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Effect of single dose administration of diuretics on the blood sugar of alloxan-diabetic mice or mice made hyperglycaemic by the acute administration of diazoxide

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Summary

1. Frusemide produced hyperglycaemia in mice when administered together with diazoxide. The interaction between the drugs in elevating the blood sugar was shown to be additive.

2. The diuretic, natriuretic and kaliuretic effects of frusemide were very markedly attenuated by diazoxide.

3. Neither ethacrynic acid nor hydrochlorothiazide exerted any effect upon blood sugar when administered together with diazoxide.

4. Bilateral nephrectomy completely prevented the hyperglycaemic effect of frusemide in normal mice and in mice treated with diazoxide. Diazoxide itself still produced hyperglycaemia in nephrectomized mice.

5. Frusemide, ethacrynic acid and diazoxide, but not hydrochlorothiazide, each produced hyperglycaemia in alloxan-diabetic mice, this being prevented by bilateral nephrectomy.

Introduction

In a previous report (Foy & Furman, 1971) it was shown that frusemide, but not hydrochlorothiazide, could produce hyperglycaemia in the mouse following the acute administration of large doses. Ethacrynic acid produced a hyperglycaemia that was shown to depend upon the route of administration and which was probably attributable to the irritant effects of the drug solutions. In the work presented in this paper the effects of these diuretics on blood sugar have been examined in mice made hyperglycaemic with diazoxide or alloxan. Both alloxan-diabetes (Senft, Losert, Schultz, Sitt & Bartelheimer, 1966) and diazoxide treatment (Wolff & Parmley, 1963 ; Wolff, Langdon, Ruebner, Hollander & Skoglund, 1962) have been shown to reveal or increase the hyperglycaemic effect of benzothiadiazine diuretics. It was considered therefore that the use of these agents might reveal some occult effect of hydrochlorothiazide or ethacrynic acid on carbohydrate metabolism and might provide additional information concerning frusemide hyperglycaemia. Moreover, the possible interaction between diuretics and diazoxide in influencing blood sugar has practical implications. Diazoxide may be very useful in the treatment of severe hypertension which is refractory to other agents (Pohl & Thurston, 1971).

However, the inherent sodium retaining properties of the drug demand the concurrent use of a powerful diuretic. The combination of diazoxide with benzothiadiazine diuretics is known to be diabetogenic (Dollery, Pentecost & Samaan, 1962) and it would be interesting to know whether other types of diuretics could be more safely combined with diazoxide.

Methods

Female albino mice (30–35 g) were used throughout. The animals were allowed free access to food and water prior to the experiment. Drugs were injected intraperitoneally or intravenously into a tail vein in a volume of 5 ml/kg. In nephrectomized or sham-operated mice, all drugs were injected intravenously. Blood samples were removed from the femoral vein under light ether anaesthesia. In most experiments not more than one blood sample was removed from any one mouse.

Urine was collected (from pairs of mice housed together) using modified mouse metabowls (Jencons Ltd.). Bilateral nephrectomy was performed, 4–6 h before use, through a dorsal skin incision, care being taken to preserve intact the adrenal glands and their blood supply. Sham-operations were performed in a similar manner, the kidneys being manipulated but not removed. Alloxan-diabetes was produced by injection of alloxan (80 mg/kg i.v.) three days before use.

Where two treatment groups were employed (i.e. drug and control) each treatment was administered alternately to mice selected at random. Where four treatments were employed (e.g. diazoxide+frusemide, diazoxide+control solution for frusemide, control solution for diazoxide+frusemide, control solution for diazoxide +control solution for frusemide), the treatment order was randomized.

Blood sugar was determined by the micro-colorimetric copper reduction technique of Haslewood & Strookman (1939). Liver glycogen was determined on 200 mg liver samples, after potassium hydroxide digestion and ethanolic precipitation, by the phenol sulphuric acid method of Montgomery (1957). Urinary sodium and potassium were determined using an Eel flame photometer.

