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Effect of diuretics on mouse blood sugar following single dose administration

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Summary

1. Frusemide, in doses which were maximal or supramaximal in respect of its natriuretic, diuretic and kaliuretic properties, produced a dose dependent, transient hyperglycaemic response in the mouse.

2. Bilateral nephrectomy resulted in a complete abolition of the hyperglycaemic response.

3. The hyperglycaemic response was accompanied by a decrease in liver glycogen and was significantly attenuated by adrenalectomy, adrenal demedullation and pretreatment with dihydroergotamine (DHE).

4. In the nephrectomized mouse frusemide treatment produced a marked reduction in intravenous glucose tolerance and a reduction in the plasma concentration of immunoreactive insulin following glucose administration. The drug had no detectable effect upon insulin sensitivity.

5. Hydrochlorothiazide exerted no detectable effect on mouse blood sugar.

6. Ethacrynic acid hyperglycaemia was obtainable after intraperitoneal or intramuscular injection, but not after intravenous injection. Intravenously administered ethacrynic acid exerted no effect upon intravenous glucose tolerance or insulin sensitivity in the nephrectomized mouse.

Introduction

The ability of the benzothiadiazine diuretics to impair glucose tolerance in some individuals is well documented (Wilkins, 1959 ; Goldner, Zarowitz & Akgun, 1960 ; Shapiro, Benedek & Small, 1961 ; Wolff, Parmley, White & Okun, 1963 ; Weller & Borondy, 1965 ; Zamrazil, Dvorak, Oskera & Zamarazilova, 1967 ; Breckenridge, Dollery, Welborn & Fraser, 1967). A similar effect has also been reported following treatment with ethacrynic acid (Lebacq & Marcq, 1967 ; Andersen & Persson, 1968) or frusemide (Toivonen & Mustala, 1966 ; Jones & Pickens, 1967 ; Hutcheon & Leonard, 1967), although reports to the contrary appear in relation to both drugs (Dige-Petersen, 1966 ; Ozen, Sandalci & Berker, 1966 ; Feldman & Diamond, 1967 ; Jackson & Nellen, 1966 ; Schaefer, 1964 ; Morét, 1966 ; McKenzie, Fairley & Baird, 1966). Attempts have been made to investigate these effects using single dose administration in the experimental animal.

Ethacrynic acid (Tabachnick, Gulbenkian & Yannell, 1965 ; Foy, 1967 ; Wales, Grant & Wolff, 1968) and frusemide (Foy, 1967 ; Wales *et al.*, 1968) produce hyperglycaemia after acute intraperitoneal administration to the intact rat, and ethacrynic

acid is hyperglycaemic in the mouse (Foy & Furman, 1967). Formanek & Kenner (1966) showed oral glucose tolerance and insulin sensitivity to be impaired in the rat after acute administration of frusemide. The underlying mechanisms in the production of the acute hyperglycaemic response to ethacrynic acid and frusemide are not yet established and this paper describes results of an investigation into their effects on blood sugar in the mouse.

The effects of hydrochlorothiazide have also been investigated, since conflicting reports appear in the literature concerning the ability of diuretic benzothiadiazine derivatives to produce hyperglycaemia in the normal rat after acute administration (Watson, van Pelt & Winter, 1964 ; Senft, Losert, Schultz, Sitt & Bartelheimer, 1966 ; Tabachnick *et al.*, 1965 ; Foy, 1967 ; Guidox, 1969).

Methods

Female, white mice (Tuck No. 1 strain) weighing approximately 30 g were used throughout, the animals being allowed food and water *ad lib.* until the beginning of the experiment.

Blood samples were removed under light ether anaesthesia, from the femoral vein, not more than two blood samples (that is, one pretreatment and one post-treatment) being removed from any one animal. In some mice adrenalectomy was performed one week before use ; in others adrenal demedullation was performed 3 weeks before use. Adrenalectomized mice were given 0·9% sodium chloride solution in place of drinking water. Adrenal demedullated mice were similarly treated during the first week after surgery and thereafter given normal drinking water. Bilateral nephrectomy was performed, 4–6 h before use, through a dorsal skin incision, great care being taken to preserve intact the adrenals and their blood supply. Urine was collected (from pairs of mice housed together) using modified mouse Metabowls (Jencons, Ltd.).

Urinary sodium and potassium were determined using an Eel flame photometer. Blood sugar was determined by the microcolorimetric copper reduction technique of Haslewood & Strookman (1939) using 0·05 ml samples. Plasma immunoreactive insulin was estimated by the method of Hales & Randle (1963) using iodine-125 labelled insulin and insulin antibody precipitate obtained from the Radiochemical Centre, Amersham. Plasma was assayed using beef insulin standards. Although the use of beef insulin standards would probably result in an underestimate of the true immunoreactive insulin concentrations, it would appear that the method could be validly applied to mouse plasma as the various pretreatments used (fasting, glucose loading and alloxan administration) resulted in changes in plasma insulin concentrations in the anticipated directions (Fig. 1).

