THE PHARMACOLOGICAL PROPERTIES OF GLYCYRRHETINIC ACID HYDROGEN SUCCINATE (DISODIUM SALT)

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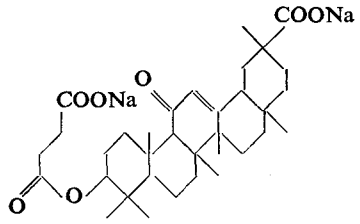
The anti-inflammatory activity, pharmacological properties and biochemical effects of the disodium salt of glycyrrhetinic acid hydrogen succinate have been tested in laboratory animals. Under the conditions of the tests this drug is an active anti-inflammatory agent of low toxicity, while its solubility in water renders it a more versatile drug than the parent compound, glycyrrhetinic acid.

IN a previous paper Finney and Somers[1] demonstrated the anti-inflammatory activity of glycyrrhetinic acid and some of its derivatives in laboratory animals. The pharmacological properties of these compounds were subsequently described by Finney, Somers and Wilkinson[2]. The substances then available were sparingly soluble in body fluids, and it was suggested that this would limit the anti-inflammatory activity which, by the cotton pellet test[3], was shown to be about one-eighth that of hydrocortisone by weight. Soluble derivatives of glycyrrhetinic acid have since been synthesised, and in this paper we describe our results with one of these, the disodium salt of glycyrrhetinic acid hydrogen succinate (GAHS-Na), a substance readily soluble in water.



A summary of part of this paper was read at a meeting of the British Pharmacological Society[4]; early results together with an indication of the clinical investigations now being undertaken have been the subject of a preliminary communication[5].

EXPERIMENTAL

Material

GAHS has an equivalent weight of 285, m.p. 315–7° and specific rotation $[\alpha]_D$ + 128° in chloroform (1 per cent). The disodium salt of GAHS (specific rotation $(\alpha)_D$ + 117° in water (1 per cent) was prepared from the acid by Professor E. E. Turner, F.R.S., assisted by Mr. D. E. M. Wotton, and supplied to us through the courtesy of Dr. S. Gottfried of

Biorex Laboratories Ltd. Hydrocortisone was used as a suspension in the form of commercial preparations of the acetates Cortelan (Glaxo) and Cortef (Upjohn) and hydrocortisone hemisuccinate (Biorex) as an aqueous solution.

Methods

The anti-inflammatory activity of GAHS–Na was determined in rats by the cotton pellet method of Meier, Schuler and Desaulles[3], using hydrocortisone and its hemisuccinate for comparison. For the test, small cotton wool dental pellets, weighing about 5 mg., were weighed and then implanted under the skin of male Wistar rats weighing 150 to 200 g. one in the region of each groin and axilla. The rats were injected subcutaneously daily with the preparations under test for 5 days and killed on the sixth day. The pellets were removed, trimmed of extraneous tissue, dried in an oven at 60° and weighed again. The difference between the initial and final weight was taken as the amount of granulation tissue formed. The results were assessed statistically.

The acute toxicity of GAHS–Na was determined in albino mice weighing 20 g., by the oral, subcutaneous, intraperitoneal and intravenous routes. For all routes of administration, where possible, the regression of mortality per cent as probits on the logarithm of the dose was found and the LD50 and limits of error ($P = 0{\cdot}95$) calculated by the method of Finney[6].

The chronic toxicity of GAHS–Na was determined in weanling rats by the intraperitoneal and oral routes. In the first experiment one group of nine weanling rats was injected intraperitoneally with 2·5 mg. GAHS–Na twice weekly and the growth compared with a similar group of untreated controls. In the second experiment groups of eight weanling rats were injected subcutaneously or dosed orally with 25 mg. or 50 mg. GAHS–Na daily. The subcutaneous route had to be abandoned because of a necrotic action of GAHS–Na at the site of injection. This is reported more fully under local reactions. Histopathological examinations were made of the major organs and tissues.

