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The effect of fourteen day treatment with diuretics on mouse blood sugar and glucose tolerance

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Treatment of mice for 14 days with ethacrynic acid or frusemide (100 mg/kg daily orally) increased the fasting blood sugar and liver glycogen. Oral glucose tolerance was altered so that blood sugar concentrations in drug-treated groups were significantly above control values. The failure of the drugs to affect intravenous glucose tolerance and sensitivity to insulin or to tolbutamide suggested that the decrease in the oral glucose tolerance was attributable to some effect of the drug treatments on the rate of absorption of glucose from the gastrointestinal tract. Treatment with hydrochlorothiazide (100 mg/kg daily orally) exerted no effect on blood sugar, liver glycogen or glucose tolerance.

The ability of benzothiadiazine diuretics (Goldner, Zarowitz & Akgun, 1960; Shapiro, Benedek & Small, 1961), ethacrynic acid (Lebacq & Marcq, 1967) and frusemide (Jones & Pickens, 1967; Hutcheon & Leonard, 1967) to impair glucose tolerance in some patients has been reported but the underlying mechanism is not clear. Attempts to investigate the phenomenon in experimental animals have produced conflicting results; according to Weller & Borondy (1965) administration of thiazide diuretics to rats over a period of days impaired glucose tolerance, but other workers (Watson, van Pelt & Winter, 1964; Senft, Losert & others, 1966; Guidox, 1969) found no change in either glucose tolerance or blood sugar. Little information has appeared in the literature on the effects of administration of ethacrynic acid or of frusemide for a period of days on blood sugar or glucose tolerance in the experimental animal. Fourteen day oral treatment with frusemide has been reported to diminish oral glucose tolerance in rats and to reduce the *in vitro* utilization of glucose by epididymal adipose tissue (Weller & Borondy, 1967). On the other hand, Senft & others (1966) found 28-days of oral treatment with frusemide did not affect glucose tolerance in the rat. We have investigated the effects upon blood sugar and glucose tolerance of 14-day administration of ethacrynic acid, frusemide or hydrochlorothiazide to mice. Some of these results were presented in preliminary form to the British Pharmacological Society (Foy & Furman, 1969).

METHODS

Female mice, 30–35 g were randomly distributed into groups of 10, for assignment to drug or control treatment. Drugs were suspended in 5% acacia and administered orally, by stomach tube, in a volume of 5 ml/kg. Control animals received the suspending agent alone. The animals were allowed free access to food and water during treatment; pair- or force-feeding techniques were not used. All tests were made 18–20 h

after the final drug administration, during which time the mice were deprived of food but allowed water freely.

A fasting blood sample was removed from the femoral vein under light ether anaesthesia and 2 h later the animals were subjected to one of the following tests.

Oral glucose tolerance test (OGTT). The mice were treated with glucose (5 g/kg, orally) and blood removed 30, 60, 90 or 120 min later, a different group of animals (10 drug treated and 10 control) being used for each time interval.

Intravenous glucose tolerance test (IVGT). The mice were treated with glucose (1 g/kg, i.v. into a tail vein, 10 ml/kg of a 10% w/v solution being given over 1 min), blood being removed 10, 20 or 30 min later; a different group of mice (10 drug treated and 10 control) was used for each time interval.

Insulin or tolbutamide sensitivity. The mice were treated with insulin (0·5 units/kg, i.v.) or tolbutamide (100 mg/kg, i.v.), blood being removed 30 min later. Blood sugar was determined on 0·05 ml samples by the microcolorimetric copper reduction technique of Haslewood & Strookman (1939).

Liver samples, of about 200 mg (wet weight) were rapidly removed from mice killed by cervical dislocation and liver glycogen was determined by the phenolsulphuric acid method of Montgomery (1957) after potassium hydroxide digestion and ethanol precipitation.

Urine was collected from mice housed individually in mouse metabolism cages. The animals were treated with drug or control and urine was collected for an 8 h period, after which the mice were returned to their normal cages to feed and drink, because the design of the metabolism cages did not permit these activities without contamination of the urine. This procedure was repeated on each of the 14 days of treatment. Urinary sodium and potassium concentrations were determined using an Eel flame photometer.

Statistical significance was assessed using Student's *t*-test or analysis of covariance. A P value of 0·05 or less was assumed to indicate statistical significance.