Liver glycogen was determined on 200 mg samples using the phenol-sulphuric acid method of Montgomery (1957) after ethanol precipitation from potassium hydroxide liver digests.

The following drugs were used and, unless otherwise stated, were dissolved in 0·9% sodium chloride solution at the stated pH: ethacrynic acid (Merck, Sharp & Dohme), pH 7·6 ; frusemide (Hoechst Pharmaceuticals), pH 8·5 ; hydrochlorothiazide (Ciba), pH 10·2 ; diazoxide (Allen & Hanburys), pH 10·8 ; bovine crystalline insulin (Calbiochem), pH 3·5 ; dihydroergotamine methane sulphonate (Sandoz) dissolved in propylene glycol and diluted in 0·9% sodium chloride solution. Control solutions

consisted of solvents for the above drugs having the same pH and composition. Control experiments were performed alongside all experiments in which drugs were used. Statistical significance was assessed using Student's *t* test, significance being accepted where $P<0.05$.

Results

Blood sugar

Table 1 shows the effect of various doses of frusemide and hydrochlorothiazide on mouse blood sugar. Frusemide in doses of 50, 100 and 200 mg/kg intraperitoneally produced a dose-dependent hyperglycaemic response. Lower doses exerted no effect on mouse blood sugar. The hyperglycaemic response to 200 mg/kg was transient, being at a maximum 1 h, and undetectable 3 h after injection.

Hydrochlorothiazide, in doses between 1 and 200 mg/kg, exerted no detectable effect upon mouse blood sugar up to 5 h after intraperitoneal injection.

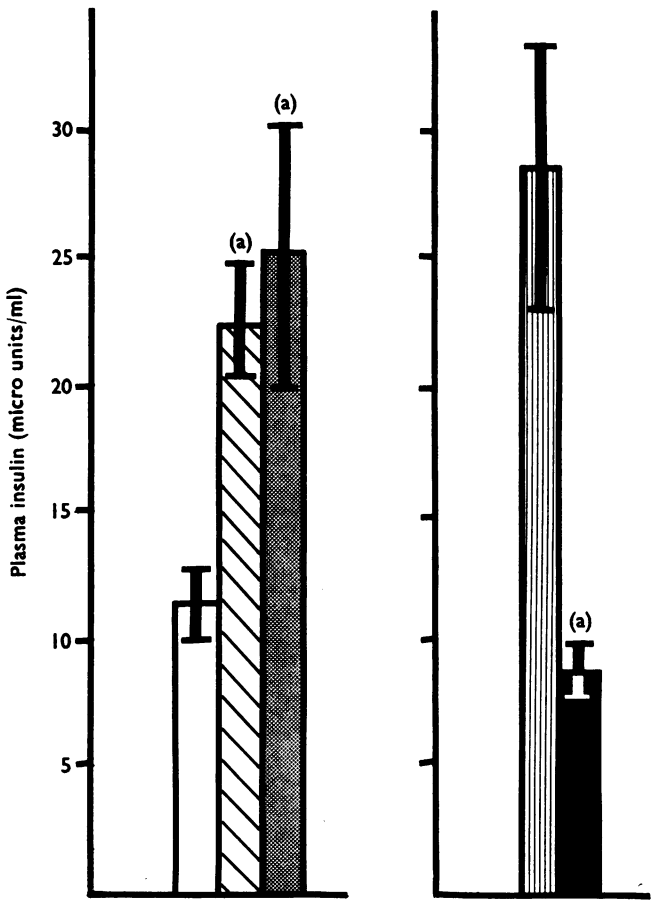

FIG. 1. Plasma concentrations of immunoreactive insulin in mice subjected to various treatments. The insulin values are expressed in terms of a beef insulin standard. (□), Fasted; (▩), glucose treated—oral; (⁘), glucose treated—intravenous; (|||), normal—fed *ad lib.*; (■), alloxan (diabetic). Each column represents the mean (±S.E.) of ten observations. (a), Statistically significant difference between insulin concentrations in treated and control mice ($P<0.05$).

From Table 2 it can be seen that ethacrynic acid was capable of producing hyperglycaemia after intraperitoneal or intramuscular injection but not after intravenous injection. Ethacrynic acid solutions appeared to be irritant when administered intraperitoneally or intramuscularly. Frusemide hyperglycaemia was manifest after administration by any of the three routes.