The following drugs were used dissolved in 0·9% sodium chloride solution with the use of sodium hydroxide to produce the stated pH where appropriate.

Alloxan monohydrate (B.D.H.); 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. Control solutions consisted of 0·9% sodium chloride solution having the same pH as the drug solution.

Statistical significance was assessed, where appropriate, by means of Student's *t* test or a two-way factorial analysis of variance. Statistical significance was accepted where $P<0·05$.

Results

Diazoxide treated mice

Diazoxide (50 mg/kg i.p.) produced hyperglycaemia in mice. The response was marked at one and two hours after injection and had practically disappeared by three hours (Fig. 1).

Frusemide alone (200 mg/kg i.v.) produced a small hyperglycaemic response, but 2 h after the concurrent administration of frusemide (200 mg/kg i.v.) and

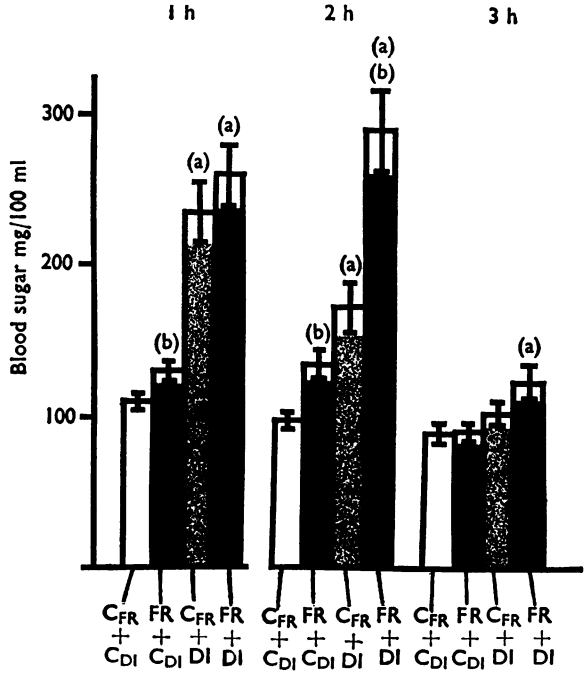

FIG. 1. The effect of the concurrent administration of frusemide (FR, 200 mg/kg i.v.) and diazoxide (DI, 50 mg/kg i.p.) on mouse blood sugar at 1, 2 and 3 h after injection. Each column represents the mean (±S.E.) of ten observations. C_{FR} and C_{DI} refer to blood sugar values of mice receiving control solutions for frusemide and diazoxide respectively. (a) Represents a statistically significant difference between diazoxide and control treatments ($P<0.05$). (b) Represents a statistically significant difference between frusemide and control treatments ($P<0.05$).

