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THE EFFECTS OF GLYCYRRHETINIC ACID ON SALT AND WATER METABOLISM

BY

E. E. GALAL*

From the Department of Pharmacology, University of Edinburgh

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Revers (1948) observed that succus liquiritiae caused water retention in some peptic ulcer patients. Molhuysen, Gerbrandy, de Vries, de Jong, Lenstra, Turner, and Borst (1950) confirmed this and found an extract consisting mainly of glycyrrhizin to be equally effective.

In normal persons, the authors reported that the succus made the potassium balance markedly negative and the sodium balance strongly positive. They failed to produce similar results in a patient with Addison's disease and concluded that the drug acted through the adrenals.

Groen, Willebrands, and Kamminga (1951), and Groen, Frenkel, Kamminga, and Willebrands (1952) found liquorice to have a DOCA-like effect on sodium and potassium metabolism in normal people and in Addisonian patients. They found glycyrrhizinic acid to be the active principle and used it for the treatment of Addison's disease.

Borst, ten Holt, de Vries, and Molhuysen (1953) suggested that liquorice activity on salt and water metabolism is potentiated by the endogenous corticoids. No conclusive evidence of such a potentiation has been found in the treatment of rheumatoid arthritis (Hart and Leonard, 1954) or in the increase in the urinary output of 17-ketosteroids (Hudson, Mittelman, and Mann, 1953).

Calvert (1954) successfully maintained a patient with Addison's disease on liquorice extract for over a year. The daily maintenance dose decreased from 60 g. to 3 g. The author concluded that the drug has a place in long-term therapy of this disease.

Glycyrrhizinic acid is a conjugate of glycuronic acid and glycyrrhetinic acid ; the latter was found to have a DOCA-like effect in normal people and in Addisonian patients (Card, Mitchell, Strong, Taylor, Tompsett, and Wilson, 1953).

Nelemans and Stamperius (1949) and Card *et al.* (1953), however, found no evidence of any effect of liquorice or glycyrrhetinic acid on water and electrolyte metabolism in laboratory animals.

Glycyrrhetinic acid is not a steroid and therefore it would be of great interest if it could be shown to possess some of the more important properties of the corticosteroids.

METHODS

Urinary Sodium Excretion

The details of the method used for assaying mineralo-cortical activity will be reported separately. Young female albino rats, adrenalectomized under ether anaethesia three days before the assay, were used. The rats were divided into groups of 5–7 animals each. On the day of the experiment, each rat was given two loads of the electrolytes investigated. Each group received either a known quantity of glycyrrhetinic acid dissolved in propylene glycol or a known quantity of DOCA in the same volume of the solvent. A comparable group of animals was included in every experiment and received the same quantity of solvent alone.

The urine of each animal was collected separately over a period of six hours and its sodium or potassium content was estimated by the flame-photometer. In some experiments, the urine was collected for 4 hr. only.

Blood Electrolytes

Male albino rats weighing 300 g. were adrenalectomized under ether anaesthesia and kept on rat cubes and a drinking solution containing sodium chloride (1%) and glucose (5%) for 4 days. On the 5th day, the animals were weighed and divided into three groups of equal mean weight. Injections were started and continued daily for five days. No saline drink was administered during this period.

Group 1 rats received subcutaneous injections of 0.25 ml. propylene glycol. Group II received 3 mg. glycyrrhetinic acid dissolved in 0.25 ml. propylene glycol. Group III received 1 mg. of DOCA in 0.25 ml. propylene glycol. Group IV rats were non-adrenalectomized, from the same colony, and of the same weight. Each rat received 0.25 ml. of propylene glycol subcutaneously.

On the 5th day, 4 hr. after the injection, the animals were anaesthetized with an intraperitoneal injection of 0.7 ml. 6% pentobarbitone sodium solution. The chest was opened and the heart punctured. The blood was collected in a centrifuge tube and allowed to clot ;

* In receipt of a scholarship from Cairo University.

the serum was pipetted off later. After dilution, the sodium and potassium contents were estimated with the flame photometer.

Diuresis

(*a*) *Anaesthetized Rats.*—Male rats above 250 g. were used. They were fasted overnight, but given free access to tap water. At about 10 a.m. anaesthesia was produced by administering 5 ml./100 g. body weight of 10% alcohol in tap water into the stomach by a thin rubber catheter, and the rat was kept in a warm place. If the rat was not unconscious in 45 min., another dose was given (1.5 ml. of the same solution/100 g. body weight). A small suprapubic cut was made, the bladder secured and a thin polythene tube (3 mm. internal diameter) was introduced into the bladder through the apex and tied in securely. The penis was tied to prevent any urine escaping except through the polythene tube. A thin polythene cannula was put into the jugular vein. A fine thermometer was introduced into the rectum and the animals were placed on a box warmed by electric bulbs and their temperature kept as constant as possible at 37°. The end of the catheter was fixed to a graduated centrifuge tube and a reading of the urine collected was taken every 10 min. When diuresis started, a new water load of 10–14 ml. of water, or of 3% alcohol in tap water if anaesthesia was getting light, was introduced by stomach tube. The degree of anaesthesia was kept deep enough to prevent the animals from moving or responding to external stimuli, but not so deep as to inhibit diuresis. Whenever 8 ml. of urine had been collected, it was replaced by 10 ml. of water by stomach tube or of 3% alcohol, if required. The solvent of the drug was administered intravenously in the same manner as the drug, which was given as a clear solution in propylene glycol (0.05–0.2 ml.) and washed down the cannula with a minimum amount of saline. Care was taken, as all these factors affected the flow of urine.

