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THE EFFECT OF FASTING ON THE HYPERGLYCAEMIC RESPONSES TO CATECHOL AMINES IN RATS

BY

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The relative activities of adrenaline, noradrenaline and isoprenaline in producing hyperglycaemia and glycogenolysis in skeletal muscle have been studied in both fed and fasted rats, 1 hr after subcutaneous injection of the catechol amines. The relative hyperglycaemic activities of the three catechol amines depended greatly upon the prandial state of the rats and on the dose range used. In fed rats the relative potencies were in the descending order of potency, adrenaline—noradrenaline—isoprenaline, irrespective of the dose range. Isoprenaline had no hyperglycaemic activity in fed rats even at doses as high as 2 mg/kg. In fasted rats the order of potency depended on the dose. At low doses (0.005 to 0.02 mg/kg) the descending order was isoprenaline—adrenaline—noradrenaline. At higher doses (0.1 to 1 mg/kg) the descending order was adrenaline—isoprenaline—noradrenaline. The relative activities of the three catechol amines in causing glycogenolysis in muscle was independent of the dose range or the prandial state of the rats. Under all conditions the descending order of potency was isoprenaline—adrenaline—noradrenaline. The results are discussed with reference to Ahlquist's (1948) hypothesis of α- and β-receptors and were consistent with the concept that, in the rat, liver glycogenolysis is mediated predominantly by α-receptors and muscle glycogenolysis mainly by β-receptors. In general the hyperglycaemic response in the fed rat is mediated predominantly by α-receptors and in the fasted rat the response is mainly due to the activation of β-receptors. A drug possessing both α- and β-receptor activity elicits an exception to this rule in the fasted rat. Several perturbing problems in the literature, particularly with regard to the hyperglycaemic activity of isoprenaline and to the difficulty in blocking the hyperglycaemic response, can now be explained in the light of these findings.

A survey of the literature concerned with the effects of catechol amines on carbohydrate metabolism reveals several conflicting observations. For example, Ellis & Anderson (1951) reported that isoprenaline had no hyperglycaemic activity in the rat, even at doses as high as 25 mg/kg, while other investigators have reported definite hyperglycaemic effects in the rat (Pol, 1956; Claassen & Noach, 1960). Discrepancies may also be found in blocking experiments. Although Schwartz (1962) obtained relatively good block by phenoxybenzamine of the hyperglycaemic response to adrenaline in rats, Tobe, Kurihara & Takeuchi (1961) failed to get any block with dibenamine, a related drug.

We have found that 1 hr after the subcutaneous injection of the catechol amines, adrenaline, noradrenaline and isoprenaline, the relative hyperglycaemic activity

depends on the prandial state of the rat and the dose range. Many of the inconsistencies in previously published material, such as the examples already cited, may now be understood in the light of these findings. A preliminary report of this work has appeared (Fleming & Kenny, 1961).

METHODS

Drugs. The sympathomimetic amines used were (−)-adrenaline hydrochloride (Parke Davis), (−)-noradrenaline hydrochloride (Delta Chemical Works), and (±)-isoprenaline hydrochloride (Sterling-Winthrop). The drugs (all concentrations) were dissolved in 0.9% saline containing ascorbic acid (0.1 mg/ml.). All drugs were injected subcutaneously.

Blood glucose experiments. Male Holtzman rats, from 6 to 8 weeks old, were maintained on a commercial laboratory diet throughout the study. When the rats were fasted, food was withheld during a period of from 16 to 20 hr before the start of the experiment. The rats were lightly anaesthetized with ether and injected subcutaneously either with the sympathomimetic drug or with the saline containing ascorbic acid. At 1 hr after the injection the rats were bled by cardiac puncture under light ether anaesthesia. Blood glucose concentrations were determined by the method of Nelson (1944). The various aspects of the use of ether as an anaesthetic in this type of experiment have been discussed at greater length elsewhere (Kenny & Morey, 1962). Although it was possible to show that the blood glucose concentra-

