can be sensed under certain circumstances. Two individuals who received a substantial beta particle dose to the hands reported a local "tingling sensation" during exposure. Another individual receiving several thousand rads from an electron beam experienced a burning sensation although there was probably no temperature rise greater than 0.05°C. In animals relatively small doses of penetrating radiation have produced later avoidance behavior(1) in cats, rats, and mice. A direct radiation avoidance has been reported(2) in albino rats at dose rates of 1-4 r/minute after a total dose of 360 r had been delivered. Our experiments were designed to determine whether mice were capable of sensing the presence of an X-ray beam, and if so, whether it was recognized as something sufficiently unpleasant to be avoided.

Methods. During each run the test animal was in a plastic cage 25 x 9 x 6 cm pierced with many small holes to permit free air circulation. This cage was mounted on a knife