# THE FATE OF TRITIUM-LABELLED β-GLYCYRRHETIC ACID IN THE RAT

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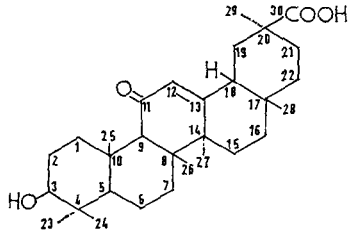
Tritium-labelled β-glycyrrhetic acid was prepared and administered orally, subdermally and intraperitoneally to male and female rats. Most of the radioactivity was excreted in the faeces, only traces being found in the urine. Administered to rats with biliary cannulae most of the radioactivity was excreted in the bile. Three metabolites of β-glycyrrhetic acid have been detected in the bile, but these have not yet been identified.

β-Glycyrrhetic acid (3-hydroxy-11-oxo-18-β-olean-12-en-30-oic acid) is a triterpenoid which occurs as the diglucuronide, glycyrrhizic acid, in liquorice root. β-Glycyrrhetic acid and its derivatives have been used in the treatment of gastric ulcer (Doll, Hill, Hutton and Underwood, 1962) and of dermatitis (Colin-Jones, 1960) and it has been shown to reduce hypercholesteraemia (Shibata, 1961). The metabolism of orally administered tritium-labelled glycyrrhetic acid and monoammonium glycyrrhizate in man has been studied by Carlat, Margraf, Weathers and Weichselbaum (1959), who suggested that both these compounds were poorly absorbed from the gastrointestinal tract, since most of the material was excreted in the faeces. We have now studied the fate of tritium-labelled β-glycyrrhetic acid in rats and have found that the compound is absorbed well from the alimentary canal. Most of the radioactive material is excreted in the bile and is then eliminated in the faeces, mainly as metabolites.




β-Glycyrrhetic Acid

## Experimental

### *Materials*

β-Glycyrrhetic acid randomly labelled with tritium was prepared from tritiated water and unlabelled β-glycyrrhetic acid, m.p. 284 − 7°, $[\alpha]_D^{19}$ + 163° ± 1° (*c*, 1 in $CHCl_3$) by the method of catalytic exchange (see Gould, 1958). 3-Acetyl-β-glycyrrhetic acid (1·2 g.) together with 660 mg. of tritiated water (2·5 c, Radiochemical Centre, Amersham), 3·3 ml.

acetic acid and 67 mg. of pre-reduced platinum oxide catalyst (Adams, Voorhees and Shriner, 1941) were heated at 127° for 72 hr. in a sealed tube. The product, dissolved in 5 ml. water and 135 ml. of a 2·5 per cent w/v solution of potassium hydroxide in methanol, was then hydrolysed by refluxing under nitrogen for 2 hr. The hydrolysed solution was acidified with 100 ml. 2N hydrochloric acid and the white precipitate of tritium-labelled $\beta$-glycyrrhetic acid which formed was extracted into 150 ml. chloroform. The aqueous layer was further extracted with two 50 ml. portions of chloroform and the combined extracts washed twice with 100 ml. water, dried over anhydrous sodium sulphate and the solvent evaporated. The crude product (yield, 1·07 g., m.p. 270–275°) was recrystallised from a mixture of methanol and chloroform to give pure tritium-labelled $\beta$-glycyrrhetic acid (0·8 g.), m.p. 281–5° $[\alpha]_D^{22} + 169° \pm 2°$ (*c*, 1 in $CHCl_3$), 3 mc/g. The product gave a single spot when run on a paper chromatogram by descending chromatography on Whatman No. 1 paper in a solvent consisting of light petroleum (b.p. 80–100°): methanol: water (5:4:1 by vol.) (Bush, 1952). The infra-red spectra was identical with that of authentic $\beta$-glycyrrhetic acid.