TABLE 1. *Effect of intraperitoneally administered frusemide or hydrochlorothiazide upon mouse blood sugar*

Drug	Dose (mg/kg) [*]	Time after injection (h)	Blood sugar (mg/100 ml) Initial	Final	Level of significance
Frusemide	0 [10]	1	132·0±4·0	140·0± 5·6	—
	10 [10]		130·0±5·3	142·0± 5·1	
	0 [10]		126·4±4·8	134·5± 4·8	—
	25 [10]		125·9±5·3	140·8± 6·4	
	0 [10]		129·7±3·2	121·5± 4·7	$P<0·02$
	50 [10]		128·0±5·1	143·8± 7·3	
	0 [10]		125·3±5·1	138·1± 3·2	$P<0·005$
	100 [10]		120·3±5·4	160·8± 5·3	
	0 [10]		123·8±4·9	144·2± 2·9	$P<0·001$
	200 [10]		124·9±5·3	215·9±15·9	
Frusemide	0 [10]	2	118·2±3·4	113·4± 6·9	$P<0·05$
	200 [10]		127·7±3·8	140·9± 9·9	
Frusemide	0 [10]	3	123·0±4·8	103·0± 3·7	—
	200 [10]		122·3±5·6	109·0± 4·6	
Hydrochlorothiazide	0 [10]	1	116·0±4·5	129·9± 9·1	—
	1 [10]		112·0±7·2	124·9± 5·6	
	0 [10]		123·2±6·1	144·1± 8·0	—
	5 [10]		125·9±5·6	140·8± 8·5	
	0 [10]		125·2±5·5	140·0± 8·4	—
	50 [10]		124·9±6·8	138·4± 7·1	
	0 [10]		107·5±4·6	126·2± 8·7	—
	100 [10]		106·5±5·1	123·9± 7·7	
	0 [10]		119·4±5·3	128·7± 7·6	—
	200 [10]		115·9±5·8	124·3± 5·7	
Hydrochlorothiazide	0 [10]	2	119·0±5·6	119·3± 5·7	—
	200 [10]		114·5±6·0	116·2± 5·3	
Hydrochlorothiaxide	0 [10]	3	129·9±7·1	113·9± 4·7	—
	200		130·0±7·6	112·5± 4·9	
Hydrochlorothiazide	0 [10]	4	117·8±4·1	95·4± 3·1	—
	200 [10]		119·5±5·6	96·0± 3·5	
Hydrochlorothiazide	0 [10]	5	122·1±4·6	90·1± 2·9	—
	200 [10]		117·0±3·9	91·5± 3·1	

[*] Indicates the number of observations.

TABLE 2. *Influence of the route of administration upon the hyperglycaemic response to ethacrynic acid or frusemide*

Drug		Dose (mg/kg) [*]	Route	Blood sugar (mg/100 ml) mean±S.E. Initial	Final	Level of significance
Ethacrynic acid		0 [10]	i.p.	118·9±5·2	122·4± 4·9	$P<0·001$
		50 [10]	i.p.	129·7±6·2	200·0±15·0	
	1 h	0 [10]	i.v.	114·8±4·3	101·1± 3·7	—
		50 [10]	i.v.	106·4±5·8	109·2± 6·1	
	2 h	0 [10]	i.v.	121·0±4·1	122·0± 4·9	—
		50 [10]	i.v.	117·2±4·8	115·3± 2·4	
		0 [10]	i.m.	118·1±4·5	104·0± 3·5	$P<0·001$
		50 [10]	i.m.	114·3±3·3	232·0±20·6	
Frusemide		0 [10]	i.p.	124·0±4·3	133·1± 3·3	$P<0·001$
		200 [10]	i.p.	127·8±6·1	224·0±14·9	
		0 [10]	i.v.	115·3±6·0	117·1± 5·5	$P<0·001$
		200 [10]	i.v.	119·0±5·1	198·8±14·1	
		0 [10]	i.m.	118·7±6·2	126·7± 5·7	$P<0·005$
		100 [10]	i.m.	109·5±5·6	166·8±11·1	

[*] Indicates the number of observations.

TABLE 3. *Effect of intraperitoneally administered ethacrynic acid, frusemide or hydrochlorothiazide upon mouse urine volume and the urinary excretion of sodium and potassium*

Drug	Dose (mg/kg) [*]	Urine Volume (ml/100 g mouse) mean±S.E. 1 h	5 h	Urinary Na^+ (μ Eq/100 g mouse at 5 h)	Urinary K^+ (μ Eq/100 g mouse at 5 h)
Ethacrynic acid	0 [10]	0·0	0·4±0·2	198±37	70±18
	1 [6]	2·4±0·5	3·8±0·3	639±50	94±9
	5 [6]	3·1±0·4	5·4±0·5	834±54	165±26
	10 [5]	5·3±0·2	7·8±0·1	1259±33	317±27
	20 [5]	4·5±0·3	7·5±0·4	1217±85	273±19
	50 [6]	2·6±0·3	6·8±0·5	974±72	211±17
		(3·6±0·3)	(7·4±0·4)	(1087±55	(231±13)
Frusemide	0 [10]	0·0	0·2±0·8	162±72	84±38
	2·5 [5]	1·7±0·2	1·8±0·2	290±34	110±18
	5 [5]	3·0±0·2	3·0±0·2	446±22	150±12
	10 [5]	3·2±0·5	3·5±0·4	530±61	137±20
	25 [5]	4·1±1·5	4·7±0·4	626±67	186±25
	50 [6]	4·3±0·2	6·9±0·4	1010±82	193±21
	100 [6]	4·9±0·2	8·4±0·6	1250±49	312±20
	200 [6]	4·3±0·2	7·6±0·8	1097±57	239±14
		(4·3±0·4)	(7·6±0·2)	(1128±58)	(260±16)
Hydrochlorothiazide	0 [10]	0·0	0·8±0·3	193±63	77±23
	1 [5]	0·5±0·2	2·1±0·5	533±91	150±22
	2 [5]	1·2±0·4	2·9±0·4	729±80	214±13
	10 [5]	1·2±0·2	3·0±0·5	709±82	167±23
	20 [5]	1·0±0·2	3·0±0·5	654±27	191±15
	50 [5]	1·6±0·2	3·0±0·5	712±65	190±16
	200 [5]	0·9±0·2	3·6±0·5	803±95	230±22

