Human Metabolism of Orally Ingested Glycyrrhetinic Acid and Monoammonium Glycyrrhizinate.*† (25207)

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Triterpene glycyrrhetinic acid (G.A.) and the ammonium salt of its diglucuronide, monoammonium glycyrrhizinate (MAG) have been studied in view of their reported adrenocortical-like activity effects on electrolyte metabolism(1,2,3,4). Other authors found G.A. had potential anti-inflammatory properties(5,6,7) and blocked *in vitro* the metabolism of certain steroids(8). Since there are no known chemical methods of determining G.A. or its metabolites, and since we have previously shown (9) that totally adrenalectomized human subjects, maintained solely on MAG, excreted significant amounts of steroid-like materials in urine, as indicated by formation of Porter Silber (phenylhydrazone) chromogens, we studied the *in vivo* metabolism of G.A. and MAG randomly labelled with tritium.

Methods, apparatus and materials. Both MAG and G.A.‡ were labeled with tritium by the New England Nuclear Corp., following which they were recrystallized to constant specific activity (15.7 μc/mg and 63.6 μc/mg respectively. All countings were done using a Packard Tri-Carb liquid scintillation counter. Complete urine collections from subjects receiving labeled compounds were pooled, concentrated *in vacuo* to about 1/20th original volume, and extracted with ethyl acetate to give the "free" fraction. Urines were then brought to pH 11 and exhaustively extracted with n-butanol to remove the "conjugates." Butanol was removed *in vacuo,* the residue taken up in pH 4.5 acetate buffer and incubated with beta-glucuronidase (Ketodase, Warner-Chilcott), then extraction with ethyl acetate was repeated to remove materials formerly present as glucuronic-acid conjugates. Residual aqueous phase was brought to pH 0.4 with sulfuric acid and allowed to stand 2 days at 37°C to bring about further hydrolysis, and similarly extracted. Radioassay of aliquots of all organic extracts, as well as all aqueous phases was carried out. Fecal samples were extracted twice by homogenizing with alcohol in a Waring Blendor, and filtered. (Soxhlet extraction of dry residue remaining after second hemogenization gave negligible additional activity.) The column chromatographic system used, both on urinary and fecal extracts, was a modification of that described by Van Katwijk and Huis in't Veld (10), in which the crude material is placed on a florisil column in benzene solution and eluted with increasing concentrations of alcohol in benzene. In all cases, when counting aqueous samples (blood and bile) or highly colored extracts of organic solvents (from urine and feces), known amounts of tritium standard were added to the samples after counting to obtain a correction for the quenching caused by color and/or solvent.

Results. Values of urinary excretion of radioactivity following oral administration of labeled MAG and G.A. to 4 human subjects are shown in Table I. Urines of female subjects, subsequently concentrated and used for isolation studies, showed the same degree of activity both before and after concentration, thus eliminating the possibility of large-scale formation of H^3OH which would have been lost in the distillate upon concentration. Excretion of a very minute percentage of administered radioactivity in urines was confirmed by results with normal male subjects, and, in addition, presence of practically all radioactivity in feces within 48 hours following ingestion of tagged compounds was conclusively demonstrated. Purification of the fecal extract of the male subject,

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TABLE I. Metabolic Fate of H^3-Labeled MAG and G.A.

Age	Sex	Diagnosis	Amt/day MAG	Amt/day G.A.	Duration, days	% adm. activity found in Urine	% adm. activity found in Feces
48	♀	Breast cancer—adrenalectomized	*1 mC H^3		5	.3	
50	♀	11 yr Addison's disease		†1 mC H^3	5	.4	
35	♂	Normal	*1 mC H^3		1	.8	98
40	♂	"		†2 mC H^3	1	.4	"

* Plus 4 g carrier. † Plus 3 g carrier.

fed G.A., resulted in isolation of a small quantity of radioactive, crystalline material whose infrared spectrum was the same as that of the administered compound (Fig. 1). This shows that at least a portion of G.A. is excreted unchanged in the feces. A complete characterization of all radioactive fractions has not yet been accomplished.