Experiments were also made in cats which were given 500 mg. of GAHS–Na orally for 30 days.

Some indications of the absence of hepatotoxicity were obtained in two rabbits. They were injected daily with 2·5 mg./kg. intraperitoneally for 8 days and determinations made of the serum glutamic-oxaloacetic (S.G.O.T.) and glutamic-pyruvic (S.G.P.T.) transaminase by Dr. J. H. Wilkinson, using the spectrophotometric methods of Karmen, Wróblewski and LaDue[7] and of Wroblewski and LaDue[8] described by Pryse-Davies and Wilkinson[9].

The local toxicity was studied in rats injected subcutaneously with GAHS–Na; specimens of the skin and surrounding tissues being taken for histological examination 24 hours after injection.

The pharmacodynamics of this compound were studied in anaesthetised cats and the effects of the drug on the intestine *in vitro* on the isolated duodenum of the rabbit, and *in vivo* by the charcoal meal test using mice.

The renal effects of the compound were determined in rats previously given an oral dose of water (5 mg./100 g.) and in anaesthetised cats in which the bladder was cannulated. Determinations were made of the blood and the urine electrolyte levels by the methods described by Tárnoky[10] and blood levels of glycyrrhetinic acid by a modification of the method of Van Katwijk and Huis in't Veld as previously described[2]. The effect of GAHS–Na on blood glucose levels of rabbits was studied by means of colorimetric glucose estimations[10].

The effect of GAHS–Na on the urinary excretion of neutral 17-keto-steroids were determined by the method of Norymberski, Stubbs and West[11], and 17-hydroxycorticosteroids by that of Appleby, Gibson, Norymberski and Stubbs[12] on 24-hour urine specimens from rabbits.

The possibility of a haemolytic effect of GAHS–Na was investigated in three ways; by adding a drop of packed, washed human red blood cells from freshly taken normal blood to aqueous solutions of the drug, by allowing aqueous solutions to act on freshly taken normal whole blood in silicone-coated glass tubes at room temperature, and by repeating the second method at 37° with solutions of GAHS–Na in 20 per cent water: 80 per cent serum taken from the same donor on the day before the test.

Results

Anti-inflammatory Activity

GAHS-Na is seen to have a significant anti-inflammatory action in the cotton pellet test (Table I). The results show that a subcutaneous dose of 12·5 mg./200 g. rat was as effective as 5 mg. of hydrocortisone. The response was linearly related to the logarithm of the dose, a greater depression of granulation tissue being obtained with increasing doses of GAHS–Na (Fig. 1).

TABLE I

COTTON PELLET TEST IN RAT

Treatment	No. of pellets	Granulation tissue mg.	Standard error mg.	Per cent of control	Significance to control
Controls	16	13·3	±0·34	—	—
GAHS-Na 12·5 mg./day S.C.	16	7·8	±0·19	58·6	P = 0·005
Controls	16	9·8	±0·23	—	—
Hydrocortisone 5·0 mg./day S.C.	16	5·8	±0·17	59·2	P = 0·001

Results of a quantitative comparison of GAHS–Na with hydrocortisone hemisuccinate are shown in Table II. This was determined by injecting subcutaneously groups of eight rats at ascending dose levels on a logarithmic scale with GAHS–Na or hydrocortisone hemisuccinate. One group was kept as an untreated control. The validity of the results and the relative potencies together with their limits of error (P + 0·95) were calculated by the method of Bliss and Marks[13] after Pugsley[14]. The results showed that GAHS–Na had 0·23 times the activity of hydrocortisone hemisuccinate, with limits of error 87·6 and 114 per cent. There was no significant deviation from parallelism.