## METHODS

*Animal experiments.* $^3$H-$\beta$-Glycyrrhetic acid was administered orally to albino rats (weighing *ca.* 150 g.) as an aqueous suspension or as a solution in arachis oil, and intraperitoneally as a solution in propylene glycol.

*Determination of radioactivity.* In preliminary experiments, the faeces, urines and tissues were dissolved in formamide (Kinnory, Kanabrocki, Greco, Veatch, Kaplan and Oester, 1958) and the radioactivity counted in a Panax refrigerated scintillation counter type SC–LP at 7°. A solution of a mixture of naphthalene, 2,5-diphenyloxazole (PPO), 1,4-bis-[2′-(5′-phenyloxazole)]-benzene (POPOP) (800:50:1, by weight) in toluene: ethanol: dioxane (5:4:3, by volume) (TED/5 Panax) was used as phosphor. In subsequent experiments, tissues, faeces, urine and bile were dried in cellophane packets under infra-red lamps, and then ignited in an atmosphere of oxygen (Kelly, Peets, Gordon and Buyske, 1961). The $^3H_2O$ formed by the combustion was dissolved in TED/5 phosphor (Panax) and the solution counted as before. Results obtained by these two different methods showed no significant differences, but the latter method eliminated the high tissue blanks observed in the formamide method.

*Biliary cannulation.* To investigate the possibility of biliary excretion of glycyrrhetic acid or its metabolites, $^3$H-$\beta$-glycyrrhetic acid was administered orally or intraperitoneally to albino rats after surgical insertion of a polythene cannula into the common bile duct (Stewart and Harrison, 1961). The rats were kept in wire restraining cages (Bollman, 1948) and were allowed free access to an aqueous solution of glucose (5 per cent w/v) -saline (1 per cent w/v), with or without addition of bile salt (0·04 per cent w/v). The bile, urine and faeces were collected at intervals over a period of three days and together with tissues, were combusted to $^3H_2O$ and the radioactivity measured.

*Chromatography of metabolites.* The bile obtained from cannulated rats dosed with $^3$H-β-glycyrrhetic acid was first chromatographed on Whatman No. 1 paper in solvent A, i.e. chloroform: acetic acid: water (2:1:1 by vol.). Radioactive material remaining at the origin was eluted and further chromatographed on Whatman 3 MM paper in solvent B, i.e. the lower phase of 70 per cent v/v aqueous acetic acid: 1,2-dichloroethane: n-butanol (10:9:1 by vol.) by the descending technique (Sjovall, 1955). The chromatograms were cut into strips and the positions of the radioactive metabolites determined by absorption in ultra-violet light and by combusting 1 cm. bands of the paper and counting the $^3H_2O$ produced.

*Estimation of unchanged β-glycyrrhetic acid in faeces.* The unchanged β-glycyrrhetic acid in the rat faeces was determined by a reverse isotope dilution procedure. β-Glycyrrhetic acid (250 mg.) was added to dried faeces (1·0 g.) and the mixture extracted with chloroform in a Soxhlet apparatus for 1·5 hr. The chloroform extract was concentrated to 5 ml. and an aliquot (one-fifth) was chromatographed on Whatman 3 MM paper in solvent A. The area of the chromatogram between $R_F$ 0·6–1·0 was cut out and eluted with chloroform: methanol (9:1 by vol.) and the eluate re-chromatographed on Whatman No. 1 paper in light petroleum (b.p. 80–100°): methanol: water (5:4:1) (solvent C). The area between $R_F$ 0·0–0·17 of the second chromatogram was cut out and eluted as before and the eluate evaporated to dryness (52 mg.). An aliquot (ca. 1 mg.) of the residue was ignited and the radioactivity of the water of combustion determined.

