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THE ROLE OF THE BLOOD GLUCOSE LEVEL IN DETERMINING VOLUNTARY ETHANOL CONSUMPTION IN THE LACG AND DIABETOGENIC C57BL STRAINS OF MICE

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Abstract—The influence of the blood glucose level (BGL) on the voluntary consumption of ethanol by two strains of mice has been investigated. LACG mice show an aversion towards ethanol whereas C57BL mice which are mildly hyperglycaemic show a preference for ethanol. Chronic compulsory ethanol drinking produced hypoglycaemia only in C57BL mice. The oral antidiabetic drugs phenformin and glibenclamide lowered the BGL of C57BL mice which then showed an aversion to ethanol. Raising the BGL of LACG mice by acute alloxan or streptozotocin treatment did not reduce ethanol aversion significantly, but alloxan significantly increased total fluid intake. Diazoxide chronically increased the BGL and slightly increased ethanol consumption in LACG mice. It is concluded that the BGL in C57BL mice may be a factor in determining their innate preference for alcohol. The relationship between alcoholism and diabetes is discussed.

The study of voluntary ethanol consumption by animals has been facilitated by the development of inbred strains of mice which show widely differing degrees of preference for alcohol [1], and by this means it has been possible to investigate the biochemical and behavioural determinants of ethanol preference within a single species. The diabetogenic C57BL10/ScSn (C57) strain used in the present study has been shown to possess a higher blood glucose level (BGL) than mice of other strains and also to exhibit a significant preference for ethanol solutions when presented with a choice of 12% ethanol or water [2, 3].

Since ethanol is known to produce hyperglycaemia followed by hypoglycaemia after acute doses [4], the present work has sought to examine the relationship between the BGL, chronic ethanol consumption, and ethanol preference.

Ethanol also has profound effects on glucose metabolism, not only at the level of the pancreas [5], but also in the liver where the $NADH_2/NAD$ ratio is increased following ethanol consumption [6]. In addition, ethanol can increase brain glucose levels and the uptake of glucose into the brain [7]. These actions may all be contributory in the relatively high incidence of diabetes found in confirmed alcoholics [8], although pancreatitis is also a feature of alcoholism. Chronic liver disease, whether alcohol-induced or not, can also result in impaired glucose tolerance [9].

The aims of the present work were therefore, first, to compare the effects of ethanol on glucose utilization in strains of mice which exhibited either spontaneous preference for, or aversion to, drinking ethanol solutions and, second, to manipulate the BGL by use of drugs in order to examine whether or not this tendency to alcohol consumption might be related to the BGL. A preliminary account of part of this work has already been reported [10].

MATERIALS AND METHODS

General. The mice used in all the experiments were adult LACG or C57 strains of either sex derived from inbred colonies. They were kept at 20–22° and provided with Oxoid laboratory animal diet in either pelleted or powdered form and tap water *ad lib.* Prior to the chronic feeding or drinking experiments the mice were familiarised with cages containing appropriate food hoppers and pairs of water bottles.

Ethanol tolerance was induced by use of a drinking schedule of gradually increasing concentrations of ethanol in water as the sole source of drinking fluid [2]. The final ethanol concentration was 20% (w/v) and the mice could be maintained on this dose indefinitely.

In the fasting experiments, food was removed from the cages at 18.00 hr; the mice were weighed the following day at 09.00 hr and at 24- and 48-hr intervals subsequently.

Drug administration. Alloxan and streptozotocin (Sigma Chemical Co., Poole, U.K.) were administered i.p. in physiological saline (0.9% w/v). 1-Phenylbiguanide [phenformin (Aldrich Chemical Co., Milwaukee, WI) and glibenclamide were added to the drinking water of the mice. Diazoxide [eudemine (Allen & Hanbury's Ltd, London, U.K.)] was added to the powdered diet since it did not dissolve below pH 9 and could not therefore be added to the drinking water. The ethanol used throughout was obtained from James Burroughs Ltd (London, U.K.)

Ethanol preference testing. The mice were placed in cages containing two water bottles and provided with a choice of water or 12% (w/v) ethanol solution.

The position of the bottles was reversed every 24 hr and the relative volumes of the fluid consumed measured between 24 and 72 hr following the presentation of 12% ethanol. It was assumed that the fluid loss due to leakage would not be different between the two solutions. Evaporation was negligible since the bottles were equipped with ball valves on the drinking nozzles.