(*b*) *Conscious Rats.*—Female albino rats around 150 g. weight were used. Food was withdrawn at 5 p.m. the previous night; water was withdrawn at 9 a.m. on the day of the experiment. The animals were divided into two equal groups, one to act as control and the other as test. At 11 a.m. each control rat was given 1 ml. of propylene glycol by stomach tube. The test group had 100 mg. glycyrrhetinic acid dissolved in 1 ml. of propylene glycol. The rats were kept in a quiet place for 3 hr. At 2 p.m. each animal got 12 ml. of tap water by stomach tube; its bladder was emptied by a gentle pulling of the tail, and the rat was placed in a metabolism cage which consisted of a waxed tin funnel covered by a waxed wire mesh and surmounted by a cylinder of wire mesh with a tin cover. A graduated cylinder was placed under each cage to receive the urine. Two hours after placing the animals in the cages the bladder was emptied by pulling the tail gently, and the urine was allowed to run into the cylinder; then a reading was taken. The whole experiment was carried out at constant temperature (22° C.±1°) in a quiet room. Five days later, after 4 days of complete rest, the experiment was repeated and the groups were crossed so that each animal acted as its own control. To eliminate any source of emotional stress that may interfere with the diuresis, it was necessary first to train the animals to the conditions of the experiment, particularly the use of the stomach tube, for a week.

(*c*) *Normal Dog.*—A collie bitch of 10 kg. weight was used. A period of 3 weeks' training was necessary before the full co-operation of the dog was gained.

The night before the experiment, food was removed from the cage; the dog had free access to water. At 9 a.m. the next morning, 70 ml. of tap water, divided into three portions, was given by stomach tube. Glycyrrhetinic acid (350 mg.) was suspended in the first portion and washed down by the last two. The dog was then allowed free movement with no access to food or water for 2 hr. At 11 a.m. a self-retaining catheter was introduced and the bladder emptied. The dog was placed in a Pavlov stand. At 11.15 a.m. 250 ml. of tap water was given by stomach tube, followed by 300 ml. at 11.55. From 12 noon, urine flow was recorded at 5 min. intervals for 1 hr. and 40 min. The total urine excreted from 11.15 till 3.05 was also measured. Throughout the experiment, everything was avoided which might antagonize the dog and all external interferences were cut down as far as possible. A constant temperature and complete quietness were essential.

In one experiment in which the dog received no glycyrrhetinic acid, 2.5 mg. DOCA was injected subcutaneously at 9 a.m. and the rest of the experiment continued as before.

(*d*) *Rats with Diabetes Insipidus.*—Female albino rats (120–150 g.) were used. The posterior lobe of the pituitary gland was sucked out under ether anaesthesia, most of the anterior lobe being left intact. The operation was kindly performed by Dr. M. Vogt. The rats were given 5% glucose drinking solution for 2 days and ordinary rat cubes for food. Penicillin was administered as a protective measure for 2 days. In order to discover which rats were diabetic, 2 days after the operation each rat was put in a metabolism cage and was freely supplied with water and cubes. Urine was collected over 24 hr. and the volume compared with that from intact rats of the same batch. Later, collection of urine was carried out over-night for 18 hr. with water only supplied, the feeding being done late every afternoon. The urine volume was again compared with that of intact control rats. Overnight urine collections were continued during the glycyrrhetinic acid tests to measure the degree of diabetes insipidus present in every test rat during the whole procedure. This was necessary because in some of the operated rats only a transient phase of diabetes was achieved.

The test for the antidiuretic action of glycyrrhetinic acid was carried out only on those rats in which the operation proved successful. All the rats used in these tests were diabetic throughout the test period.

The method used to test the effect of glycyrrhetinic acid on diuresis in the rats with diabetes insipidus was the same as that employed for the non-anaesthetized intact rats. The same dose (100 mg.) of the acid was introduced by stomach tube 3 hr. before the 12 ml. water load. A 2 hr. urine collection was carried out. Each rat acted alternately as control and as test animal. Two control values and two test values were obtained from each rat whenever possible. Before subjecting the rats to the operation they were trained for one week to get them accustomed to the stomach tube and urine collection procedures.