[*] Indicates the number of pairs of mice in a treatment group.
Values in parentheses indicate values obtained after intravenous administration of the same dose of drug.

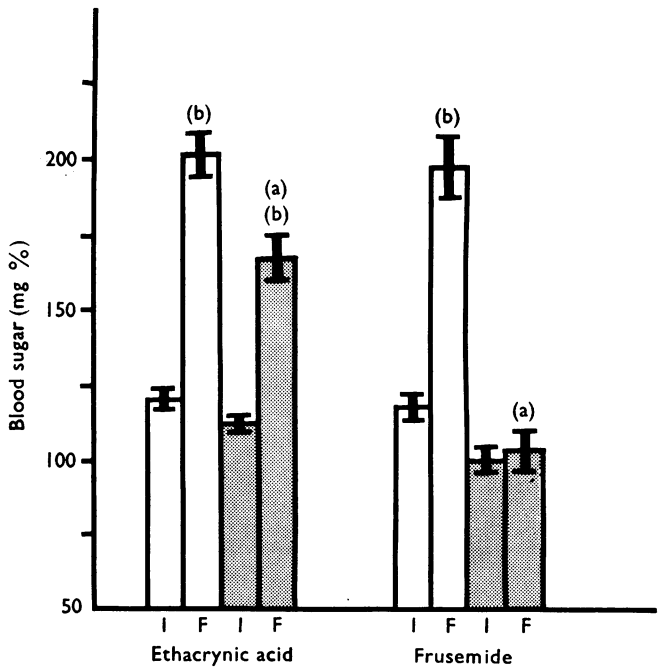

FIG. 2. Effect of bilateral nephrectomy (4–6 h before use) upon the hyperglycaemic response evoked by ethacrynic acid (2 h after 50 mg/kg i.p.) or frusemide (1 h after 200 mg/kg i.p.). (□), Sham-operated; (⁘), nephrectomized. Each column represents the mean (±S.E.) of ten observations. (I), Initial (pretreatment) blood sugar values; (F), final (post-treatment) blood sugar values. (a), Statistically significant difference between the effect of the two procedures ($P<0·05$); (b), statistically significant difference between pre- and post-treatment values ($P<0·05$).

Natriuresis, diuresis and kaliuresis

Table 3 shows the effects of various doses of ethacrynic acid, frusemide and hydrochlorothiazide upon the volume of mouse urine and the urinary excretion of sodium and potassium. Maximal natriuretic, diuretic and kaliuretic responses were evoked by 10 mg/kg ethacrynic acid, 50 mg/kg frusemide and 2 mg/kg hydrochlorothiazide.

The maximal responses elicited by ethacrynic acid and frusemide were quantitatively very similar and with both drugs at least 60% of the total diuretic response was achieved within 1 h of injection. The maximal diuresis evoked by these latter two agents was at least twice that evoked by hydrochlorothiazide. Sodium excretion under the influence of hydrochlorothiazide, although less than that under the influence of ethacrynic acid or frusemide, was not proportionately less in relation to the lower urine volume. Potassium excretion differed little between the three drugs.

Bilateral nephrectomy

Bilateral nephrectomy (performed 4–6 h before use) significantly reduced the hyperglycaemic response elicited by ethacrynic acid and completely abolished the hyperglycaemic response to frusemide, the responses being fully manifest in sham-operated animals (Fig. 2).