Assay of blood glucose and tissue glucose uptake. BGLs were assayed by the glucose oxidase–peroxidase method of Fleming and Pegler [11]. Mice were killed by decapitation and blood from the trunk collected on a heparinized watch glass at 0°. A 100-μl aliquot was added to 1.2 ml of 3% (w/v) perchloric acid, mixed rapidly and centrifuged for 10 min at 3000 *g*. A 1.2-ml aliquot of the supernatant was neutralized with 1 N sodium hydroxide and 0.5 ml aliquots taken for duplicate glucose assays.

The glucose oxidase, peroxidase and *O*-dianisidine used in the subsequent assays were obtained from the Sigma Chemical Co. A set of standard glucose solutions was assayed for each experiment.

Tissue glucose uptake *in vitro* was determined using D-[U-^{14}C]glucose by the method described previously [12] for rat tissues. In the present experiment each incubation contained either 15–30 mg wet weight of cerebral cortex as 0.5-mm slices or 25–30 mg diaphragm tissue. The uptake of glucose was expressed as μmoles glucose/wet weight tissue/min. The D-[U-^{14}C]glucose (291 mCi/mmole) was obtained from the Radiochemical Centre (Amersham, U.K.)

Statistical analysis of results. BGLs and tissue glucose uptake valves were analysed by the Student *t*-test (one-tailed) for measuring the significance of the difference between the means of independent groups. Ethanol preference was analysed by the Wilcoxon matched-pairs signed-ranks test [13].

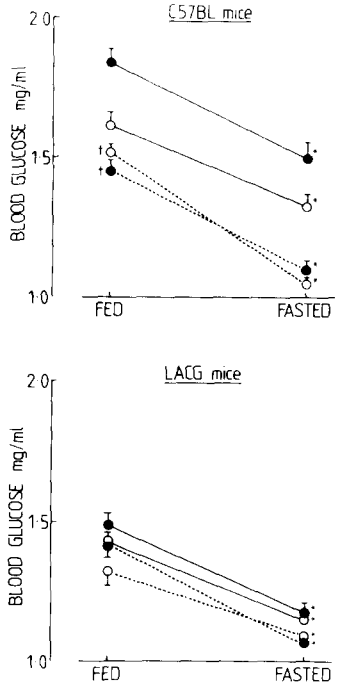

Fig. 1. Effects of chronic ethanol drinking and fasting on blood glucose level (BGL). BGLs were determined before or after 48 hr fasting. Male controls (●——●), female controls (○——○), male drinkers (●---●), female drinkers (○---○). *Fasted BGL < control, $P < 0.01$. †Ethanol-drinking BGL < control, $P < 0.01$.

RESULTS

Effects of ethanol consumption on BGL

The BGLs of control, that is untreated, mice showed significant differences between the two strains and, in the case of the C57 mice, between the two sexes (see Table 1). Male and female C57 mice had significantly higher BGLs than their respective sexes of the LACG strain. In mice rendered tolerant to ethanol and then provided with 20% ethanol solution as their sole source of drinking fluid for at least 21 days (chronic drinkers), the BGLs were, in all cases, reduced from their control values (see Table 1). However, the hypoglycaemic action of the ethanol was of greater magnitude in the C57 mice than in the LACG mice where the difference was small and, in the case of the males, not statistically significant.

In order to determine whether or not the effect of the ethanol was mediated to any significant extent by a reduction in total calorific intake, the BGLs were determined in control and chronic drinking mice after 48 hr fasting. The results are shown in Fig. 1; fasting significantly reduced the BGL in both sexes of both strains, and this reduction was superimposed upon the hypoglycaemic action of ethanol.

Table 1. Mouse blood glucose levels (mg/ml)*

Mouse strain	Sex	Control	Ethanol drinkers
C57	Male	1.84 ± 0.026(14)	1.46 ± 0.044(6)‡
	Female	1.62 ± 0.022(12)	1.52 ± 0.019(6)§
LACG	Male	1.49 ± 0.030(8)†	1.41 ± 0.031(8)
	Female	1.43 ± 0.019(14)†	1.32 ± 0.034(8)§

* Values are expressed as means ± S.E.M.; the number of mice in each group is shown in parentheses. Chronic ethanol drinkers had been rendered tolerant to ethanol and then maintained on 20% ethanol as their sole drinking fluid.

† LACG < corresponding C57, $P < 0.001$.

‡ Ethanol < control, $P < 0.01$.

§ Ethanol < control, $P < 0.05$.