Water Absorption from the Alimentary Canal

Normal Rats.—Adult albino rats of about 170 g. weight were trained for one week to being given water by stomach tube. The actual experiment consisted in fasting the rats overnight, allowing them free access to drinking water. They were then divided into pairs, selected to be identical in sex and weight. At 11 a.m. 1 ml. of propylene glycol was administered by stomach tube to one rat of each pair and 100 mg. of the acid suspended in the same volume of propylene glycol to the other. The rats were kept in a quiet, warm place for 3 hr., when they received 5% of the body weight of warm tap water by stomach tube. They were killed 15 to 60 min. later and their intestinal tracts were dissected out and weighed after removing the formed contents of the rectum and caecum.

Throughout the experiment, the two rats of each pair were handled together and kept in the same cage; conditions for the members of the same pair were therefore as identical as possible and the results obtained for the control and the glycyrrhetinic-treated rats comparable.

The dose of the acid, its mode of administration, and the period elapsing until the administration of the water load were chosen so as to duplicate the procedures in the diuresis experiments reported before, in the hope that such an arrangement would facilitate the assessment of the results.

In another series of experiments, a 300-mg. dose of the acid was given to the test animals, the contents of the caecum were not emptied before weighing the gut, and the liver was dissected out and weighed. The times allowed for water absorption were 15, 30, and 75 min.

RESULTS

Urinary Sodium Excretion

In glycyrrhetinic-acid-treated adrenalectomized rats (Table I) sodium excretion was not inhibited or altered to any significant degree if the collection of urine was continued for a period sufficiently long to allow urine to flow. With doses of 0.2 and 0.4 mg. glycyrrhetinic acid a 6 hr. collection was sufficient. When DOCA, 1.0 μg./rat, was administered under the same conditions significant sodium retention was achieved (P=0.03).

TABLE I

SODIUM EXCRETION IN ADRENALECTOMIZED RATS

Expt. No.	Time of Collecting Urine (hr.)	Propylene Glycol Only		Glycyrrhetinic Acid (GA) or DOCA			
		No. of Rats	Mean Na Excretion (mg.)	No. of Rats	Dose (μg.)	Mean Na Excretion (mg.)	Na Excretion (% of Control)
1	6	7	3·45	8	400 GA	3·78	109·6%
2	6	6	0·61	6	200 GA	1·45	240·0%
3	6	7	2·66	7	200 GA	2·25	84·6%
				7	1·0 DOCA	0·899	29·6%
4	6	5	2·53	6	400 GA	2·23	89·2%
				5	5·0 DOCA	0·573	22·6%
5	6	6	1·58	6	400 GA	1·29	84·3%
6	4	7	1·48	4 and 3*	400 GA	0·56	37·8%
7	4	7	1·66	4 and 3*	400 GA	0·39	23·5%
				7	1·5 DOCA	0·43	26·0%

* Number of animals which excreted no urine at all. They are included in the calculation of the mean Na excretion for the whole group.

When urine was collected over 4 hr. only, and a dose of 0.4 mg. of the acid was administered to each rat, the mean sodium excretion for the whole group was appreciably below that of the control group. Under these conditions, however, it was observed that a number of the glycyrrhetinic-acid-treated rats did not excrete any urine at all and thus did not contribute to the total excretion of sodium by the group. With a 4 hr. collection, 1.5 μg. DOCA caused a significant sodium retention ($P<0.01$), while the volume of urine collected from any rat was not appreciably smaller than that collected in 6 hr. In other experiments, the second sodium load was replaced by a load of 3.8 mg. potassium chloride in 2.0 ml. water. The group of rats receiving 400 μg. of glycyrrhetinic acid showed a significant increase in their mean urinary potassium output compared with the control group ($P<0.02$). See Table II.

TABLE II

POTASSIUM EXCRETION IN ADRENALECTOMIZED RATS AFTER A KCl LOAD OF 3·8 MG.

Controls				Glycyrrhetinic Acid (400 μg.)			
No. of Rats	Mean Na Excretion (mg.)	Mean K Excretion (mg.)	$\frac{K}{Na} \times 100$	No. of Rats	Mean Na Excretion (mg.)	Mean K Excretion (mg.)	$\frac{K}{Na} \times 100$
6	1·05	0·86	81·9	6	1·62	2·41	148·8

Blood Electrolytes

The results are shown in Table III. DOCA in 1.0 mg. doses given for five days successfully maintained the serum sodium of adrenalectomized rats above the normal level for the non-adrenalectomized rats of the same colony ($P<0.01$). The

TABLE III

EFECTS OF GLYCYRRHETINIC ACID (GA) ON SERUM ELECTROLYTES IN RATS

	Group	No. of Rats/ Group	Mean Serum Na (% of Normal)	Significance of Na Change	Mean Serum K (% of Normal)	Significance of K Change	Mean Gain in Weight (g.)	Survival %
Normal	0·25 ml. propylene glycol	8	100	—	100	—	+0·1	100
Adrenalectomized	0·25 ml. propylene glycol	10	88·7	From normal $P<0.001$	166·7	From normal $P=0.01-0.02$	−10·5	30
	1 mg. DOCA	8	105·9	From adrenalectomized non-treated $P<0.001$; From normal $P<0.01$	144·7	From adrenalectomized, non-treated—not significant; From normal—not significant ($P=0.1-0.2$)	+0·5	100
	3 mg. GA	10	91·3	From adrenalectomized non-treated—not significant; From normal $P<0.01$	163·5	From adrenalectomized non-treated—not significant; From normal $P=0.2$	−8·6	50