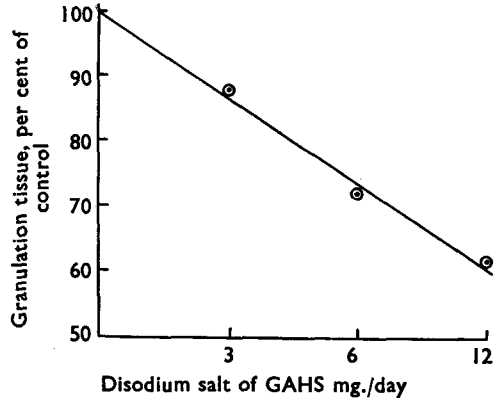


FIG. 1. Regression of granulation tissue to doses of disodium salt of GAHS.

TABLE II

COMPARISON OF GAHS–Na AND HYDROCORTISONE HEMISUCCINATE BY COTTON PELLET METHOD

Treatment	Mean weight granulation tissue mg.	Mean weight as per cent control
1. Controls	11·4	
2. GAHS–Na 4 mg.	9·4	80
3. GAHS–Na 8 mg.	7·8	68·5
4. GAHS–Na 16 mg.	5·9	51·5
5. Hydrocortisone hemisuccinate 1 mg. ..	9·0	79
6. Hydrocortisone hemisuccinate 2 mg. ..	7·6	66·5
7. Hydrocortisone hemisuccinate 4 mg. ..	5·6	49·0

Toxicity

Acute. The acute toxicity was low. In mice, single oral and subcutaneous doses as high as 250 mg./kg. have been given without causing deaths. By the intraperitoneal route the LD50 was determined as 101 mg./kg.; limits of error, P = 0·95, 93 mg. to 111 mg./kg., and by the intravenous route 43 mg./kg. (38–49). Single doses of 16 mg./kg. have been injected intravenously into rabbits and single doses of 23 mg./kg. into anaesthetised cats without ill effects.

Chronic. Rats treated with GAHS–Na intraperitoneally increased in weight more quickly than the controls (Fig. 2). By the end of the twelfth week the mean weight of the treated rats was 194 g. (± 8·3) compared with 174 g. (± 5·1) for the controls. This increase in weight was probably caused by oedema, for the drug has an antidiuretic action, and D'Arcy and others[15] reported swelling of the face in rats chronically treated with glycyrrhetinic acid. Evidence of this was further obtained by injecting the controls with GAHS–Na from 12 weeks onwards, by which time their natural growth was slow; the controls then rapidly increased in weight so that at the end of the sixteenth week there was no significant difference between the weights of the two groups.

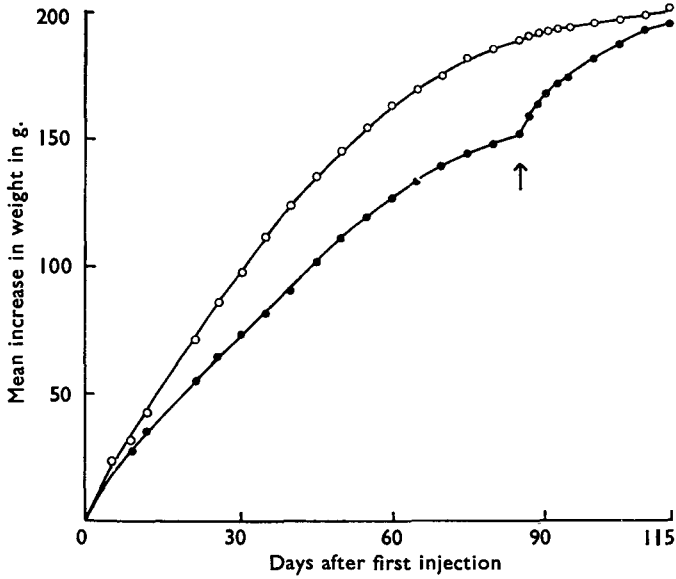


FIG. 2. The chronic toxicity of the disodium salt of GAHS in rats. ●—● Controls. ○—○ 2·5 mg. GAHS–Na intraperitoneally twice weekly. At arrow, controls placed on same regime.