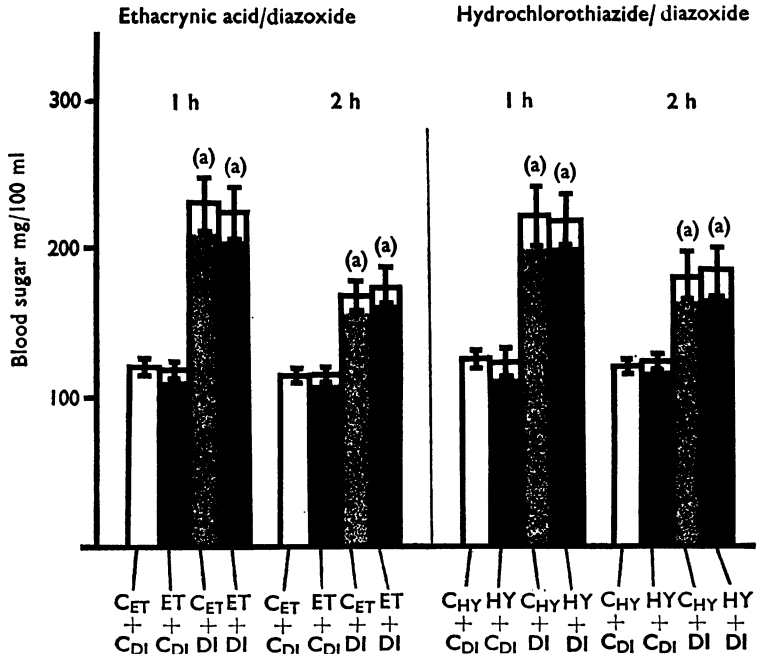

FIG. 2. The effect of the concurrent administration of ethacrynic acid (ET, 50 mg/kg i.v.) or hydrochlorothiazide (HY, 200 mg/kg i.v.) and diazoxide (DI, 50 mg/kg i.p.) on mouse blood sugar at one or two hours after injection. Each column represents the mean (±S.E.) of ten observations. C_{ET}, C_{HY} and C_{DI} refer to blood sugar values of mice receiving control solutions for ethacrynic acid, hydrochlorothiazide and diazoxide respectively. (a) Represents a statistically significant difference between diazoxide and control treatments ($P<0.05$).

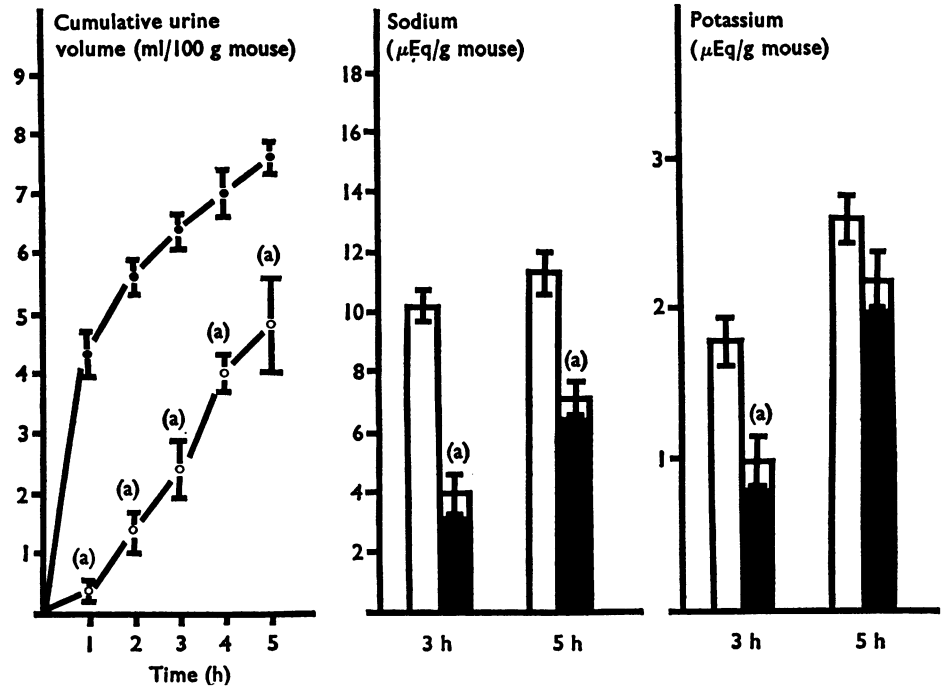

FIG. 3. The effect of the concurrent administration of diazoxide (50 mg/kg i.p.) on the diuretic natriuretic and kaliuretic responses evoked by frusemide (200 mg/kg i.v.). Each point or column represents the mean (±S.E.) of six observations. (a) Represents a statistically significant difference between the effects of diazoxide or alkaline saline administered together with frusemide ($P<0.05$). ○——○, Frusemide+diazoxide—shaded column. ●——●, Frusemide+saline—open column.