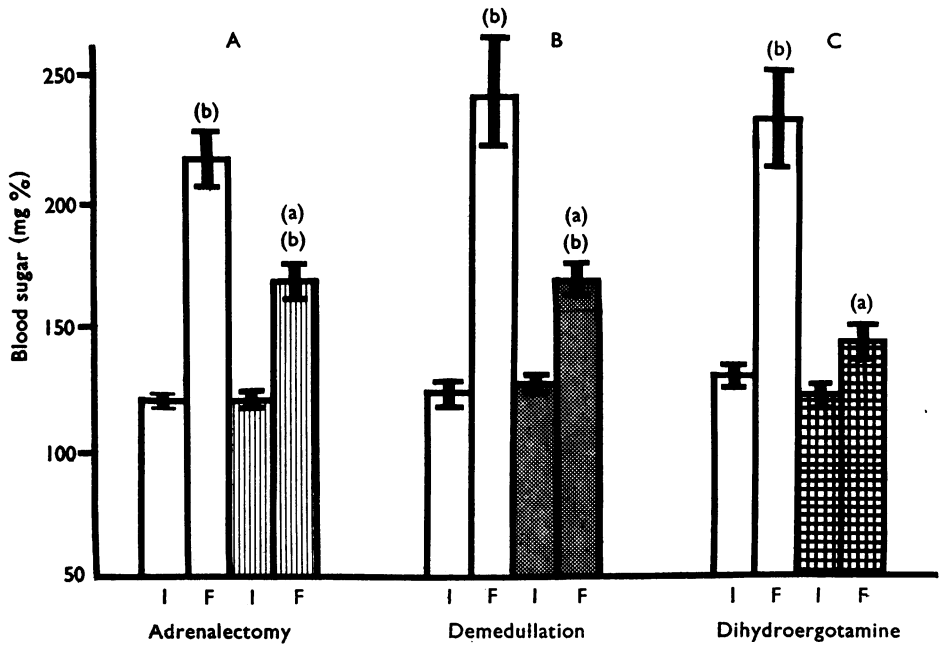

FIG. 3. Influence of adrenalectomy, adrenal demedullation or pretreatment with dihydroergotamine (10 mg/kg s.c. 60 min before diuretic injection) on the hyperglycaemic response evoked by frusemide (1 h after 200 mg/kg i.p.). A: (□), Sham-operated; (|||), Ad-x. B: (□), Sham-operated; (∴). demed. C: (□), Control; (▒), DHE. Each column represents the mean (±S.E.) of ten observations. (I), Initial (prefrusemide) blood sugar value; (F), final (postfrusemide) blood sugar value. (a), Statistically significant difference between the effects of different treatments (that is between DHE and control, or operated and sham-operated) ($P<0.05$); (b), statistically significant difference between pre- and postfrusemide blood sugar values ($P<0.05$).

Adrenalectomy, adrenal demedullation or pretreatment with dihydroergotamine (DHE)

Figure 3 shows that the hyperglycaemic response to frusemide was significantly attenuated by adrenalectomy or by adrenal demedullation, and very markedly and significantly attenuated by pretreatment with DHE.

Liver glycogen

In the intact animal frusemide treatment resulted in a significant reduction in liver glycogen 1 h after injection ($P<0{\cdot}05$), the values obtained being as follows:

Liver glycogen in controls (mean $\pm$ S.E.) $37{\cdot}5 \pm 3{\cdot}1$ mg/g wet weight; [$n=10$].

Liver glycogen in frusemide (mean $\pm$ S.E.) $21{\cdot}0 \pm 3{\cdot}5$ mg/g wet weight treated mice (200 mg/kg intraperitoneally); [$n=10$].

Intravenous glucose tolerance and insulin sensitivity

In order to eliminate the complications in interpretation which would arise in the intact animal from diuretic-induced alterations in extracellular fluid volume and possible alterations in the renal excretion of glucose, the effects of ethacrynic acid and frusemide upon intravenous glucose tolerance and insulin sensitivity were assessed in the nephrectomized mouse. Treatment with ethacrynic acid (50 mg/kg intravenously) had no effect upon glucose tolerance or insulin sensitivity (Fig. 4). Frusemide treatment produced a marked reduction in intravenous glucose tolerance without affecting the hyperglycaemic action of exogenous insulin (Fig. 4).