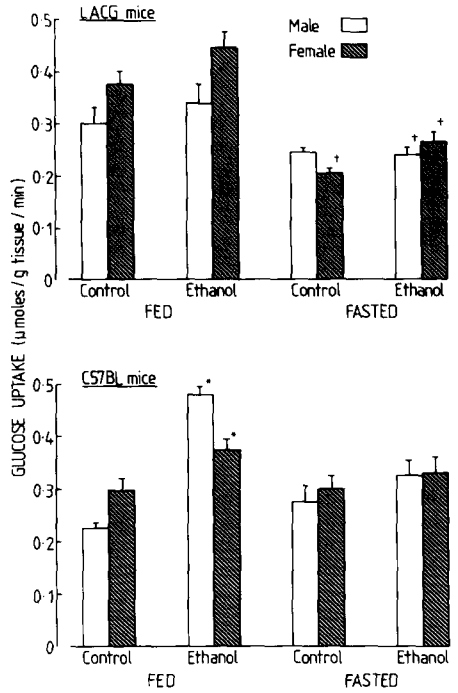

Fig. 2. Effects of chronic ethanol drinking and fasting on glucose uptake into cerebral cortical slices *in vitro*. Mice were fasted for 48 hr prior to assay. Results are means ± S.E.M. of at least five observations. *Ethanol drinkers > controls, $P < 0.02$. †Fasted < fed, $P < 0.05$.

The effects of ethanol on tissue glucose uptake in vitro

The results from determining the effects of ethanol drinking on glucose uptake into cerebral cortical slices are shown in Fig. 2. Although there were slight differences between the sexes under the different experimental conditions, none of them were significant. In LACG mice, chronic ethanol drinking did not significantly alter the rate of glucose uptake either under fed or fasted conditions. Fasting, however, produced a significant fall in glucose uptake in control female mice and ethanol drinkers of both sexes. In C57 mice, ethanol drinking produced a significant increase in brain glucose uptake in both sexes. This increase was not observed in fasted mice, and, in addition, the fasting did not appear to reduce brain glucose uptake as had been observed in the LACG mice, although the fasted male drinkers had a significantly lower rate of glucose uptake than the control drinkers.

The results of the assays of glucose uptake into the diaphragm *in vitro* are shown in Fig. 3. There were no significant differences between the sexes under any of the experimental conditions, although male C57 controls showed a significantly lower glucose uptake compared to the corresponding LACG mice. Also, in male C57 controls fasting produced a significant increase in uptake. In contrast to the results obtained with brain slices, ethanol drinking caused no change in the rates of glucose uptake into the diaphragm in either strain of mouse under either fed or fasted conditions.

The influence of BGL on ethanol preference

The effects of two hypoglycaemic drugs, phenformin and glibenclamide, on the BGL of C57 mice were determined by measuring the BGL after 24 hr and 72 hr of drug administration either in the drinking water (phenformin 375 mg/l) or in the powdered diet (glibenclamide 300 mg/100 g). The BGL was significantly reduced from control values with both drugs at both time intervals (see Table 2). Ethanol preference was therefore determined over the 48 hr period commencing 24 hr following addition of the drug to the water or food. In the case of phenformin both the water and the 12% ethanol solution contained the drug. The results shown in Table 2 clearly indicate that the preference for ethanol is diminished when the BGL is lowered to the value normally found in the LACG mice.

The reverse experiment, that is, increasing the BGL of LACG mice to match that of the C57 mice was performed by treating the mice either with a single dose of alloxan or streptozotocin or by administering diazoxide chronically in the food. The results are shown in Table 3. Alloxan had an all-or-none effect in that the mice either appeared unaffected by the drug or showed very marked hyperglycaemia. A dose of alloxan sufficient to produce chronic hyperglycaemia in all the mice thus treated caused a large number of behavioural side effects. The mice became aphagic and lost weight and their water