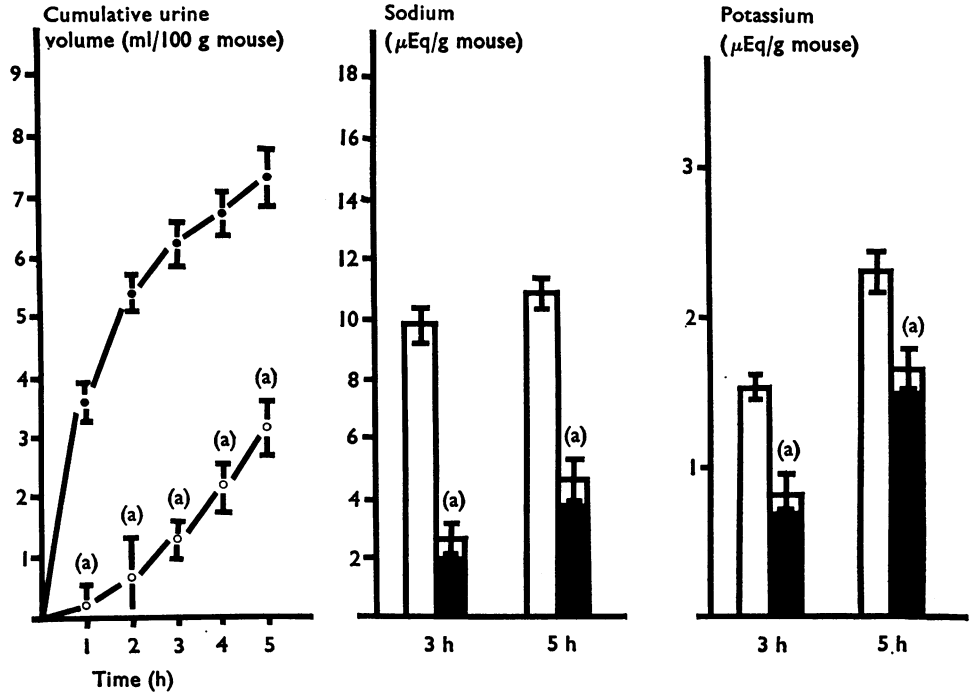

FIG. 4. The effect of the concurrent administration of diazoxide (50 mg/kg i.p.) on the diuretic, natriuretic and kaliuretic responses evoked by ethacrynic acid (50 mg/kg i.v.). Each column or point represents the mean (±S.E.) of six observations. (a) Represents a statistically significant difference between the effects of diazoxide and alkaline saline administered together with ethacrynic acid ($P<0.05$). ○——○, Ethacrynic acid+diazoxide—shaded columns. ●——●, Ethacrynic acid+saline—open columns.

diazoxide (50 mg/kg i.p.) there was a marked elevation of blood glucose relative to control mice receiving diazoxide and alkaline saline (Fig. 1). No increased hyperglycaemic effect of frusemide was evident in diazoxide-treated mice at 1 or 3 h after injection. A lower dose of frusemide (25 mg/kg) had no effect on the blood glucose of normal or diazoxide-treated mice. Two way factorial analysis of variance showed that the interaction between frusemide and diazoxide was no more than additive ($P>0{\cdot}05$).

Neither ethacrynic acid (50 mg/kg i.v.) nor hydrochlorothiazide (200 mg/kg i.v.) had any effect on blood glucose, either alone or when administered together with diazoxide (50 mg/kg i.p.) (Fig. 2).

Diazoxide (50 mg/kg i.p.) caused a very marked reduction in the diuresis, natriuresis and kaliuresis evoked by frusemide (200 mg/kg i.v.) (Fig. 3) or ethacrynic acid (50 mg/kg i.v.) (Fig. 4). In mice that were bilaterally nephrectomized, frusemide produced no elevation in blood sugar, either alone, or in combination with diazoxide, although the responses were fully manifest in sham-operated animals (Fig. 5).