TABLE I

PAPER CHROMATOGRAPHY OF THE GLYCYRRHETIC ACIDS AND SOME DERIVATIVES

Chromatograms were run on Whatman No. 1 paper in solvent A: chloroform: acetic acid: water (2:1:1 by volume); B: the lower phase of 70 per cent v/v aqueous acetic acid: 1,2-dichloroethane: n-butanol (10:9:1) (Sjovall, 1956); and C: light petroleum (b.p. 80–100°): methanol: water (5:4:1, by volume) by descending technique (Bush, 1952).

| Compound | $R_F$ value in solvent | | |
|---|---|---|---|
| | A | B | C |
| β-Glycyrrhetic acid | 0·98 | 0·95 | 0·1 |
| α-Glycyrrhetic acid | 1·0 | 1·0 | 0·0 |
| 3-Keto-β-glycyrrhetic acid | 1·0 | 1·0 | 0·0 |
| 3-Acetyl-β-glycyrrhetic acid | 1·0 | 1·0 | 0·57 |
| Ammonium glycyrrhizinate | 0·06 | 0·60 | 0·0 |
| Metabolite I | 0·0 | 0·40 | 0·0 |
| Metabolite II | 0·0 | 0·46 | 0·0 |
| Metabolite III | 0·44 | 0·77 | 0·0 |

Chromatography in solvent A separates β-glycyrrhetic acid from its metabolites, which are found at the origin and at $R_F$ 0·1–0·4 (see Table I) and chromatography in solvent C separates β-glycyrrhetic acid from less polar compounds naturally present in faeces.

## RESULTS

After administration of an oral dose of 60 mg./kg. of finely ground $^3$H-β-glycyrrhetic acid suspended in water (3 ml.) to female rats, an

average of 86 per cent of the administered radioactivity was recovered in 1–3 days, 83 per cent being found in the faeces, 4 per cent in the liver and 1 per cent in the urine (see Table II). After sub-dermal injection of an aqueous suspension of $^3$H-β-glycyrrhetic acid (60 mg./kg.) to female rats, an average of 74 per cent of the administered radioactivity was recovered in 5 days, 73 per cent being in the faeces and 1 per cent in the urine. Of the radioactivity present in the faeces, only 7·4 per cent was found to be β-glycyrrhetic acid when the triterpenoid was administered orally, and only 5·2 per cent when it was administered sub-dermally (see Table II). The levels of radioactivity found in the liver and gastrointestinal contents after oral administration were found to vary with the duration of the experiment in a manner suggestive of an entero-hepatic circulation of β-glycyrrhetic acid.

TABLE II

FATE OF $^3$H-β-GLYCYRRHETIC ACID IN RATS

| Expt. No. | Sex | Duration of expt. (days) | Dose per cent of radioactivity found in | | | Total per cent of dose accounted for |
|---|---|---|---|---|---|---|
| | | | Faeces | Urine | Liver | |
| Oral doses of 60 mg./kg. in aqueous suspension: | | | | | | |
| 1 | F | 1 | 70* | — | 7·5 | 78 |
| 2 | F | 1·5 | 112* | — | 2·6 | 115 |
| 3 | F | 2 | 82** | 1·0 | — | 83 |
| 4 | F | 3 | 56 | 1·2 | — | 57 |
| 5 | F | 3 | 94 | — | 3 | 100† |
| 6 | M | 2 | 46* | — | 9·3 | 56 |
| Sub-dermal doses of 60 mg./kg. in aqueous suspension: | | | | | | |
| 7 | F | 5 | 84‡ | 0·8 | — | 85 |
| 8 | F | 5 | 62 | 1·2 | — | 63 |

* Also includes gastrointestinal contents.
† Includes 3 per cent found in gastrointestinal contents.
** 7·4 per cent present as β-glycyrrhetic acid.
‡ 5·2 per cent present as β-glycyrrhetic acid.

*Biliary excretion.* After oral administration of $^3$H-β-glycyrrhetic acid as a solution in arachis oil at a dose level of 25 mg./kg. to female rats, more than 70 per cent of the dose of radioactivity was excreted in the bile in 2–3 days, 13–20 per cent was found in the faeces and 2–3 per cent in the urine (see Table III). After oral administration of aqueous suspensions (25 mg./kg.) to male rats an average of 53 per cent of the dose of radioactivity was excreted in the bile in 3 days, a further 11 per cent was excreted in the faeces and 2 per cent in the urine. Female rats in the same time excreted 54 per cent in the bile, 15 per cent in the faeces and 9 per cent in the urine.