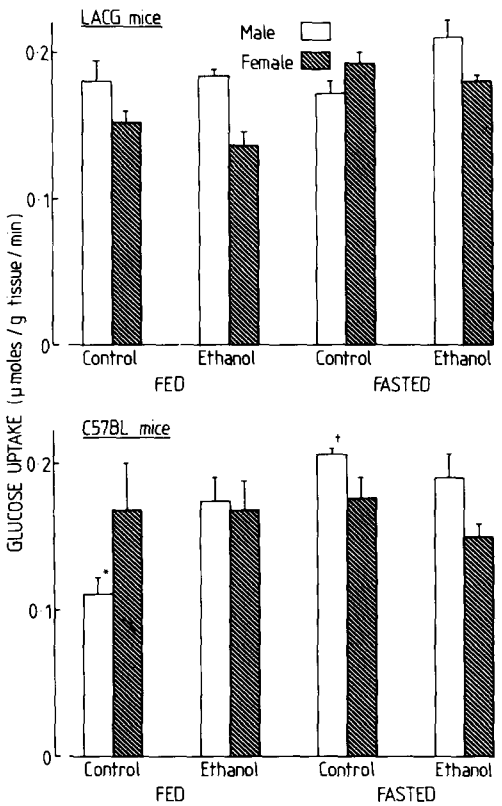

Fig. 3. Effects of chronic ethanol drinking and fasting on glucose uptake into the diaphragm. Mice were fasted for 48 hr prior to assay. Results are means ± S.E.M. of at least five observations. *Control < ethanol drinkers, $P < 0.05$. †Fasted > control, $P < 0.02$.

Table 2. Effects of phenformin and glibenclamide on blood glucose level and voluntary ethanol consumption*

	Mouse strain			
	LACG		C57BL	
	Male	Female	Male	Female
	Controls			
Blood glucose (mg/ml)	1.49 ± 0.03(8)	1.43 ± 0.019(14)	1.84 ± 0.026(14)	1.62 ± 0.022(12)
Ethanol solution consumed (% of total fluid intake)	11.6	8.6	66.7	51.0
	Phenformin (375 mg/l in drinking water)			
Blood glucose (mg/ml)				
24 hr	1.27 ± 0.03(12)†	1.11 ± 0.03(12)†	1.42 ± 0.04(12)†	1.41 ± 0.04(12)†
72 hr	1.21 ± 0.04(6)	1.12 ± 0.05(6)†	1.48 ± 0.04(6)†	1.40 ± 0.06(6)†
Ethanol solution consumed (% of total fluid intake)	17.4	13.2	16.5‡	20.2‡
	Glibenclamide (300 mg/100 g food)			
Blood glucose (mg/ml) (72 hr)	—	—	1.13 ± 0.04(6)†	1.33 ± 0.04(6)†
Ethanol solution consumed (% of total fluid intake)	—	—	38.7‡	28.0‡

* Consumption of the 12% ethanol solution was measured between 24 hr and 72 hr following administration of the two drugs. Blood glucose levels are expressed as means ± S.E.M.

† Drug-treated < control, $P < 0.01$ (Student's t-test). Ethanol consumption is expressed as the median value from four experiments involving six mice in each group.

‡ Drug-treated < control, $P < 0.01$ (Wilcoxon matched-pairs signed-ranks test).

intake was much increased (see Table 4). For these reasons it was not feasible to determine alcohol preference.

Streptozotocin and chronic diazoxide both produced moderate increases in BGL (Table 3) in LACG mice compared to controls. In these mice there was a slight but not significant increase in the proportion of ethanol solution consumed.

Drug effects on total fluid intake

In expressing the ethanol preference as a percentage of the total fluid intake, there is an inherent assumption that the total fluid intake (ml/kg/day) is a relatively constant figure. The ethanol preference experiments in themselves do not produce any significant change in the total fluid intake of any of the mice tested (see Table 4). However, acute alloxan and chronic phenformin treatments produced large increases in the fluid intake and diazoxide tended to reduce the fluid intake compared to control animals offered a choice of 12% ethanol solution or water. The chronic glibenclamide treatment did not produce a significant change in fluid intake in the C57 mice.

DISCUSSION

The numerous determinants of alcohol preference in animals have been reviewed by Myers and Veale [14] and include environment, acclimation, dietary state, endocrine function, age, sensory function, level of stress and genotype.

The alcohol-preferring C57BL mouse strain has

Table 3. Effects of increasing blood glucose level (BGL) on voluntary ethanol consumption in LACG mice*

Drug treatment	Blood glucose level (mg/ml)		Ethanol consumed (% of total fluid intake)	
	Male	Female	Male	Female
Control (saline i.p.)	1.49 ± 0.03(8)	1.43 ± 0.019(14)	11.6	8.6
Alloxan (250 mg/kg i.p.)	3.82 ± 0.53(5)†	3.49 ± 0.32(10)†	—	—
Streptozotocin (70 mg/kg i.p.)	1.81 ± 0.058(6)‡	1.65 ± 0.104(5)§	15.2	14.8
Diazoxide (1.6 g/100 g food)	1.99 ± 0.048(8)‡	1.87 ± 0.07(6)‡	15.6	13.1

* Blood glucose levels were determined 72 hr after alloxan and streptozotocin or after 48 hr of diazoxide feeding. Ethanol preference was determined over a 48 hr period from these times.