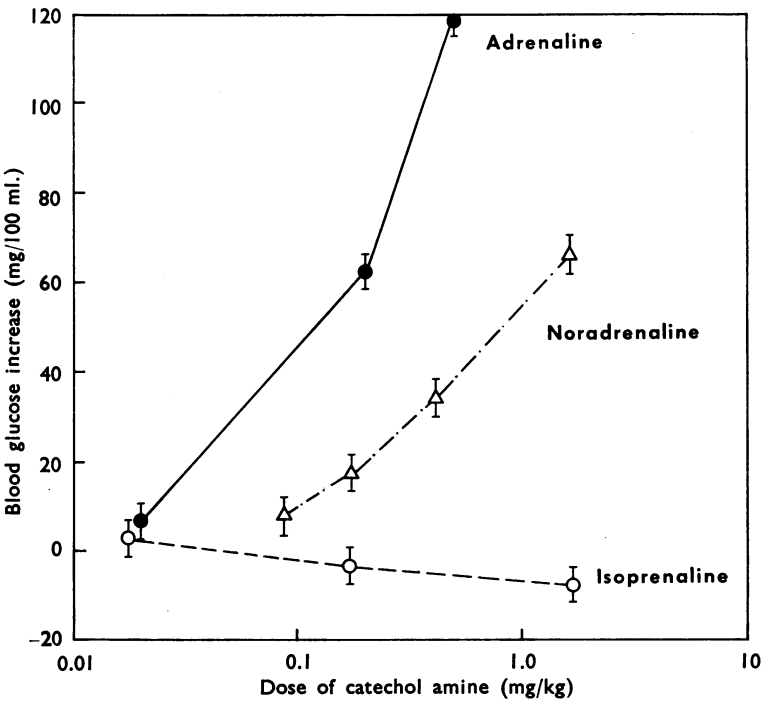

Fig. 1. Dose/response curves for the hyperglycaemic responses to adrenaline, noradrenaline and isoprenaline in fed rats. The responses are expressed as the increases in blood glucose compared with the blood glucose level (mean and standard error, 110·4±2·5 mg/100 ml.) of forty-six control rats injected with 0.9% saline. The drugs were injected subcutaneously and blood glucose levels were determined 1 hr later. The doses are plotted (abscissa, log scale) in terms of free base of drug. Each point represents the mean value from at least fifteen rats. The vertical lines indicate standard errors of the means.

tions in the control rats were significantly ($P<0.05$) higher if the rats had been bled at 1 hr after an initial anaesthetization with ether than if they had been bled without any earlier treatment (82.6 compared with 76.9 mg/100 ml.), the standard deviation was such that a total of 114 rats was needed to uncover the statistical significance ($P<0.05$) of this difference.

Muscle glycogen experiments. The rats were treated as in the blood glucose experiments except that at 1 hr after injection each rat was lightly anaesthetized with ether and approximately 1 g of muscle was removed from the lateral surface of the thigh and quickly placed in 4.0 ml. of 30% potassium hydroxide solution in a weighed, 25 ml., stoppered test-tube. Glycogen was determined by the method of Good, Kramer & Somogyi (1933); the results were expressed in mg of glucose/g of wet tissue.

RESULTS

Blood glucose experiments. The dose/response curves for the hyperglycaemic effects of the three catechol amines in fed rats are plotted in Fig. 1. The descending order of potency was adrenaline—noradrenaline—isoprenaline, the last drug having no demonstrable hyperglycaemic action even at a dose of 2 mg/kg. The dose/response curves for these drugs in fasted rats are plotted in Fig. 2. Very different results were obtained with fasted rats. At low doses, 0.02 mg/kg or less, the descending order of potency was isoprenaline—adrenaline—noradrenaline. At higher doses, isoprenaline and noradrenaline (40 and 16 mg/100 ml. respectively)