RESULTS

Fasting blood sugar. Treatment with ethacrynic acid (10 or 50 mg/kg daily) produced no effect upon fasting blood sugar. A dose of 100 mg/kg daily produced a consistent elevation of fasting blood sugar (e.g. control — 77 $\pm$ 3 mg/100 ml, ethacrynic acid — 105 $\pm$ 4 mg/100 ml) which was statistically significant in six of seven repeat experiments. Treatment with frusemide (10 or 25 mg/kg daily) produced no effect upon fasting blood sugar in any of five repeat experiments. A daily dose of 100 mg/kg resulted in an elevation of the fasting blood sugar (e.g. control 71 $\pm$ 3 mg/100 ml, frusemide 87 $\pm$ 7 mg/100 ml) which was significant in three of six repeat experiments. Hydrochlorothiazide (50 or 100 mg/kg daily) exerted no effect upon fasting blood sugar.

Liver glycogen concentrations. Treatment with ethacrynic acid or frusemide (100 mg/kg daily) but not hydrochlorothiazide (100 mg/kg daily) produced a significant increase in fasting glycogen concentrations (Table 1).

Oral glucose tolerance (OGT) (Fig. 1). Ethacrynic acid or frusemide (100 mg/kg daily) altered the OGT, the blood sugar concentration being significantly elevated relative to the controls at 30 and up to 60 min (ethacrynic acid) or at 60 and 90 min

(frusemide) after the glucose load. At 50 mg/kg daily, ethacrynic acid produced a similar trend with a significant elevation in blood sugar at 30 min post-glucose but not at 60 min. Doses of ethacrynic acid less than 50 mg/kg and of frusemide less than 100 mg/kg exerted no significant effect on OGT. Hydrochlorothiazide (50 and 100 mg/kg daily) had no effect.

Table 1. *Effect of 14-day oral treatment with diuretics on fasting liver glycogen concentrations.*

Treatment	Liver glycogen mg/g wet weight tissue ± s.e. Control	Diuretic treated	*P*
Ethacrynic acid	8·4 ± 1·2	18·3 ± 2·4	<0·001
Frusemide	8·1 ± 1·8	17·9 ± 1·9	<0·005
Hydrochlorothiazide	6·7 ± 1·8	6·4 ± 1·6	—

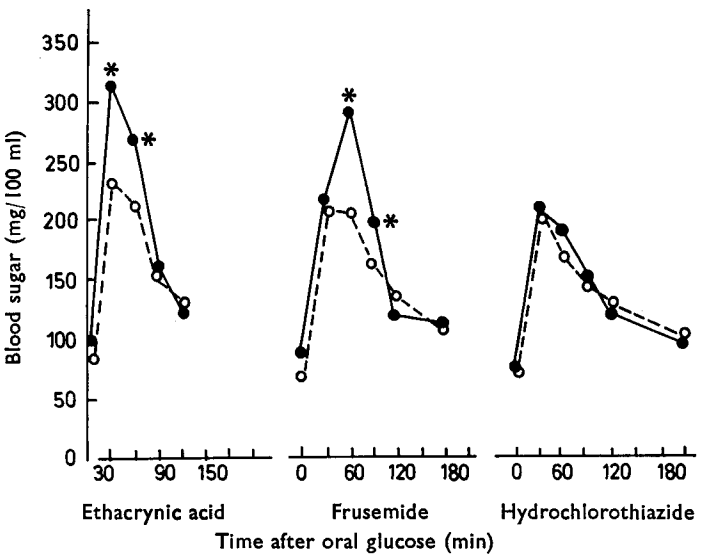

FIG. 1. The effect of 14-day oral treatment with ethacrynic acid, frusemide or hydrochlorothiazide (100 mg/kg daily) on oral glucose tolerance. Each point represents the mean of ten observations. The standard errors have been omitted for clarity. * Indicates a statistically significant difference between the effects of drug and control treatment as assessed by analysis of covariance.

Intravenous glucose tolerance (IVGT), insulin sensitivity, tolbutamide sensitivity. Oral daily treatment with ethacrynic acid or frusemide (100 mg/kg daily), exerted no effect on IVGT (Table 2). The insulin or tolbutamide sensitivities of the mice were similarly unaffected by treatment with these diuretics or with hydrochlorothiazide (Fig. 2).

Urine volume and the urinary excretion of sodium and potassium. The urine volume and urinary excretion of sodium and potassium produced under the influence of ethacrynic acid or frusemide, in the 8 h after drug administration, were similar, while for hydrochlorothiazide these parameters were much less (Fig. 3). With each drug the responses were repeatable on each day of administration, although the daily responses to ethacrynic acid appeared to increase over the first week.