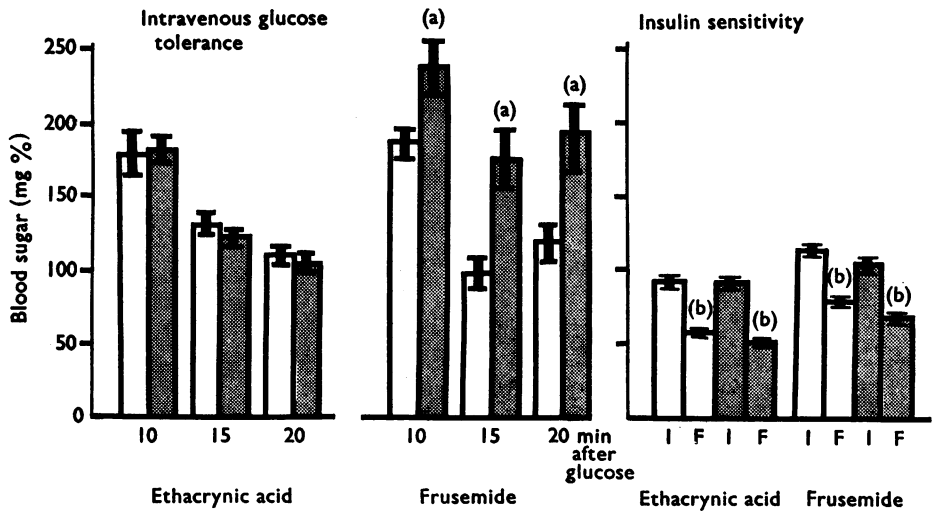

FIG. 4. Effect of ethacrynic acid (50 mg/kg i.v.) or frusemide (200 mg/kg i.v.) upon intravenous glucose tolerance and insulin sensitivity. Ninety minutes after diuretic administration, glucose (1 g/kg i.v.) or insulin (0·1 u/kg i.v.) was injected, blood samples being removed at the time intervals shown after glucose injection and at 30 min after insulin injection (□), Control; ( ), diuretic treated. Each column represents the mean (±S.E.) of ten observations. (I), initial (prediuretic) blood sugar values; (F), final (postinsulin) blood sugar values. (a), Statistically significant difference between diuretic and control treatment ($P<0{\cdot}05$); (b), statistically significant difference between pre- and post-treatment values ($P<0{\cdot}05$).

Plasma concentrations of immunoreactive insulin

In these experiments frusemide was compared with diazoxide, since this agent is known to block insulin secretion in other species (Loubatières, Mariani & Alric, 1968). Frusemide and diazoxide were examined in the nephrectomized and intact mouse, respectively. It is evident (Fig. 5) that neither diazoxide nor frusemide had any real effect upon 'normal fed' insulin concentrations. However, diazoxide-treated mice are markedly hyperglycaemic 90 min after injection and thus a relative suppression of insulin release is inferred. Both drugs caused a significant reduction, compared with the controls, of plasma immunoreactive insulin concentrations following glucose administration.

Discussion

From the results it can be seen that frusemide treatment in the mouse was capable of producing an acute hyperglycaemic response as previously demonstrated in the rat (Foy, 1967; Wales *et al.*, 1968).

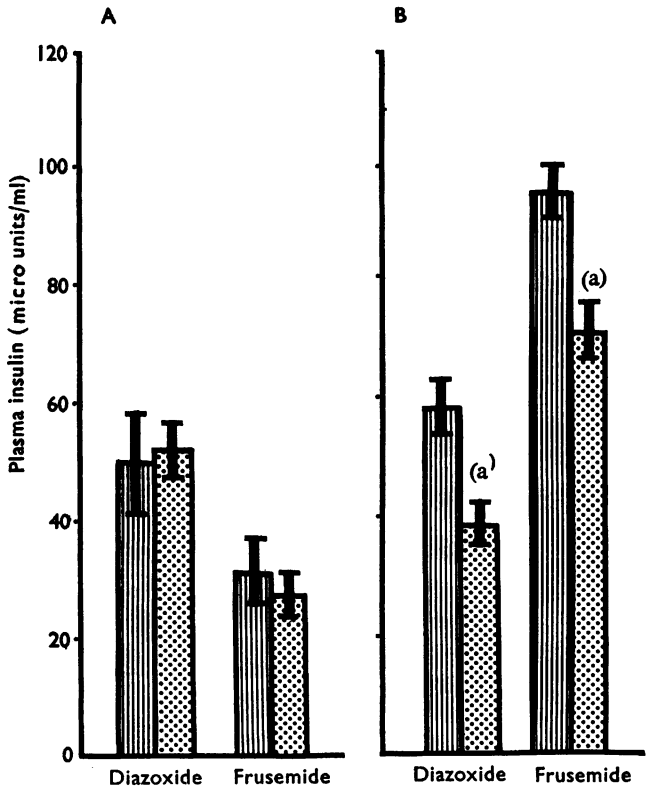

FIG. 5. Effect of diazoxide (50 mg/kg i.p. in normal mice) or frusemide (200 mg/kv i.v. in nephrectomized mice) upon plasma concentrations of immunoreactive insulin. In experiment A blood was removed 90 min after injection of drug (⁙) or control solution (|||). In experiment B blood was removed 2 min after the injection of glucose (1 g/kg i.v.), the glucose being injected 90 min after administration of drug or control solution. Each column represents the mean (±S.E.) of ten observations in experiment A or six observations in experiment B. (a), Statistically significant difference between drug and control treatments ($P<0{\cdot}05$).

Hydrochlorothiazide treatment failed to exert any detectable effect upon mouse blood sugar, this being in agreement with the findings, in the normal rat, of Watson *et al.* (1964), Senft *et al.* (1966) and Guidox (1969) although, using the rat, other workers (Tabachnick *et al.*, 1965 ; Foy, 1967) have shown hydrochlorothiazide to produce an acute hyperglycaemic response.