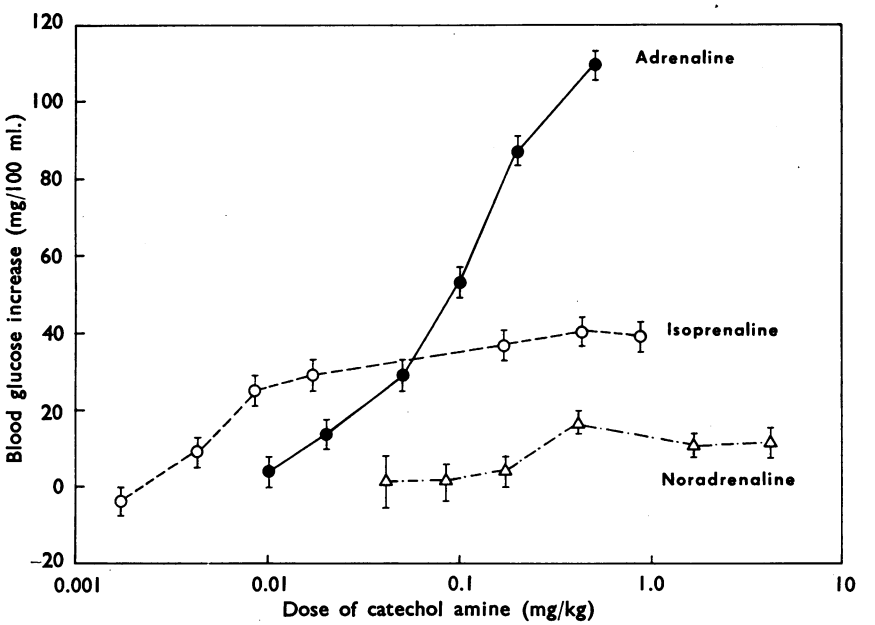

Fig. 2. Dose/response curves for the hyperglycaemic responses to adrenaline, noradrenaline and isoprenaline in fasted rats. The responses are expressed as the increases in blood glucose compared with the blood glucose level (mean and standard error, 82.6±1.4 mg/100 ml.) of 103 control rats injected with 0.9% saline. The drugs were injected subcutaneously and blood glucose levels were determined 1 hr later. The doses are plotted in terms of the free base of drug. Each point represents the mean values from five to twenty-five rats for the noradrenaline curve and from at least fifteen rats for the curves of the other two drugs. The vertical lines indicate standard errors of the means.

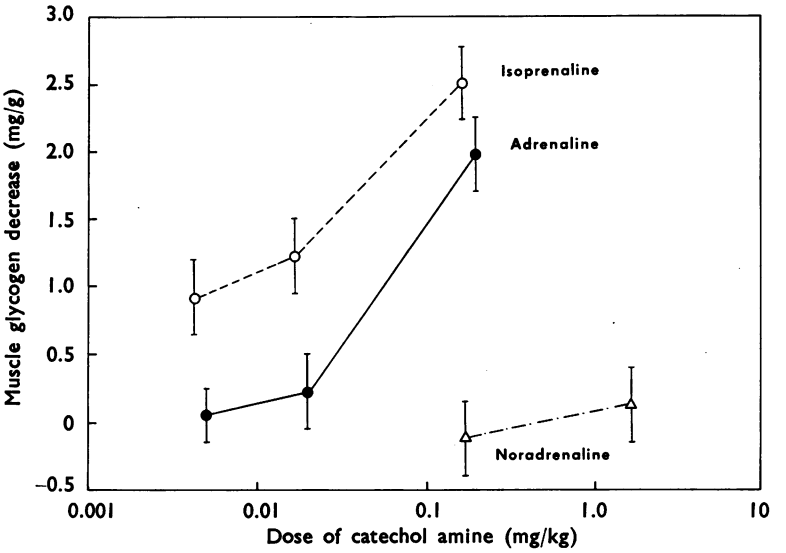

Fig. 3. Dose/response curves for the glycogenolytic responses in muscle to adrenaline, noradrenaline and isoprenaline in fed rats. The responses are expressed as the decreases in muscle glycogen compared to the muscle glycogen content (mean and standard error, 5.70±0.18 mg/g of wet tissue) of twenty-five control rats injected with 0.9% saline. The drugs were injected subcutaneously and muscle glycogen levels were determined 1 hr later. The doses are plotted in terms of the free base of drug. Each point represents the mean value from at least ten rats. The vertical lines indicate the standard errors of the means.