Adrenal weight. The mean adrenal weight in ethacrynic acid-treated mice (100

mg/kg daily for 14 days) was 8·6 ± 0·3 mg wet wt/mouse (n = 8) compared with 6·9 ± 0·5 mg wet wt/mouse (n = 8) in the controls. The difference was statistically significant ($P < 0·02$). The mean adrenal weight in frusemide treated mice (100 mg/kg daily for 14 days) was 8·6 ± 0·7 mg wet wt/mouse (n = 6) compared with 7·0 ± 0·6 wet wt/mouse (n = 6) in the controls. The difference just failed to reach statistical significance ($P < 0·1 > 0·05$).

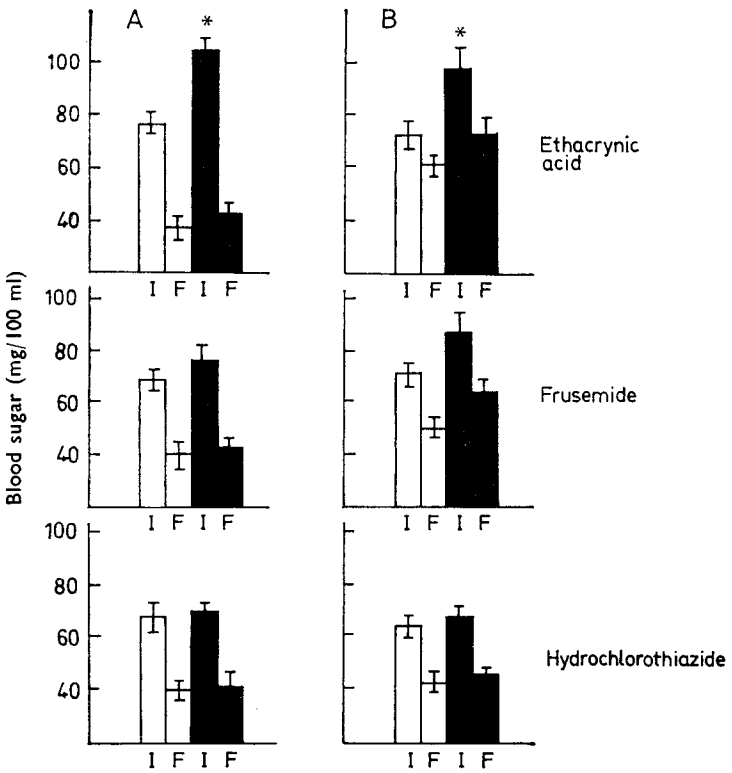

FIG. 2. The effect of 14-day treatment with ethacrynic acid, frusemide or hydrochlorothiazide (100 mg/kg daily) on the blood sugar response to (A), insulin (0·5 units/kg, i.v.) or (B), tolbutamide (100 mg/ kg, i.v.). Each column represents the mean (±s.e.) of ten observations. Open columns refer to control groups. Solid columns refer to drug-treated groups. I refers to fasting blood sugar concentrations. F refers to blood sugar values 30 min after the injection of insulin or tolbutamide. * Indicates a statistically significant difference between fasting blood sugar concentrations in drug and control treated groups ($P < 0·05$).

Table 2. *Effect of 14-day oral treatment with ethacrynic acid or frusemide on intravenous glucose tolerance.*

Treatment	Blood sugar values mg/100 ml (±s.e.) at time after glucose load							
	Fasting	10 min	Fasting	15 min	Fasting	20 min	Fasting	35 min
Control	81 ± 5	237 ± 13	82 ± 3	190 ± 10	73 ± 3	151 ± 9	79 ± 6	120 ± 5
Ethacrynic acid 100 mg/kg daily	*101 ± 4	260 ± 16	94 ± 5	222 ± 14	*90 ± 5	160 ± 11	96 ± 6	132 ± 7
Control	75 ± 3	263 ± 20	69 ± 5	225 ± 19	72 ± 4	164 ± 8	71 ± 4	145 ± 6
Frusemide 100 mg/kg daily	*92 ± 3	269 ± 9	85 ± 5	234 ± 12	80 ± 4	171 ± 5	*90 ± 3	126 ± 7

* Statistically significant, drug and control, $P < 0·05$.