The next problem was to determine whether MAG and G.A., under these conditions, pass directly through the GI tract without being absorbed (except to a very small degree) or whether they are absorbed into the blood and later excreted via the bile. To answer this question, 5 normal human subjects were fed the labeled compounds and radioassays were performed on simultaneously collected blood and urine samples, and, in 2 of these subjects, on bile. The results (Table II) show that values are all 1% or less of total dose, so that absorption into the blood and re-excretion via the bile may be dismissed as a major metabolic pathway of these ingested materials.

Finally, we were able to isolate and identify by infrared spectroscopy pure glycyrrhetinic acid from the "free" urine fractions of both female subjects, who were fed G.A. and MAG (Table I). Thus it is apparent that the carbohydrate moiety of MAG is hydrolyzed in the body to give the free G.A. as an excretion product. The infrared spectra of these urinary isolation products are the same as those of authentic G.A. and the fecal

TABLE II. Urine, Blood and Bile Levels of H^3-Labeled MAG and G.A.

Age	Sex	Adm. cpd.	% excreted in urine in 4 hr	% found in blood at 4 hr*	% excreted in bile in 4 hr
30	♂	1 mC MAG	.6	.09	
54	♂	*Idem*	.4	.0	
40	♂	"	.3	.2	.0
27	♂	2 mC G.A.	.2	1.2	
70	♀	*Idem*	.05	.4	.15

* Assumed blood vol = 4.2 l and HCT = 46%.

product shown in Fig. 1. These compounds isolated from urine do not, however, comprise the entire amount of radioactive material excreted in urine. Other radioactive "free" fractions are present which show different column chromatographic characteristics, so it is assumed that these represent other compounds

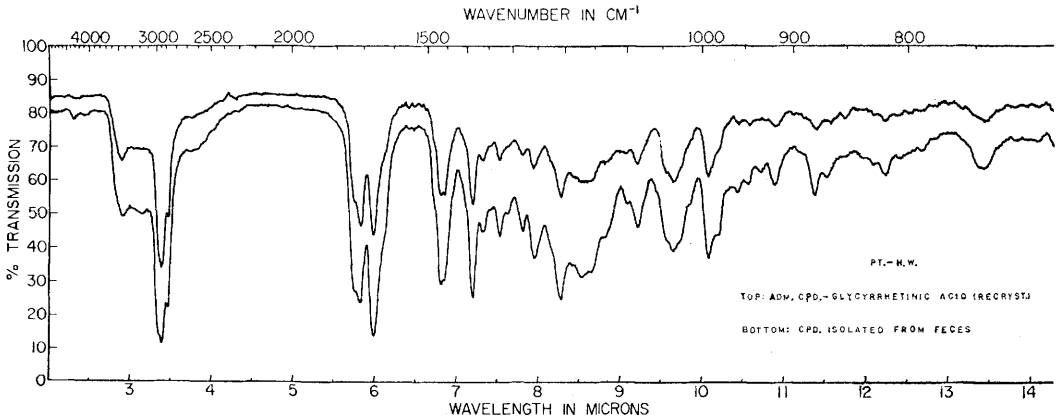

FIG. 1. Infra-red spectra in KBr of glycyrrhetinic acid, administered and isolated from feces.

not yet identified. In addition, identity of the conjugated material is unknown.

Discussion. Throughout the literature dealing with the physiological effects of G.A. and its glucuronide derivative MAG, it was necessary to give large oral doses each day for these effects to become manifest, and parenteral administration has not been feasible in view of their high degree of insolubility in aqueous media. From our results, it seems likely that the necessity for such large oral doses is due to the fact that MAG and G.A., when orally administered, are very poorly absorbed, so that oral doses of 3 to 4 g/day are probably necessary to result in very small circulating levels of parent compounds or their metabolites. Thus the actual circulating "effective amounts" of these compounds or their metabolites may be as small as those of many active steroids. If so, the problem may resolve itself into one of availability rather than one of physiological effectiveness. Variations among several G.A. samples in this regard, as reflected in greater or lesser degrees of absorption, may be related to the stero characteristics of the molecule, as discussed below. However, in view of the wide range of biological activities of MAG and G.A. demonstrated by many researchers, further studies on more efficient modes of administration and/or changing the molecule so that it may be more easily absorbed when given orally would seem to be indicated.