The biliary excretion was much greater and much more rapid after intraperitoneal administration of a solution of $^3$H-β-glycyrrhetic acid in propylene glycol (see Fig. 1). At a dose level of 25 mg./kg., both male and female rats excreted an average of 100 per cent of the administered radioactivity within 12 hr. In fact, 95 per cent of the dose was excreted within 6 to 8 hr. in both sexes. The addition of 0·04 per cent w/v of ox bile salt to the liquid diet did not appear to alter significantly the total

biliary excretion or absorption of the triterpenoid, although in some experiments the initial rate of biliary excretion is slightly lower when bile salt is present in the diet.

*Biliary metabolites of β-glycyrrhetic acid.* The pooled bile obtained from three female rats, each of which had received 4 mg. of $^3$H-β-glycyrrhetic acid in propylene glycol intraperitoneally, was chromatographed on Whatman No. 1 paper in solvent A. Two radioactive areas were detected with $R_F$ values of 0–0·1 and 0·15–0·30.

TABLE III

FATE OF $^3$H-β-GLYCYRRHETIC ACID IN RATS AFTER BILIARY CANNULATION

| Expt. No. | Sex | Duration of expt. (days) | Dose per cent of radioactivity found in | | | Alimentary canal and contents | Total accounted for |
|---|---|---|---|---|---|---|---|
| | | | Faeces | Bile | Urine | | |
| Oral doses of 25 mg./kg. in arachis oil: | | | | | | | |
| 9 | F | 1·5* | 7 | 36 | 2·4 | — | 46* |
| 10 | F | 2 | 13 | 72 | 2·7 | — | 88 |
| 11 | F | 3 | 20 | 73 | 1·6 | — | 95 |
| Oral doses of 25 mg./kg. as aqueous suspension: | | | | | | | |
| 12† | F | 2·5 | 14 | 56 | 6·6 | 1·1 | 77 |
| 13 | F | 3 | 17 | 51 | 12 | 0·3 | 81 |
| 14† | F | 3 | 31 | 27 | 1·1 | — | 60** |
| 15 | M | 3 | 5 | 55 | 1·6 | 0·3 | 62 |
| 16† | M | 3 | 17 | 56 | — | — | 73 |
| 17† | M | 3 | — | 48 | — | — | 48 |
| Intraperitoneal doses of 25 mg./kg. in propylene glycol: | | | | | | | |
| 18 | F | 2 | — | 93 | — | — | 93 |
| 19 | F | 2 | — | 102 | — | — | 102 |
| 20 | F | 2 | — | 105 | — | — | 105 |
| 21 | M | 2 | — | 102 | — | — | 102 |
| 22 | M | 2 | — | 83 | — | — | 83 |
| 23† | M | 2 | — | 109 | — | — | 109 |

* Animal died before completion of expt.
** Flow rate of bile impeded, 0·6 per cent in the liver.
† Bile salt included in diet.

The material of $R_F$ 0–0·1 was eluted with a mixture of methanol-chloroform (9 : 1 by volume) and the eluate was found to contain radioactivity equivalent to 4·5 mg. of the administered $^3$H-β-glycyrrhetic acid or to 38 per cent of the dose. Further chromatography of this material on Whatman 3 MM paper in solvent B by descending chromatography revealed two ultra-violet-absorbent, radioactive spots with $R_F$ values of 0·40 (metabolite I) and 0·46 (metabolite II). These two substances were separated by allowing the solvent to run off the front of the paper, the chromatograms being run for a period of 31 hr. The two spots were cut out, eluted with methanol : chloroform (9 : 1 by volume) and the solvent evaporated to give a semi-crystalline residue in each case (24·6 mg. containing metabolite I and 27·9 mg. containing metabolite II).