The doses are per day per rat of approx. 300 g. All injections were given subcutaneously for 5 days.

serum sodium of the DOCA-treated adrenalectomized rats was thus very much higher than that of the adrenalectomized propylene-glycol-injected controls ($P<0.001$). These controls showed the classical picture of adrenal deficiency; their serum sodium was below the normal level ($P<0.001$) and their serum potassium higher ($P<0.001$). The serum potassium of the DOCA-treated group showed a slight return towards normal, but was not significantly different from that of untreated adrenalectomized or normal rats.

Glycyrrhetinic acid did not cause a significant rise of serum sodium or a fall of serum potassium. The daily dose administered was equivalent to 600 mg. glycyrrhetinic acid for a 60 kg. man, a dose well within the range reported to be effective in man.

DOCA successfully maintained all the animals in the group alive and in good shape. The glycyrrhetinic acid group started to show weakness much earlier even than the adrenalectomized controls. The survival of the group, however, was slightly, but not significantly, longer than that of the controls.

The adrenalectomized animals treated with glycyrrhetinic acid or left without treatment showed a marked loss of weight; this was prevented by DOCA.

Diuresis

(*a*) *Anaesthetized Rats.*—Glycyrrhetinic acid caused an appreciable inhibition of diuresis. In a dose of 1 mg. intravenously to a 300 g. rat, it caused almost complete suppression of urine flow from which recovery was very slow, taking about 3–4 hr. The response, however, was not uniform. Some rats responded to a small dose of 200 μg. more markedly than other rats to bigger doses.

When given intravenously (Fig. 1) 0.1 ml. propylene glycol alone had no effect, whereas, when the same fluid contained 100 μg. of the acid, the diuresis diminished appreciably for 4 hr. and then did not regain the initial level, although this had previously been maintained practically constant for 2 hr.

The smallest effective dose for a 280 g. rat was around 40 μg. (Fig. 2); 150 μg. had a marked effect on this rat.

When the drug was injected intravenously, a slight haemorrhagic tinge was sometimes noticed in the urine. This tinge, however, was also noticed with propylene glycol alone.

(*b*) *Conscious Rats.*—The results are shown in Table IV. A variable degree of water retention

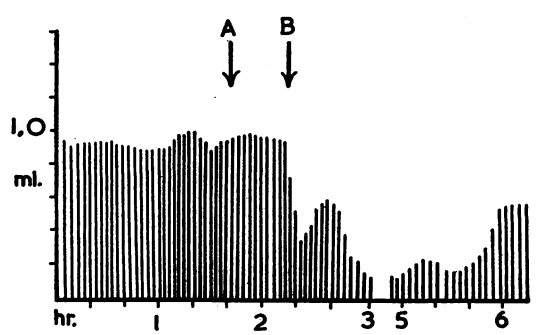

FIG. 1.—Effect of intravenous glycyrrhetinic acid on diuresis in a rat. Ordinate: Urine volume in ml./10 min. Abscissa: Time in hr. A: 0·1 ml. propylene glycol washed in with 0·6 ml. saline. B: same as A, but containing 100 μg. glycyrrhetinic acid.

TABLE IV

EFFECT OF GLYCYRRHETINIC ACID (GA) BY MOUTH ON DIURESIS IN NORMAL UNANAESTHETIZED RATS

Urine collected over 2 hr. after a load of 12 ml. H_2O.

No. of rat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
Controls (ml. urine)	8	10	10	7·5	8	6·5	8·5	9	7·5	8·5	9·5	6	9	8	8·29
100 mg. GA (ml. urine)	3·5	4·75	3·5	6·5	6·5	6·5	5	1·5	7	4·75	7·25	4	7·25	5·5	5·25
$\frac{GA}{Control} \times 100$	44	48	35	87	81	100	59	17	94	56	76	67	81	69	65

was observed after the administration of 100 mg. of glycyrrhetinic acid, each rat being used as its own control.

The mean water retention of the whole group was 35% ; only one out of 14 rats showed no inhibition of diuresis.