The failure of intravenously administered ethacrynic acid to produce hyperglycaemia in the intact mouse, or to affect glucose tolerance or insulin sensitivity in the nephrectomized mouse, together with the observed irritancy of ethacrynic acid solutions when administered intraperitoneally or intramuscularly, would suggest that, in the mouse, ethacrynic acid exerts no real effect on carbohydrate metabolism following acute treatment. The hyperglycaemia resulting from intraperitoneal or intramuscular injection seems to be associated with the irritant effect of the drug solutions, such an irritant effect possibly provoking the release of adrenal medullary catecholamines. A role for the adrenal medulla in the production of hyperglycaemia by ethacrynic acid has been suggested previously (Foy, 1967 ; Foy & Furman, 1967).

The abolition of frusemide hyperglycaemia by nephrectomy provided strong evidence that this response was in some way associated with the marked natriuretic, diuretic and kaliuretic properties of the drug. This would agree with the findings of Senft *et al.* (1966) who, using nephrectomized alloxan-diabetic rats, could not obtain the hyperglycaemic response that could be demonstrated in ' intact ' alloxan-diabetic animals. However, several points suggest that the renal responses, although obviously important, are not the sole factors involved. First, it is evident that some dose-effect relationship is present concerning the hyperglycaemic response, but all doses producing hyperglycaemia exert approximately the same (maximal) natriuretic, diuretic and kaliuretic effect. The observation is in agreement with the findings concerning ethacrynic acid hyperglycaemia in the mouse (Foy & Furman, 1967) and ethacrynic acid and frusemide hyperglycaemia in the rat (Wales *et al.*, 1968). Second, intravenously administered ethacrynic acid, whilst producing very similar renal responses to those produced by intravenously administered frusemide, failed to produce a hyperglycaemic response, further suggesting that the renal effects were not solely responsible for frusemide hyperglycaemia. Third, in the nephrectomized mouse, frusemide treatment produced a marked reduction in glucose tolerance. The reduction in glucose tolerance was associated with a significant reduction in the plasma concentrations of immunoreactive insulin following glucose loading. Such an effect has been demonstrated previously in relation to diazoxide hyperglycaemia in other species (Loubatières *et al.*, 1968) and diazoxide also appears to exert this effect in the mouse. The discrepancy between the results presented in this paper and those of Senft *et al.* (1966), who could not show any effect of frusemide on glucose-stimulated insulin secretion, cannot readily be explained.

The hyperglycaemic response to frusemide in the intact mouse appears to involve, at least in part, some adrenergic mechanism in view of the fairly rapid onset and transient nature of the response, the associated reduction in liver glycogen, the attenuation of the response by adrenalectomy and adrenal demedullation and the marked attenuation of the response by dihydroergotamine, an agent known to block adrenaline hyperglycaemia in the rat (Kennedy & Ellis, 1969) and in the mouse (Furman, 1970). From the foregoing it can be suggested that, in the intact mouse, the rapid reduction in extracellular fluid space accompanying frusemide diuresis

provokes the release of catecholamines. It is possible that the resultant glycogenolysis becomes manifest as hyperglycaemia in the case of frusemide but not ethacrynic acid, due to the apparent ability of the former drug to suppress the rate of disappearance of a glucose load. In view of the failure of frusemide to diminish the sensitivity of the mice to exogenous insulin, it is attractive to suggest that the impairment of intravenous glucose tolerance is due to the demonstrated suppression of glucose-stimulated insulin secretion. Further work is in progress to determine whether or not the suppression of glucose-stimulated insulin secretion by frusemide is due to a direct action of the drug on the β cells of the islets of Langerhans or to a non-specific alteration in pancreatic haemodynamics.

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REFERENCES

ANDERSEN, O. O. & PERSSON, I. (1968). Carbohydrate metabolism during treatment with chlorthalidone and ethacrynic acid. *Br. med. J.*, **2**, 798–801.

BRECKENRIDGE, A., DOLLERY, C. T., WELBORN, T. A. & FRASER, R. (1967). Glucose tolerance in hypertensive patients on long term diuretic therapy. *Lancet*, **1**, 61–64.

DIGE-PETERSEN, H. (1966). Ethacrynic acid and carbohydrate metabolism. *Nord. Med.*, **75**, 123–125.

FELDMAN, E. & DIAMOND, S. (1967). Ethacrynic acid—a non-diabetogenic diuretic. *Dis. Chest*, **51**, 282–287.

FORMANEK, K. & KENNER, T. (1966). Special features of the action of a new diuretic. *Br. J. Pharmac. Chemother.*, **26**, 27–33.

FOY, J. M. (1967). Acute diuretic induced hyperglycaemia in rats. *Life Sci.*, **6**, 897–902.

FOY, J. M. & FURMAN, B. L. (1967). Ethacrynic acid hyperglycaemia in the mouse. *Experientia*, **23**, 1039–1040.

FURMAN, B. L. (1970). Diuretic induced hyperglycaemia in the mouse. Ph.D. thesis, University of Bradford, page 81.