Unfortunately, when dealing with G.A. at the present time, one is faced with a lack of definite knowledge regarding the various stereoisomeric forms under which this compound may exist. And, since it can exist theoretically as many isomers, it is possible that these may possess different physiological as well as physical properties. This could explain the contradictory reports in the literature regarding effectiveness of G.A. in performing certain physiological functions, since the investigators often used material commercially sold by various sources as "glycyrrhetinic acid" or "Monoammonium Glycyrrhizinate," and failed to characterize their compounds physically. Thus the G.A. used in one laboratory may not have been at all the same as that used in another.

The G.A. used by us is likewise of uncertain stereoisomeric structure. Its infrared spectrum is identical with that of a commercial sample designated as beta G.A.§ as well as with that of an experimental sample of 18-beta G.A.‖ On the other hand, the specific rotation of our material is lower than that of the other 2 samples by 50 degrees, and its solubility in alcohol and chloroform is less. It is not 18-alpha G.A., since it is different in all properties, including infrared absorption, from a commercial sample of alpha G.A.§, which is probably the same as the 18-alpha compound of Beaton and Spring(11). Our compound has been purified by recrystallization, as well as by charcoal treatment of the Na salt, followed by precipitation of the free acid, and we have been unable to produce any significant change in either its infrared spectrum or specific rotation. The same procedures were carried out on both the commercial alpha and beta samples, again without change of characteristics. It may therefore be that our compound is beta oriented at the same position as are the others, but differs from them in some other position which does not cause a change in absorbance of infrared light but which affects the specific optical rotation. What this position might be we do not know.

Summary. Orally administered glycyrrhetinic acid and monoammonium glycyrrhizinate were poorly absorbed from the gastro-intestinal tract as indicated by blood, bile and urine levels of radioactivity. The bulk of fed materials are excreted directly in the feces, and, in the case of G.A., at least in part as unchanged compound. Small quantities of glycyrrhetinic acid have been isolated from urines of subjects fed each of above compounds. Many problems relating to stereoisomerism of G.A. remain to be clarified.

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Bone Sodium and Na^{22} Exchange: Relation to Water Content.* (25208)

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Reported values for sodium content of bone and for radiosodium exchangeability have shown considerable variation. Part of this variability is the result of age effects(1,2,3). for sodium content increases and exchangeability declines as animals become older. There still remains to be explained the variations noted among adult animals and men(1, 3,4,5). This report presents data on sodium content and radiosodium exchangeability for normal bone in a number of species, and demonstrates that the observed values fall into a regular pattern when allowances are made for variations in bone water content.

Materials and methods. The samples studied represent specimens of marrow-free bone obtained from normal animals. Human bone samples were obtained from subjects undergoing surgery, or from the autopsy room; none of the subjects suffered from disturbances of electrolyte metabolism. The human bone samples cover an age span from the 900 g premature infant to the adult, and those for the cat and rat from weanling to adult. The pigs were about 2 months old, the armadillo and fish of unknown age. The frogs ranged in weight from 3 to 85 g. A number of skeletal sites are represented: rib, skull, humerus and radius-ulna for the cat, pooled long bones of the rat, frog femur and tibia, pig and veal femur, armadillo rib, radius-ulna, and external bony plate, and rib, ilium, and long bones in man. Except for veal bone, all samples represent only the cortical portion. The human tooth samples† represent one each of a normal erupted, a normal unerupted, and a hypoplastic adult specimen. Enamel and dentin were carefully separated with the aid of a diamond grinding wheel. The teeth and veal bone had been frozen prior to analysis; all other samples of bone, including the goosefish (*Lophius americanus*),‡ represent fresh specimens. A number of bones were analyzed from this latter species, a marine teleost. Radiosodium^{22} was administered by the intravenous route to normal cats, and by the intraperitoneal route to normal rats, in a dose of 5 μc per 100 g body weight. Two hours later the animals were killed and samples of blood and bone removed for analysis. After removal of periosteum and marrow the bone samples were cut into small bits with an orthopedic rongeur, and dried *in vacuo* at 65°C. Fat was removed by ether extraction at room temperature, and the dry fat-free bone ground to a coarse powder in a mortar. Aliquots of this powder were then ashed in a muffle furnace at 525°C, and the ash taken up in 2N HCl. Sodium analyses were made either by a previously described column chromatographic technic(6) or by applying appropriate corrections to flame photometric readings of the ash

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