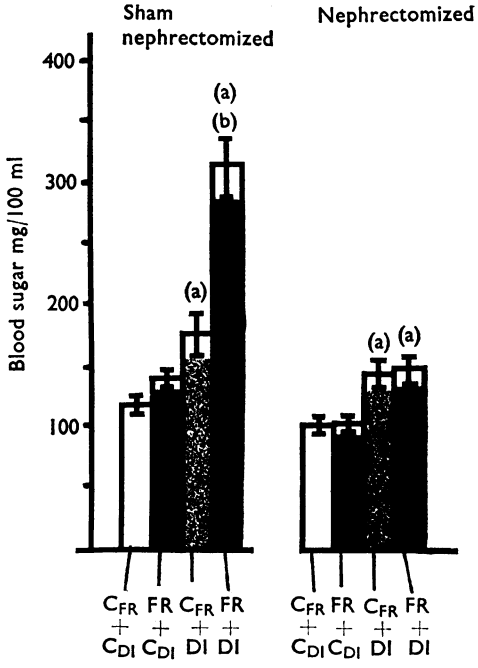

FIG. 5. The effect of the concurrent administration of frusemide (FR, 200 mg/kg i.v.) and diazoxide (DI, 50 mg/kg i.v.) on the blood sugar of nephrectomized or sham-operated mice, blood sugar being measured at 2 h after injection. Each observation represents the mean (±S.E.) of ten observations. C_{FR} and C_{DI} refer to blood sugar values of mice receiving control solutions for frusemide and diazoxide respectively. (a) Represents a statistically significant difference between diazoxide and control treatments ($P<0{\cdot}05$). (b) Represents a statistically significant difference between frusemide and control treatments ($P<0{\cdot}05$).

Alloxan-diabetic mice

In alloxan-diabetic mice both frusemide (200 mg/kg i.v.) and ethacrynic acid (50 mg/kg i.v.) produced a marked elevation of blood sugar. In these mice, the lower dose of frusemide (25 mg/kg) which had no effect on blood glucose alone,

or when administered with diazoxide, also produced marked hyperglycaemia. Hydrochlorothiazide, however, had no effect on blood glucose in alloxan-diabetic mice (Fig. 6).

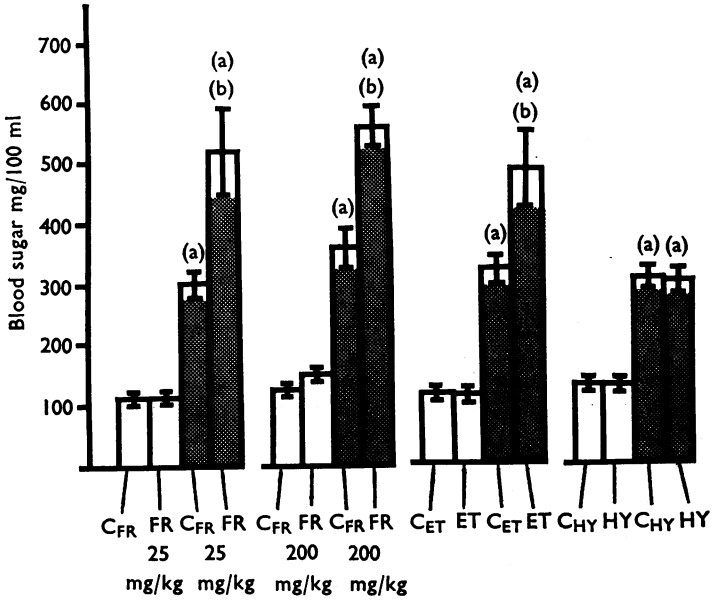

FIG. 6. The effect of frusemide (FR, 25 mg/kg or 200 mg/kg i.v.) ethacrynic acid (ET, 50 mg/kg i.v.) or hydrochlorothiazide (HY, 200 mg/kg i.v.) on the blood sugar of alloxan-diabetic (shaded columns) and normal (open columns) 2 h after the injection of diuretic or appropriate control solution (C_{FR}, C_{ET} or C_{HY}). Each column represents the mean (±S.E.) of ten observations. (a) Represents a statistically significant difference between the mean blood sugars of alloxan-diabetic and normal mice ($P<0.05$). (b) Represents a statistically significant difference between mean blood sugar values of mice receiving the diuretics and those receiving appropriate control solutions ($P<0.05$).