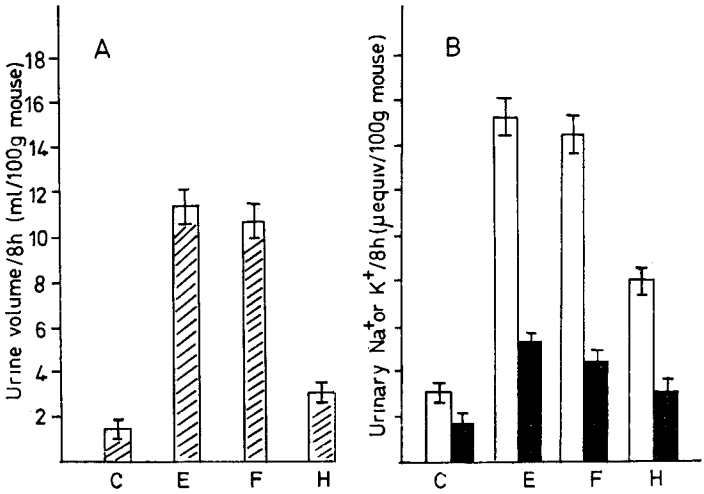

FIG. 3. The effect of orally administered ethacrynic acid (E), frusemide (F), or hydrochlorothiazide (H), (100 mg/kg) on urine volume (cross hatched columns) and the urinary excretion of sodium (open columns) and potassium (solid columns). Urine was collected in the 8 h after drug administration. The responses were repeatable on each of the 14 treatment days and a typical daily result is shown for each drug. Each column represents the mean (±s.e.) of five observations.

DISCUSSION

From the results it can be seen that treatment of mice with large daily doses of ethacrynic acid or frusemide results in elevation of concentrations of the fasting blood sugar and hepatic glycogen, and an alteration in oral glucose tolerance. Similar observations have been recorded in the rat after frusemide treatment (Weller & Borondy, 1967). However, several factors suggest that the observed alteration in glucose tolerance we found is not indicative of a diabetogenic effect of these diuretics. First, the failure of the drugs to affect IVGT, insulin sensitivity or tolbutamide sensitivity suggests that the disposal of a glucose load is probably normal and that the ability of the pancreas to secrete adequate insulin in response to a glucose load or tolbutamide is not impaired. Secondly, the observed alteration in glucose tolerance is characterized by elevations in blood sugar at early time intervals after the glucose load, with no significant delay in the return to control concentrations. This is reminiscent of the "lag-storage" type of glucose tolerance curve described by McLean (1927) in which the nature of the glucose tolerance curve is probably attributable to an increased rate of absorption of glucose from the gastrointestinal tract. The relation of the observed effects on blood sugar, liver glycogen and oral glucose tolerance to the natriuretic, diuretic and kaliuretic effects of the drugs is not clear. However, it is of interest that such effects were produced only by ethacrynic acid and frusemide, which exerted very similar effects on renal excretion, but not by hydrochlorothiazide, which produced much smaller natriuretic and diuretic responses. It is possible that the elevation in fasting blood sugar and liver glycogen seen in ethacrynic acid- or frusemide-treated mice may be explained in terms of an elevation in corticosteroid secretion in response to the intense natriuresis and diuresis occurring during treatment, although such a stimulus as sodium depletion would be expected to promote the secretion of mineralo-

corticoids rather than glucocorticoids (Eisenstein & Hartroft, 1957; Hartroft & Eisenstein, 1957). The only, albeit circumstantial, evidence for an increase in corticosteroid secretion lies in the increase in adrenal weight, relative to the controls, seen in ethacrynic acid- and frusemide-treated mice. An alternative explanation for the increase in fasting blood sugar and liver glycogen concentrations could lie in some alteration in the feeding schedule induced by the drugs. Although no marked, prolonged, diuretic-induced anorexia seems likely in view of the normal weight maintenance of the treated mice, it is possible that the intense diuresis, natriuresis and kaliuresis following drug administration resulted in a transient period of anorexia. Thus, feeding in the treated mice would be delayed relative to the controls, resulting in a shorter time interval between the last food ingestion in the drug-treated, compared with the control, mice.

The failure of hydrochlorothiazide treatment to exert any significant effect on any parameter measured is in agreement with findings in the rat (Senft & others, 1966; Guidox, 1969; Watson & others, 1964).

From our work and that of others it appears that, in rats and mice there is no simple, short-term method for reproducing a state analagous to human diuretic-induced impairment of glucose tolerance.

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