† BGL > control BGL, $P < 0.001$.

‡ BGL < control BGL, $P < 0.001$.

§ BGL < control BGL, $P < 0.01$.

Table 4. Drug effects on total fluid intake*

Drug treatment	Mouse strain LACG Male	LACG Female	C57 Male	C57 Female
Control (water only)	202 ± 18.2(3)	255 ± 13.5(3)	194 ± 14.0(3)	210 ± 16.8(3)
Ethanol choice (12% solution)	180.2 ± 5.4(5)	218.5 ± 7.6(5)	168.1 ± 4.3(8)	191.7 ± 6.1(10)
Alloxan (250 mg/kg i.p.)	327.6 ± 19.0(3)	325, 356(2)	—	—
Streptozotocin (70 mg/kg i.p)	202 ± 4.8(4)	186, 194(2)	—	—
Diazoxide (1.6 g/100 g food)	139.2 ± 8.9(4)	99.7 ± 6.2(4)	—	—
Phenformin (375 mg/l water)	—	—	254(2)	288(2)
Glibenclamide (300 mg/100 g food)	—	—	176 ± 12.2(3)	183 ± 12.6(3)

* Fluid consumption was determined over a 48 hr period from groups of six mice per cage. Results are expressed as ml consumed/kg body weight/day and are the means ± S.E.M. from the number of experiments shown in parentheses.

already been shown to have a lower brain sensitivity to the depressant effects of ethanol [15] and increased rates of hepatic ethanol metabolism [16] when compared to other strains. The C57BL lines are all diabetogenic to some extent, varying from the severely hyperglycaemic and obese ob/ob subline to the moderately hyperglycaemic line used in the present work. The basis for this hyperglycaemia is an insensitivity to insulin and an inability to release insulin in response to glucose infusion [17]. The first object of the present experiments was therefore to compare the effects of chronic ethanol consumption on BGL and tissue glucose utilization. Since the fall in BGL produced by ethanol in the C57 mice was not observed in LACG mice, it is possible that there is a BGL, corresponding to that of the normal LACG mice, below which the effects of ethanol are overcome by metabolic regulatory processes such as increased hepatic glycogenolysis. It has been found in the rat, for example, that acute ethanol is only hypoglycaemic in fed animals and the hypoglycaemic fasted animals show no response [18]. However, chronic ethanol still produced hypoglycaemia in fasted C57 mice whose BGL was comparable to that of fed LACG mice.

The hypoglycaemic action of ethanol is not thought to be due to an effect on insulin secretion and several studies in animals and man (e.g. Ref. [19]) have shown that plasma immunoreactive insulin levels are not changed in response to plasma ethanol levels of up to 300 mg/100 ml. Nevertheless, ethanol does have widespread effects on the neuroendocrine system [20] including a stimulation of adrenal corticosterone release mediated by a direct effect on the pituitary [21] but this would tend to raise the BGL. The increase in glucose uptake into the brain in C57 mice could account in part for the observed reduction in BGL. Goas *et al.* [22] suggested that the diabetogenic effect in C57 mice might be due to a "resistance of cellular glucose uptake mechanisms". The present results show no significant differences in tissue glucose uptake between the C57 and LACG strains although brain uptake was lower in both sexes of the C57s compared to LACGs.

It has been recognised that, in a free-choice situation, animals and man will tend to select a diet that is beneficial and nutritionally balanced [23]. If the high BGL found in C57 mice is considered abnormal, these mice might tend to select a diet which would tend to normalise their BGL. If this is the basis for the ethanol preference exhibited by these animals, then the manipulation of BGL should be reflected in changes in ethanol preference. Goas *et al.* [22] reported that insulin reduced voluntary ethanol consumption and increased water consumption in C57 mice in a dose-dependent manner, but did not monitor the BGL during these experiments. We have shown previously [3] that C57 mice subjected to a compulsory ethanol drinking regime lose their preference for ethanol when subsequently offered a choice. In the short term this may be due to the lower BGL induced by the chronic ethanol pretreatment (see Table 1).