The increases in urine volume and the urinary excretion of sodium and potassium produced in alloxan-diabetic animals by ethacrynic acid (50 mg/kg i.v.) or frusemide (200 mg/kg i.v.) (Table 1) were similar to those produced by these agents in non-diabetic mice (Foy & Furman, 1971).

TABLE 1. *The effect of ethacrynic acid (50 mg/kg i.p.) or frusemide (200 mg/kg i.p.) on urine volume and the urinary excretion of sodium and potassium during the 3 h period following the administration of these diuretics to alloxan-diabetic mice*

Treatment	Number of observations	Urine volume ml/100 g mouse	Urinary sodium excretion μEq/100 g mouse	Urinary potassium excretion μEq/100 g mouse
Control	6	3·2±0·4	406± 50	110±11
Ethacrynic acid	5	11·9±0·8	1,865±109	477±13
Frusemide	4	10·5±1·1	1,664±155	518±65

Removal of the kidneys from alloxan-diabetic mice abolished the hyperglycaemic effect of frusemide and ethacrynic acid, although the responses were fully manifest in sham-operated animals (Fig. 7). Diazoxide hyperglycaemia was also prevented by nephrectomy, even when very large doses (up to 200 mg/kg) were used.

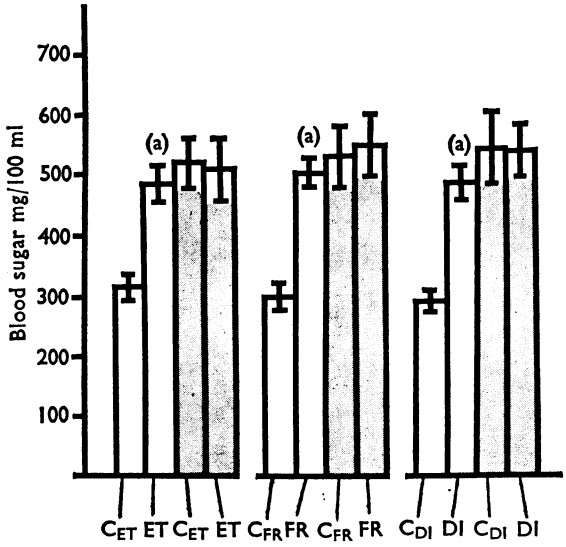

FIG. 7. The effect of ethacrynic acid (ET, 50 mg/kg i.v.) frusemide (FR, 200 mg/kg i.v.) or diazoxide (DI, 50 mg/kg i.v.) on the blood sugar of nephrectomized (shaded columns) and sham-operated (open columns) alloxan-diabetic mice (80 mg/kg i.v. three days previously) two hours after injection. Each column represents the mean (±S.E.) of ten observations. C_{ET}, C_{FR} and C_{DI} refer to blood sugars of mice receiving the control solutions for ethacrynic acid, frusemide and diazoxide respectively. (a) Represents a statistically significant difference between drug and control treatment ($P<0.05$).

Blood sugar in nephrectomized, alloxan-diabetic mice was markedly and significantly higher than in 'intact' alloxan-diabetic mice ($P<0.05$).

Liver glycogen in nephrectomized, alloxan-diabetic mice (28.5 ± 1.5 mg/g wet weight liver; $n=5$) was not significantly different ($0.1<P<0.2$) from glycogen in 'intact', alloxan-diabetic mice (38.3 ± 5.9 mg/g wet weight liver; $n=5$).