The material of $R_F$ 0·15–0·30 in solvent A was similarly eluted with a mixture of methanol : chloroform (1 : 1) and the eluate was found to contain radioactivity equivalent to 4·3 mg. of the administered $^3$H-β-glycyrrhetic acid or to 36 per cent of the dose. Evaporation of the solvent gave a semi-crystalline residue containing metabolite III (32·2 mg.).

The material containing metabolite I, but neither of the other two metabolite fractions, gave a strongly positive naphthoresorcinol reaction for glucuronic acid.

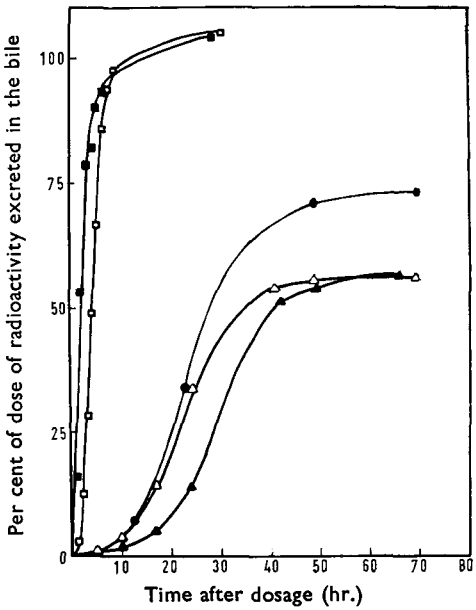



FIG 1. The rate of biliary excretion of radioactivity following administration of tritium labelled β-glycyrrhetic acid, intraperitoneally in propylene glycol to male rats, □, to female rats, ■; orally as an aqueous suspension to male rats, △, to female rats ▲; and orally as a solution in arachis oil to female rats, ●.

## DISCUSSION

Tritium-labelled β-glycyrrhetic acid administered to rats is eliminated from the animal *via* the bile, for almost the whole of an intraperitoneal dose is excreted by this route and only traces are excreted in the urine. The extent and rate of the biliary excretion of this compound is approximately the same in both sexes.

When the compound is administered orally, the biliary excretion is less and the rate of excretion slower than when it is given intraperitoneally. Moreover, the biliary excretion is less and the rate of excretion slower when the oral dose is administered as an aqueous suspension than when it is administered as a solution in arachis oil. Both of these facts suggest that absorption from the gastrointestinal tract is slow, particularly when an aqueous suspension is fed. The replacement of the bile salt lost through cannulation by supplementation with ox bile salt in the diet has no marked effect.

In the previous experiments of Carlat, and others (1959) in which tritium-labelled β-glycyrrhetic acid was fed to man, most of the compound was recovered unchanged from the faeces, only trace amounts were found in the urine, and no activity was detected in the bile. This may have been

because bile was obtained during only the first 4 hr. after dosage, and from our experiments with rats, one would expect the biliary excretion to be about one per cent of the dose, or less, during this period. We likewise found that in the normal rat without biliary fistula, most of the compound was recovered from the faeces, but only a small proportion of this was unchanged β-glycyrrhetic acid. Moreover, since the amount of β-glycyrrhetic acid present in the faeces after oral administration (7·4 per cent) was only slightly greater than that found after injection of the triterpenoid (5·2 per cent), the orally administered compound is probably almost completely absorbed.

The three products excreted in the bile have not as yet been identified but none is identical with unchanged β-glycyrrhetic acid. This may explain why β-glycyrrhetic acid has a healing action for gastric but not duodenal ulcers (see Doll and others, 1962). The β-glycyrrhetic acid, being a weak acid, should be largely absorbed from the stomach (Brodie and Hogben, 1957) and although the drug is almost completely excreted again into the duodenum *via* the bile, it is now present only as metabolites, which may have no healing activity.

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