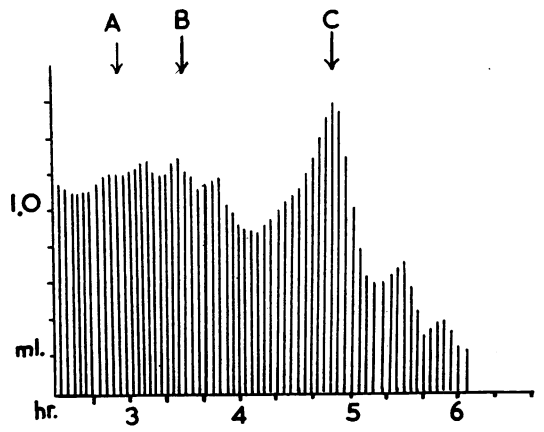

FIG. 2.—Effect of intravenous glycyrrhetinic acid on diuresis in a rat. Ordinate: Urine volume in ml./10 min. Abscissa: Time in hr. A: 0·05 ml. propylene glycol washed in with 0·1 ml. saline. B: same as A but containing 40 μg. glycyrrhetinic acid. C: Same as A but containing 150 μg. glycyrrhetinic acid.

(*c*) *Normal Dog.*—In control experiments without glycyrrhetinic acid, a mean rate of diuresis of 4 ml./min. was observed (Fig. 3). Glycyrrhetinic acid caused a depression of the rate of diuresis averaging 40% during the peak period. The volume of urine excreted over 3 hr. and 50 min. dropped by an average of 20% in the glycyrrhetinic acid experiments. In the one experiment with DOCA no such inhibition was observed.

To see whether the antidiuretic effect of glycyrrhetinic acid was due to a lowering of the blood pressure, the femoral vein of a 200 g. rat under urethane anaesthesia was cannulated and connected to a saline-filled burette ; the carotid artery was also cannulated and connected to a sensitive blood-pressure manometer. Propylene glycol produced a short-lived rise in blood pressure, and ethanol in the same dilution produced a smaller pressor effect. Glycyrrhetinic acid (0.4 mg. in 20% alcohol) certainly had no hypotensive effect ; it caused a rise in pressure similar to that due to the solvent.

Since the antidiuretic effect of glycyrrhetinic acid could not be attributed to a fall in blood pressure, attempts were made to discover what mechanisms were involved. Rats in which removal of the posterior lobe of the pituitary had been attempted were used, and their responses compared with those of conscious normal rats (Table V). In order to be able to do cross-over tests, the diuresis was followed in the unanaesthetized animal and the drug given by mouth.

TABLE V

DIURESIS IN RATS AFTER REMOVAL OF THE POSTERIOR LOBE OF THE PITUITARY.

The rats had free access to water; urine collected over 18 hr.

Rat Number	Urine Secretion as % of Control				
	6th Day after Operation	1st Experiment*		2nd Experiment*	
		Before	After	Before	After
1	190	93	311	—	—
2	141	224	239	376	372
3	190	93	710	—	—
4	270	195	533	106	286
5	1,400	1,830	350	370	330
6	2,360	3,170	510	650	400

* Before and after the corresponding glycyrrhetinic acid experiments (Table VI).

(*d*) *Rats with Diabetes Insipidus.*—In Table V the degree of diabetes insipidus attained by the operated rats is presented. The values are calculated as percentage of urine secretion by non-operated controls of the same batch. The first values given are those for the sixth night after operation ; they are followed by the figures for the night preceding, and that following, each glycyrrhetinic acid test. The figures in this table show that the degree of diabetes insipidus resulting from the operation was not steady from day to day. On the other hand, all rats showed a manifest increase in diuresis maintained till the animals died or were killed several weeks after the operation.

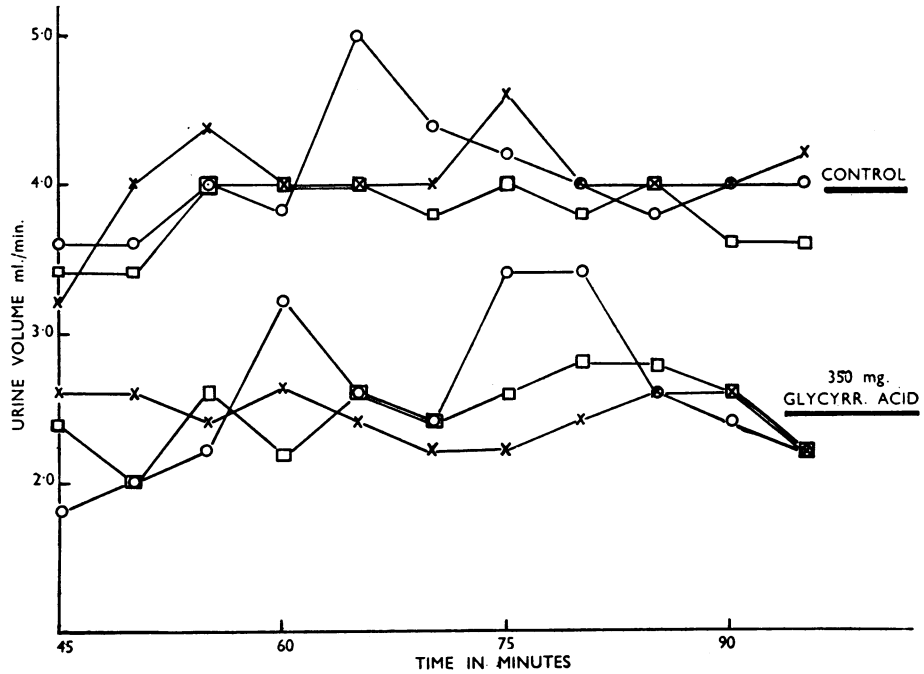

FIG. 3.—Five min. urine collections after two water loads of 250 ml. and 300 ml. Three tests and three control experiments carried out on the same dog. Ordinate: Urine volume in ml./ min. Abscissa: Time in min.