GOLDNER, M. G., ZAROWITZ, H. & AKGUN, S. (1960). Hyperglycaemia and glycosuria due to thiazide derivatives administered in diabetes mellitus. *New Engl. J. Med.*, **262**, 403–405.

GUIDOX, R. (1969). Effets diabétogènes des diurétiques thiazidiques et du solvant N-monométhylamide de l'acide acétique chez le rat. *Diabetologia*, **5**, 11–21.

HALES, C. N. & RANDLE, P. J. (1963). Immunoassay of insulin with insulin-antibody precipitate. *Biochem. J.*, **88**, 137–146.

HASLEWOOD, G. A. D. & STROOKMAN, T. A. (1939). A method for the estimation of "true" sugar in 0·05 ml blood. *Biochem. J.*, **33**, 920–923.

HUTCHEON, D. E. & LEONARD, G. (1967). Diuretic and antihypertensive actions of frusemide. *J. clin. Pharmac.*, **7**, 26–33.

JACKSON, W. P. V. & NELLEN, M. (1966). Effect of frusemide on carbohydrate metabolism, blood pressure and other modalities: a comparison with chlorothiazide. *Br. med. J.*, **2**, 333–336.

JONES, I. G. & PICKENS, D. T. (1967). Diabetes mellitus following oral diuretics. *Practitioner*, **199**, 209–210.

KENNEDY, B. L. & ELLIS, S. (1969). Interaction of catecholamines and adrenergic blocking agents at receptor sites mediating glycogenolysis in the rat. *Archs int. Pharmacodyn. Ther.*, **177**, 390–406.

LEBACQ, E. & MARCQ, M. (1967). A study of the mechanism of ethacrynic acid induced hyperglycaemia. *Revue fr. Étude clin. biol.*, **12**, 160–162.

LOUBATIÈRES, A., MARIANI, M. M. & ALRIC, R. (1968). The action of diazoxide on insulin secretion, medullo-adrenal secretion and the liberation of catecholamines. *Ann. N.Y. Acad. Sci.*, **150**, 226–241.

MCKENZIE, I. F. C., FAIRLEY, K. F. & BAIRD, C. W. (1966). A clinical trial of frusemide ("Lasix"). *Med. J. Aust.*, **1**, 879–886.

MONTGOMERY, R. (1957). Determination of glycogen. *Archs Biochem. Biophys.*, **67**, 378–386.

MORÉT, B. (1966). Zur behandlung des Diabetikers mit Saluretika. *Med. Klin.*, **61**, 382–385.

OZEN, M. A., SANDALCI, D. & BERKER, F. (1966). Ethacrynic acid and carbohydrate metabolism. *Am. J. med. Sci.*, **252**, 558–563.

SCHAEFER, H. F. (1964). Modern diuretics and their side-effects in diabetes mellitus. *Die. med. Welt*, **16**, 922–926.

SENFT, G., LOSERT, W., SCHULTZ, G., SITT, R. & BARTELHEIMER, H. K. (1966). Ursachen der Storungen im Kohlenhydratstoffwechsel unter dem Einfluss salfonamidierter Diuretica. *Naunyn Schmiedebergs Arch. exp. Path. Pharmak.*, **255**, 369–382.

SHAPIRO, A. P., BENEDEK, T. G. & SMALL, J. L. (1961). Effect of thiazides on carbohydrate metabolism in patients with hypertension. *New Engl. J. Med.*, **265**, 1028–1033.

TABACHNICK, I. I. A., GULBENKIAN, A. & YANNELL, A. (1965). Hyperglycaemic effect of benzothiadiazine and other diuretics. *Life Sci.*, **4**, 1931–1936.

TOIVONEN, S. & MUSTALA, O. (1966). Diabetogenic action of frusemide. *Br. med. J.*, **1**, 920–921.

WALES, J. K., GRANT, A. M. & WOLFF, F. W. (1968). Studies on the hyperglycaemic action of non-thiazide diuretics. *J. Pharmac. exp. Ther.*, **159**, 229–235.

WATSON, L. S., van PELT, S. M. & WINTER, C. A. (1964). Effect of chlorothiazide on blood glucose of rats. *Fedn Proc.*, **23**, 438.

WELLER, J. M. & BORONDY, P. G. (1965). Effect of benzothiadiazine drugs on carbohydrate metabolism. *Metabolism*, **14**, 708–714.

WILKINS, R. W. (1959). New drugs for the treatment of hypertension. *Ann. intern. Med.*, **50**, 1–10.

WOLFF, F. W., PARMLEY, W. W., WHITE, K. & OKUN, R. (1963). Drug induced diabetes. *J. Am. med. Ass.*, **185**, 568–574.

ZAMRAZIL, V., DVORAK, V., OSKERA, J. & ZAMARAZILOVA, E. (1967). The influence of hydrochlorothiazide upon glucose tolerance test after prednisolone. *Metabolism*, **16**, 445–450.

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