Discussion

Frusemide hyperglycaemia has been shown to depend upon the presence of the kidneys, although the impairment of intravenous glucose tolerance produced by the drug in the nephrectomized mouse suggests the involvement of some extra-renal component (Foy & Furman, 1971). The results presented in this paper appear to confirm the role of the kidneys in the production of hyperglycaemia by frusemide. However, an understanding of the role of the kidneys is rendered difficult by several observations. In the intact, diazoxide-treated animal, frusemide hyperglycaemia was manifest at a time when the renal responses to the diuretic were markedly diminished. It is possible that the small diuresis, natriuresis and kaliuresis evoked by the diuretic under these conditions was adequate to initiate the production of hyperglycaemia by, for example, provoking the release of catecholamines from the adrenal medulla. However, ethacrynic acid, despite producing similar renal effects to frusemide in diazoxide-treated (and normal) mice produced no hyperglycaemic effect. It is thus possible that the endocrine secretions of the kidneys themselves, rather than the loss via these structures of water and salt are important in the production of hyperglycaemia by frusemide. Frusemide may have some action, independent of its diuretic effect, in activating the renin-angiotensin system; angiotensin has itself been shown to produce hyperglycaemia (Wallrabe, Nitrschkoff & Gnuechtel, 1969).

Ethacrynic acid hyperglycaemia, and indeed that produced by frusemide, in alloxan-diabetic mice can probably be explained in terms of the very large diuresis evoked by the drug. Glycogenolysis, produced by catecholamines liberated from the adrenal medulla in response to volume depletion, together with haemoconcentration, would contribute towards the hyperglycaemia, the excess blood sugar being assimilated inadequately in the alloxan-diabetic animal. In this context it is interesting that a submaximal diuretic dose of frusemide (25 mg/kg; Foy & Furman, 1971) produced marked hyperglycaemia in alloxan-diabetic mice but not in normal or diazoxide-treated animals. The abolition by nephrectomy of the hyperglycaemic responses to ethacrynic acid and frusemide in the alloxan-diabetic mouse would support the above ideas. Senft *et al.* (1966) reached similar conclusions in relation to frusemide hyperglycaemia in the alloxan-diabetic rat. A role for the kidney in the production of hyperglycaemia by diazoxide in alloxan-diabetic mice is suggested in view of the abolition of the response by nephrectomy. The very high blood sugar seen in nephrectomized alloxan-diabetic mice suggests that the kidney is important in the disposal of glucose in alloxan-diabetic mice. The antidiuretic effect of diazoxide, therefore, may be important in the production of hyperglycaemia by the drug under these conditions. However, the possibility remains that the nephrectomized alloxan-diabetic mice may be unresponsive to various hyperglycaemic stimuli, although the animals did not seem to be deficient in liver glycogen, relative to their 'intact' diabetic controls.

In view of the demonstrated impairment of intravenous glucose tolerance produced by frusemide in the nephrectomized mouse (Foy & Furman, 1971), it is perhaps surprising that the drug did not influence the hyperglycaemia produced by alloxan or diazoxide under these conditions. However, Foy & Furman (1971) suggested that this impairment of glucose tolerance produced by frusemide was mediated by a reduction in the plasma insulin levels following glucose administration. Alloxan-diabetes and diazoxide treatment are associated respectively with an absence of and a decrease in the elevation of plasma insulin in response to glucose. This mechanism is therefore unavailable to frusemide in alloxan-diabetic mice and it is possible that in the nephrectomized mice diazoxide is exerting a maximum effect with respect to suppression of glucose-stimulated insulin secretion.

The observation that ethacrynic acid, in contrast to frusemide produced no elevation in blood sugar in normal or diazoxide treated mice may indicate that ethacrynic acid would be the better diuretic to use in combination with diazoxide in the treatment of hypertension. However, great care is obviously necessary in extrapolating these results to the clinical situation, especially in view of the failure of hydrochlorothiazide, known to affect blood sugar regulation in some patients, to modify blood sugar under various conditions in the mouse both in this study and in an earlier one (Foy & Furman, 1971). Moreover, the relationship between the effects of single large doses of these drugs on blood sugar and effects seen after more prolonged administration of smaller doses is not clear (compare Foy & Furman, 1971 and Foy & Furman, 1972).

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