When glycyrrhetinic acid was administered to these rats, an inhibition of diuresis was observed (Table VI). This inhibition was comparable to that met with in non-diabetic rats. The mean water retention in these experiments was 41.5% compared with 35% in the non-diabetic rats.

To see whether delayed water absorption from the gastro-intestinal tract played any part in the reduction of water excretion, the method used by Heller and Smirk (1932) was employed to investigate the effect of glycyrrhetinic acid on water absorption.

Water Absorption from the Alimentary Canal

In the first series of experiments (Table VII) the weight of the gut, when expressed as percentage of body weight, was always higher after glycyrrhetinic acid than in the control rats. This was so whether the rats were killed 15 or 60 min. after the water load. No significant difference in the degree of water retention was observed between the two groups. Glycyrrhetinic acid can be detected in the stomach as a white precipitate 4½ hr. after administration.

In the second series of experiments, the dose of glycyrrhetinic acid was trebled and three groups,

TABLE VI

EFFECT OF ORAL GLYCYRRHETINIC ACID ON DIURESIS IN RATS WITH DIABETES INSIPIDUS

Urine collected over 2 hr. after a load of 12 ml. H_2O.

No. of rat	1	2		3	4		5		6		Mean
No. of experiment		A	B		A	B	A	B	A	B	
Control day (ml. urine)	8·75	8·5	7·0	10·5	3·0	2·75	10·75	9·5	10·0	6·75	7·75
After 100 mg. glycyrrhetinic acid (ml. urine)	7·75	6·5	6·25	6·0	1·5	0·75	5·5	6·0	5·5	2·0	4·78
$\frac{\text{Test day}}{\text{Control day}} \times 100$	88·5	76·5	89·0	57·0	50·0	27·0	51·0	63·0	55·0	30·0	58·5

TABLE VII

EFFECT OF ORAL GLYCYRRHETINIC ACID (GA) ON ABSORPTION OF WATER FROM THE GUT OF RATS

No. of Pair	Time After Giving Load (min.)	Wt. of Gut as % of Body Wt.		$\frac{b}{a} \times 100$*
		Control (a)	GA (b) 100 mg.	
1	15	8·0	8·9	111
2	15	8·6	9·6	112
3	15	9·0	9·4	105
4	15	7·4	8·6	116
5	15	8·3	10·0	120
	Mean	8·26	9·3	113
1	60	6·2	7·2	117
2	60	6·6	8·5	129
3	60	5·4	5·8	107
4	60	6·2	7·5	121
5	60	6·2	6·4	103
	Mean	6·12	7·08	115

* The differences between the mean of test and control rats are significant (P=0·01–0·001 after 15 min.; P=0·02–0·01 after 60 min.), provided the differences between each pair are used as the basis of the calculations.

killed at different time intervals, were employed. Each group consisted of ten rats, five receiving the acid and five acting as controls. As is shown in Table VIII and Fig. 4, the control groups give lower mean values than the test groups. The maximal difference in the second series was similar to that observed in the first series of experiments, where the dose of the acid given to each rat was only 100 mg. compared with 300 mg. given in the second series.

The mean gut weight, in both control and test animals, decreased gradually with time, indicating the gradual adsorption of the intestinal contents. The controls show the same kind of curve as that described by Heller and Smirk (1932); a rapid rate of absorption in the first ½ hr. after which the curve flattens out. In the test group, the same pattern is maintained with two differences; the first part of the curve is less steep and the whole curve is maintained at a higher level throughout.

The weights of the livers did not show any significant difference. Heller and Smirk found the liver to show the most marked increase in weight in response to hydration; in this experiment, however, the increase was not significant.

TABLE VIII

EFFECT OF GLYCYRRHETINIC ACID ON THE ABSORPTION OF WATER FROM THE GUT AND ITS RETENTION IN THE LIVER

Time since Water Load	No. of Rats in Group	Control		Glycyrrhetinic Acid	
		Mean Gut Wt. ± S.E.	Mean Liver Wt. ± S.E.	Mean Gut Wt. ± S.E.	Mean Liver Wt. ± S.E.
15 min.	10	12·6±0·54	4·8±0·41	13·8±0·84	5·1±0·25
30 ,,	10	9·7±0.32	4·6±0·29	12·4±0·67	4·7±0·25
75 ,,	10	9·1±0·43	4·8±0·16	10·2±0·58	4·9±0·1

When the rats received 100 mg. of the acid (Table VII), the mean weight of the gastro-intestinal tract was 6.12% of the body weight in the control group 1 hr. after the water load, and 7.08% in the glycyrrhetinic acid group. This is a difference of 0.96% of body weight or 19.2% of the water load.