Rats treated orally with 25 mg. of GAHS–Na daily for 76 days increased in weight (106 g.) normally when compared with the untreated controls (99 g.). Daily injection of 50 mg. GAHS gave an initial loss of about 9 g. after 4 days and the animals then increased in weight (88 g. over 72 days) at the same rate as the controls (86 g. over 72 days). Two rats treated with 50 mg. GAHS–Na orally for 30 days were killed for pathological examination. The following organs were examined microscopically: lung, liver, spleen, stomach, small intestine, ovary, suprarenal, kidney and heart. Of these the gastrointestinal tract showed some shedding of the mucosal cells and there was evidence of kidney tubular damage. Cats were given 500 mg. orally for 30 days without apparent ill-effects.

Rabbits showed no significant increase in the glutamic-oxaloacetic (S.G.O.T.) or in the glutamic-pyruvic (S.G.P.T.) transaminase levels: after seven and eight days respectively S.G.O.T. levels rose from 13 and 10 to 17 and 18 spectrophotometric units; S.G.P.T. concentrations, initially 12 and 10 units, were unchanged at 10 units.

Local Reactions

Subcutaneous injection of 1 ml. of a solution containing 25 mg./ml. of GAHS–Na was observed in rats to have a necrotic action at the site of the injection which increased with increasing concentration of the solution. It was not present with solutions containing 12·5 mg./ml. GAHS–Na. Histological sections of the skin of rats injected with 50 mg. GAHS–Na in 1 ml. of water showed a marked polymorph and monocyte infiltration and very widespread necrosis.

Heart, Circulation and Autonomic Nervous System

GAHS–Na given intravenously had no observed untoward effect on the heart, circulation or autonomic nervous system. In anaesthetised cats doses up to 50 mg./kg. body weight have been given intravenously. These caused a transient fall in blood pressure followed by a complete recovery. Respiration was unaffected. There were no observed effects on the sympathetic and parasympathetic nerves, and the responses to acetylcholine, adrenaline and histamine were not appreciably affected.

Intestinal Effects

Like glycyrrhetinic acid[2], GAHS–Na had little effect on the isolated intestine of the rabbit, a concentration as high as 20 mg. in a 20 ml. bath having no significant action on the normal tone or rhythmic contractions of the duodenum. There was a slight potentiation of the response to acetylcholine and depression of the response to histamine. The rate of transport of a charcoal meal in mice was unaffected by administration of 50 mg. GAHS–Na/kg. by mouth 30 minutes before the meal. The drug therefore has no effect on intestinal motility.

Action on the Kidney

A species variation was seen in the renal effects of GAHS–Na. In the rat given water an intraperitoneal injection of 60 mg./kg. had an antidiuretic action, and after 5 hours the total volume of urine produced was only 66 per cent of that of the controls. (Fig. 3.) There was also retention of sodium, 39 per cent of the control excretion, with, by contrast, only a slight fall in the potassium excretion (92 per cent of the controls). (Fig. 3.) The electrolyte figures here refer to the absolute amounts

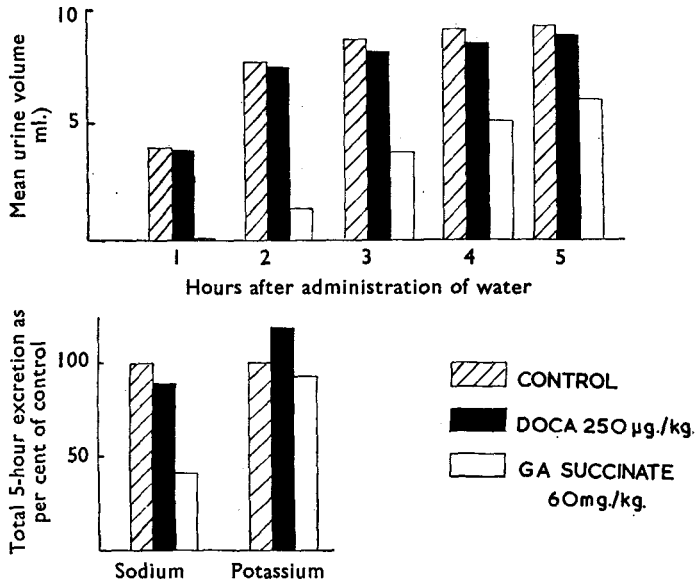


FIG. 3. Effect of DOCA and GAHS–Na on the urine volume and electrolytes in the rat.