Both phenformin, which acts peripherally to increase glycolysis, and glibenclamide, which acts by stimulating insulin release from the pancreas [24], produced a marked hypoglycaemia concurrently with a decrease in ethanol preference in C57 mice. This suggests that BGL may influence ethanol preference; however, the results obtained from LACG mice rendered diabetogenic by treatment with the pancreatic toxins alloxan and streptozotocin were less clear-cut. Both drugs, and particularly alloxan, produced considerable behavioural effects and symptoms of toxicity (locomotor ataxia, shivering, tremor, loss of appetite etc.) and under these circumstances drinking preferences cannot readily be compared with healthy control mice. Diazoxide produces hyperglycaemia [25] and was markedly less toxic than alloxan or streptozotocin when administered chronically in the food. Although it produced a moderate hyperglycaemia in LACG mice, it did not

significantly increase ethanol preference. An artificially raised BGL does not therefore appear to encourage ethanol consumption.

The present studies have clinical relevance since an abnormally high incidence of diabetes has been reported in alcoholics [26]. Chronic alcoholism can lead to pancreatitis and consequent glucose intolerance in patients [27], but it is possible that, in some cases, the diabetes may well have been a prime determinant in producing chronic ethanol consumption and eventual alcoholism.

REFERENCES

1. D. A. Rogers, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), Vol. 2, p. 107. Plenum Press, New York (1972).
2. J. W. Unwin and P. V. Taberner, *Neuropharmacology* **19**, 1257 (1980).
3. P. V. Taberner and J. W. Unwin, *Br. J. Pharmac.* **74**, 959P (1981).
4. R. A. Arky and N. Frankel, in *Biochemical and Clinical Aspects of Alcohol Metabolism* (Ed. V. M. Sardesai), p. 197. Plenum Press, New York (1970).
5. R. Metz, S. Berger and M. Mako, *Diabetes* **18**, 517 (1969).
6. G. R. Cherrick and C. M. Leevy, *Biochim. biophys. Acta* **107**, 29 (1965).
7. E. V. Flock, G. M. Tyce and C. A. Owen, *Proc. Soc. exp. Biol. Med.* **135**, 325 (1970).
8. R. A. Arky, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), Vol. 1, p. 197. Plenum Press, New York (1970).
9. C. Megyesi, E. Samols and V. Marks, *Lancet* **II**, 1051 (1967).
10. D. M. Connelly and P. V. Taberner, *Br. J. Pharmac.* **74**, 869P (1981).
11. I. D. Fleming and H. F. Pegler, *Analyst* **88**, 967 (1963).
12. P. V. Taberner, *J. Neurochem.* **20**, 669 (1973).
13. S. Siegel, *Nonparametric Statistics for the Behavioural Sciences*. McGraw-Hill, London (1956).
14. R. D. Myers and W. L. Veale, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), Vol. 2, p. 131. Plenum Press, New York (1972).
15. R. Kakihana, D. R. Brown, G. E. McLearn and I. R. Tabershaw, *Nature, Lond.* **154**, 1574 (1966).
16. P. I. Kulkosky and N. W. Cornell, *Pharmac. Biochem. Behav.* **11**, 439 (1979).
17. A. A. Like, in *The Diabetic Pancreas* (Eds. B. W. Volk and K. F. Wellman), p. 381. Tindall Bailliere, London (1977).
18. J. L. Tramill, P. E. Turner and G. Harwell, *Physiol. Psychol.* **9**, 114 (1981).
19. N. Freinkel, D. L. Singer, R. A. Arky, J. B. Sheldon, J. B. Anderson and C. K. Silbert, *J. clin. Invest.* **42**, 1112 (1963).
20. T. J. Cicero, *A. Rev. Med.* **32**, 123 (1981).
21. F. W. Ellis, *J. Pharmac. exp. Ther.* **153**, 121 (1966).
22. J. A. Goas, R. W. Pelham and A. S. Kippa, *Pharmac. Biochem. Behav.* **10**, 557 (1979).
23. C. P. Richter, *Harvey Lect.* **38**, 63 (1942).
24. J. Larner and R. C. Haynes, in *The Pharmacological Basis of Therapeutics* (Eds. L. S. Goodman and A. Gilman), fifth edition, p. 1507. Macmillan, New York (1975).
25. N. Altszuler, N. J. Hampshire and E. Morary, *Diabetes* **26**, 931 (1977).
26. D. R. Axelrod, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), Vol. 3, p. 291. Plenum Press, New York (1974).
27. V. Marks and J. Wright, *Clinics in Endocrinology and Metabolism* Vol. 7. W. B. Saunders, London (1978).