Heller and Smirk (1932), in their original experiments, showed that the weight of the gut drops in the first hour and reaches a level between 6% and 7% of the body weight and that this level is maintained after that. They reported that absorption of a 5% load is completed 15–20 min. before the height of diuresis is reached. The 19.6% difference between control and glycyrrhetinic acid groups can, therefore, be taken as an estimation of the degree of water retention in the gut caused by 100 mg. of glycyrrhetinic acid.

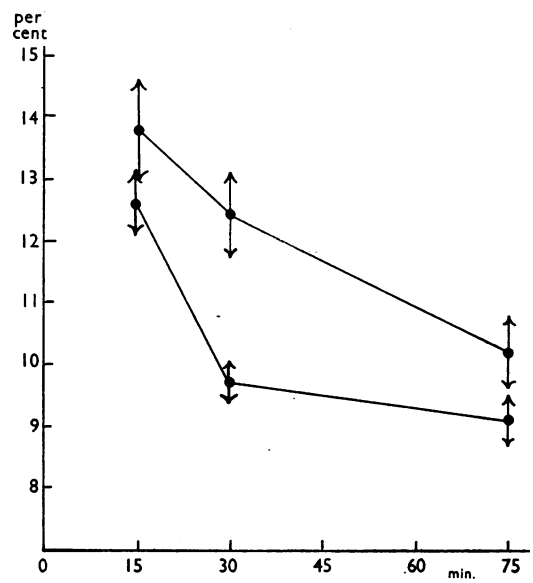

FIG. 4.—Effect of glycyrrhetinic acid on water absorption from the alimentary tract after a water load of 5% of body wt. Each point represents the mean wt. of the gut in five rats. The arrows show the standard error of the mean. Ordinate: Mean gut wt. as percentage of body wt. Abscissa: Time allowed for absorption in min. The upper curve represents the results of rats receiving 100 mg. glycyrrhetinic acid. The lower curve represents the results of control rats receiving the solvent only.

In the diuresis experiments reported in Table IV, the differences between control values and those following 100 mg. of the acid had a mean of 3.04 ml. or 25.3% of the load. Here, however, the load was almost double that used in the absorption experiments. The percentage of water retention in the gut is likely to be smaller with a bigger water load if the same dose of the acid is used. Therefore, it is unlikely that the delay in absorption of

water on giving glycyrrhetinic acid by mouth accounts for the whole 25.3% of the water retained.

To investigate further the role played by gastro-intestinal water retention in the anti-diuretic action of glycyrrhetinic acid the following experiment was carried out.

Albino rats (150 g.) were denied food for 24 hr. At 10 a.m. water was withdrawn and the animals divided into pairs of similar weight. At 12 noon, 0.2 ml. propylene glycol was injected intraperitoneally into one rat of each group. The other had 10 mg. of the acid dissolved in 0.2 ml. of propylene glycol. A water load of 12 ml. tap-water was given by stomach tube 10 min. later. The rats were transferred to metabolism cages and urine was collected for 150 min. At the end of the collection the rats were killed and their gastro-intestinal tracts dissected out. The contents of the caecum were emptied out and the gut weighed.

TABLE IX

EFFECT OF GLYCYRRHETINIC ACID ADMINISTERED INTRAPERITONEALLY ON DIURESIS AND WATER ABSORPTION

(Water load 12 ml.)

Group No.	Control		Glycyrrhetinic Acid	
	Urine % of Load*	Gut Weight % of Body Wt.	Urine % of Load*	Gut Weight % of Body Wt.
1	70	7·7	20	7·8
2	30	9·3	2·3	7·9
3	30	7·3	5	8·4
Mean	43	8·1	9·1	8·03

* Urine collection carried out over 150 min.

As shown in Table IX, 10 mg. of the acid intraperitoneally inhibited diuresis in the test rats. The water retained in these rats is not retained in the gut, as is evident by the identical weight of the gut in the control and test rats. In two of the rats, a minor degree of peritoneal effusion was noticed which, however, was not sufficient to account for the water retention.

DISCUSSION

Mineral Metabolism

The success reported by several workers (Groen, Willebrands, and Kamminga, 1951 ; Groen, Frenkel, and Kamminga, 1952 ; Card, Mitchell, Strong, Taylor, Tompsett, and Wilson, 1953 ; and Pelser, Willebrands, Frenkel, Heide, and Groen, 1953) in the treatment of adrenal deficiency with glycyrrhetinic acid and liquorice prompted the investigation of the mode of action of glycyrrhetinic acid on the salt metabolism of rats.