excreted. If referred to the rate of urine flow the decrease in potassium excretion becomes a relative rise. This action may be compared with deoxycortone (DOCA) where an intraperitoneal dose of 250 μg./kg. caused little change in urine volume, a slight drop in sodium excretion (89 per cent of the controls) and an absolute rise in potassium excretion (117 per cent of the controls: Fig. 3).

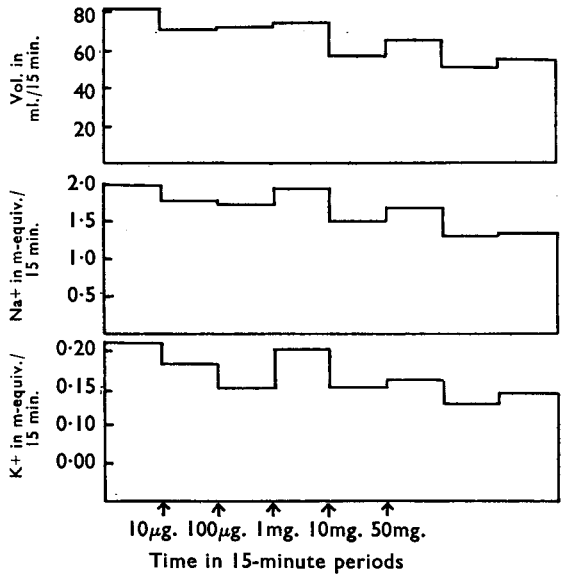


FIG. 4. Effect of GAHS–Na on urine volume and electrolytes in the cat.

In the anaesthetised cat, a dose of GAHS–Na as high as 50 mg./kg. by the intravenous route caused only a slight reduction in urine flow (Fig. 4). The slight fall in the excretion of sodium and potassium would be expected from the reduction of urine flow. Analysis of the plasma showed a high level of the drug after 84 minutes (68 μg./ml.), but little change in the plasma electrolytes.

Steroid Excretion

A 4-day and a 31-day trial have been completed to date. Two rabbits were used for each experiment and given 25 mg. GAHS–Na intraperitoneally daily. This dose had no effect either on the daily urine volumes or on the 24-hour excretions of neutral 17-ketosteroids or of 17-hydroxycorticosteriods, all of which merely showed random daily variation (Fig. 5). In the group of analyses performed on animals and man (70 hydroxysteroid determinations in all) there has been no relation between corticoid output and urine volume, and it seems that the findings of Brown and Asher[16] do not apply to this heterogeneous group.