Under experimental conditions in which 1 μg. DOCA caused a significant degree of sodium retention, 400 μg. glycyrrhetinic acid caused no sodium retention, provided the collection of urine was continued long enough to allow urinary flow. A tendency to accelerate potassium excretion was only observed when a sizable potassium load was given.

While it may be justifiable to conclude that glycyrrhetinic acid does not behave like DOCA, as far as the salt metabolism of adrenalectomized rats is concerned, it was conceivable that, like Compounds B, E, or F of Kendall, which are only weak salt retainers, glycyrrhetinic acid may have a beneficial effect on adrenocortical deficiency. No such action on weight or survival time of adrenalectomized rats was, however, found.

Glycyrrhetinic acid, in doses of 3 mg./rat/day, failed to produce any significant change either in the serum sodium or potassium of adrenalectomized rats. This dose is equivalent to 600 mg. to a 60 kg. man. Pelser *et al.* (1953) found 60–100 mg. of the acid to be effective in Addisonian patients when given intramuscularly. Card *et al.* (1953) found 50 mg. doses q.i.d. to be effective in a patient suffering from Addison's disease. The dose employed in the reported rat experiments falls well within the effective range in terms of these human doses.

These findings, therefore, confirm the findings of Nelemans and Stamperius (1949) and Card *et al.* (1953) according to which glycyrrhetinic acid did not cause significant changes in the electrolyte balance or survival times of adrenalectomized rats.

The reported favourable effects of the acid in Addisonian patients in contrast to the lack of such an effect in rats suggest two possible explanations: (*a*) the drug may be metabolized in man in a different way, giving by-products which are active in this respect ; or (*b*) the mere water retention in Addisonian patients may have favourable effects under certain conditions.

Water Metabolism

In this series of experiments, it was possible to demonstrate the water-retaining effect of glycyrrhetinic acid in albino rats and in a dog.

In all the previous reports, the water retention by glycyrrhetinic acid has been related to its reported DOCA-like effect on mineral metabolism. Since no such effect was produced in adrenalectomized animals, another explanation was called for. It may also be emphasized that in many conditions DOCA has a diuretic, rather than an anti-diuretic, action (Gaunt, Birnie, and Eversole, 1949 ; Gaunt, 1943 ; Gellhorn and Ballin, 1946 ; Winter, 1952 ;

Kuhlman, Ragan, Ferrebee, Atchley, and Loeb, 1939 ; Winter and Ingram, 1943 ; Boss, Birnie, and Gaunt, 1949).

The mechanism of the anti-diuretic action of glycyrrhetinic acid appears to be a complex one. No marked change of the blood pressure was observed when the drug was administered intravenously, which suggested that low filtration pressure in the kidneys was not the cause of antidiuresis. The drug was effective in causing water retention in rats with diabetes insipidus during the height of the diabetic phase. The rate of water retention in these diabetic rats was comparable to the rate of water retention in intact rats under the same conditions. The posterior lobe of the pituitary does not therefore seem to play a role in this mechanism.

The drug, when given by mouth in doses identical with those used in the diuresis experiments, caused a significant degree of water retention in the gut. The percentage of water retained in the diuresis experiments, however, was somewhat higher than the percentage of water retained in the gut. That other mechanisms are involved is shown by the fact that, intraperitoneally, the drug in small doses caused a marked suppression of diuresis and no water retention in the gut. In addition, minute doses administered intravenously to rats anaesthetized with alcohol caused a marked depression of diuresis. In the sodium balance experiments, the drug was observed to cause water retention when administered subcutaneously.

By mouth, glycyrrhetinic acid seems to cause water retention in the alimentary canal as well as in the internal compartments of the body. The second effect may be due to the tubular reabsorption of water in the kidneys as suggested by Molhuysen, Gerbrandy, de Vries, de Jong, Lenstra, Turner, and Borst (1950).

The acid is precipitated in the stomach when introduced in propylene glycol and can still be detected there 4½ hr. after administration. This fact provides a possible explanation of the greater efficacy of parenteral as compared with oral administration.

SUMMARY

1. The reported DOCA-like activity in man of liquorice extracts and glycyrrhetinic acid has been investigated in animals.

2. When these drugs were given to adrenalectomized rats no effect on the sodium balance was observed. Only during the period in which diuresis was suppressed was there a retention of sodium. The increase in potassium excretion was not significant unless the level of the extracellular potassium was highly elevated.

3. Prolonged treatment of adrenalectomized rats with glycyrrhetinic acid caused no significant improvement in the level of serum electrolytes, the body weight, or the survival time.

4. Given by any route, the acid had an antidiuretic effect. The posterior lobe of the pituitary is not involved in this action of the drug ; after parenteral administration, the effect is perhaps produced by a stimulation of the tubular reabsorption of water.

5. When given by mouth, the drug delays water absorption from the alimentary tract. The delay is sufficient to contribute to the water-retaining effect of the drug when administered orally.

6. It is concluded that the actions of the drug are different from those of DOCA.

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