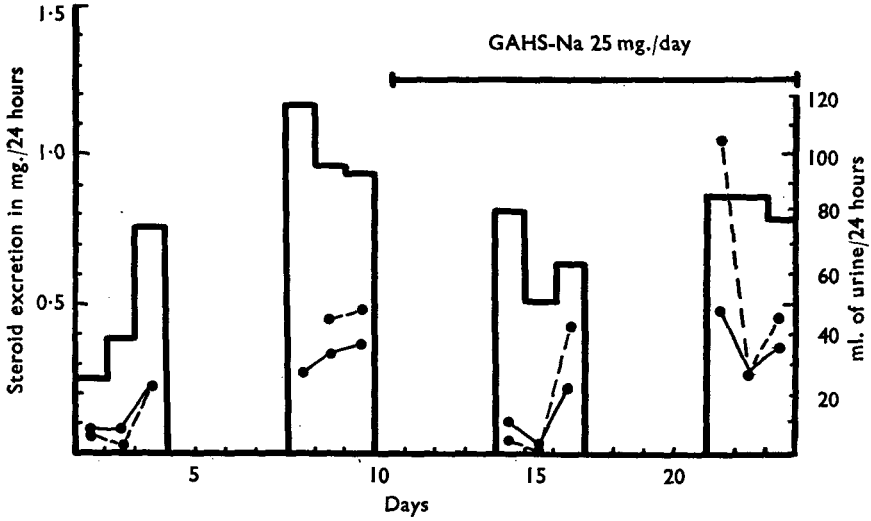


FIG. 5. Effect of GAHS–Na on urine volume and steroid excretion in a rabbit.
—— 17-ketosteroids.
– – – 17-OH-corticosteroids.

Effect on Blood Sugar Levels

Repeated tests, in which 20 mg. GAHS–Na was given intravenously to fasting rabbits and to rabbits given 5 g. glucose 5 minutes before or 1 g. glucose 10 minutes after the GAHS–Na, failed to show any definite trend in blood glucose levels for 180 minutes compared with controls. The individual variation within the treated and control groups was too great for any conclusions to be drawn, though the likelihood of a marked effect on the glucose tolerance curve or on gluconeogenesis could be discounted.

Haemolytic and Anticoagulant Effects

The investigation of a possible haemolytic effect was prompted by the structural resemblance of glycyrrhetinic acid to the triterpenoid sapogenins. Observations on the effect on blood clotting and clot retraction were incidental findings.

When left at room temperature for 24 hours, solutions containing up to 0·4 mg. GAHS–Na/ml. normal saline had no haemolytic effect on washed packed red blood cells, though lysis appeared at this concentration at 48 hours. The results were the same when drug solutions were allowed to act on whole blood; here the highest concentration which did not cause haemolysis was 0·6 mg. GAHS–Na/ml., a result unchanged after further standing at room temperature and 48 hours incubation at 37°. This experiment was conducted in siliconised tubes. The drug concentration exceeded any expected blood levels. Incubation of a series of solutions in aqueous serum at 37°, again in silicone-coated tubes, showed no haemolytic effect at 4 mg. GAHS–Na/ml. after 5 hours, and at 1·5 mg/.ml. at

24 hours. GAHS–Na thus shows no sapogenin-like properties either in its speed of action or in the concentration required to produce lysis, and its osmotic haemolytic effect is low.

An anticoagulant effect was noted in the experiments on whole blood haemolysis, at concentrations of 5 mg. GAHS–Na/ml. This strength contains sufficient hydrogen succinate to react with all the calcium present in the serum and may thus act merely in the same way as the standard sodium or ammonium oxalate anticoagulant, though it is of interest that Klosa[17] attributes an anticoagulant activity to glycyrrhizin, which he puts at 20 per cent of the activity of dicoumarol presumably on the basis of one-stage prothrombin times of treated animals, since the latter has no effect *in vitro*. When whole-blood haemolysis was tested for at room temperature, GAHS-Na concentrations of 2·5 and 1·25 mg./ml. allowed clotting but prevented clot retraction. These findings, like those of lysis, are observed well above clinical levels of drug concentration.

Conclusions

Glycyrrhetinic acid hydrogen succinate as the disodium salt has been shown to have a powerful anti-inflammatory action in experimental conditions in rats. Assessed by the cotton pellet method it had 0·23 times the activity of hydrocortisone hemisuccinate. Its actue toxicity was low and it has been given intravenously in relatively high doses. Subcutaneously, when injected in high concentrations, the drug had a local necrotic effect. In rats it affected water and mineral metabolism but not in cats. It had no effect on the 17-ketosteroid or 17-hydroxy-corticosteroid excretion in rabbits. The drug showed a slight haemolytic action *in vitro* at concentrations well above those injected clinically; at these high concentrations an anticoagulant effect also appeared. The results suggest that the disodium salt of glycyrrhetinic acid hydrogen succinate may be a valuable drug in the treatment of inflammatory conditions in man.

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