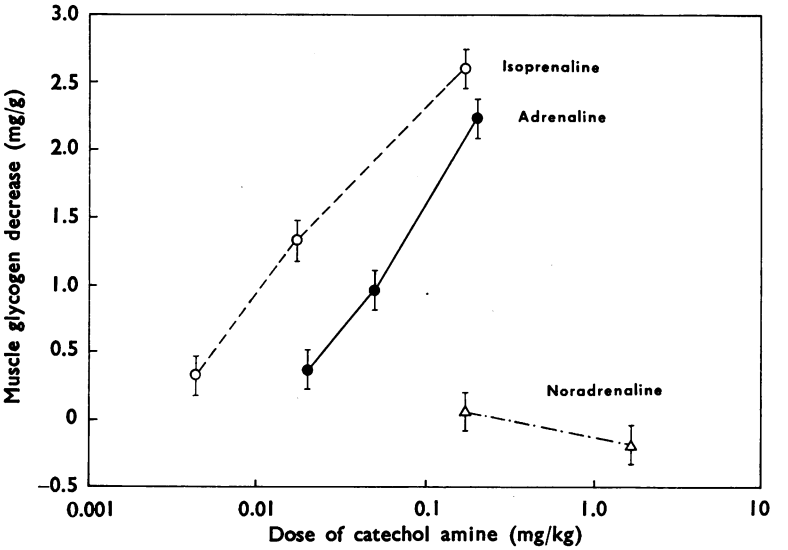

Fig. 4. Dose/response curves for the glycogenolytic responses in muscle to adrenaline, noradrenaline and isoprenaline in fasted rats. The responses are expressed as the decreases in muscle glycogen compared to the muscle glycogen content (mean and standard error, 4.50±0.11 mg/g of wet tissue) of fifteen control rats. The drugs were injected subcutaneously and muscle glycogen levels were determined 1 hr later. The doses are plotted in terms of the free base of drug. Each point represents the mean value from at least nine rats. The vertical lines indicate the standard errors of the means.

gave maximal responses. The responses to adrenaline continued to increase with dose so that at 0.1 mg/kg or more the descending order of potency was adrenaline—isoprenaline—noradrenaline. In interpreting the orders of potency, particularly from Figs. 2, 3 and 4, it must be remembered that isoprenaline was given as the racemic-form whereas the other drugs were administered as their laevo-isomers.

When the values in Figs. 1 and 2 are compared it is evident that the response to adrenaline was little affected by fasting. At lower doses, 0.02 and 0.2 mg/kg, the response was slightly greater in the fasted rats. At the highest dose, 0.5 mg/kg, the response was slightly less in the fasted rats. On the other hand the responses to the other two catechol amines were decisively influenced by the prandial state of the rats. Whereas feeding enhanced the response to noradrenaline it completely abolished the response to isoprenaline.

Muscle glycogen experiments. The dose/response curves for adrenaline, noradrenaline and isoprenaline on muscle glycogen in fed and fasted rats are plotted in Figs. 3 and 4 respectively. With rats in both conditions the descending order of potency was isoprenaline—adrenaline—noradrenaline. Noradrenaline had no glycogenolytic activity even at a dose of 2 mg/kg. In contrast to the hyperglycaemic response the effect on muscle glycogen was little influenced by the prandial condition of the rats. Of the doses compared, the only significant difference between the response in fasted rats and that in fed rats was that 0.005 mg/kg of isoprenaline caused a greater response in the fed rats.

DISCUSSION

It is clear that the relative hyperglycaemic activities of the three catechol amines depend on the prandial state of the rat and the dose range. It is equally clear that the hyperglycaemic action cannot itself be conveniently classified into either a simple α- or a simple β-receptor response, according to the system proposed by Ahlquist (1948) for other responses. This system is based on the relative responsiveness of smooth and cardiac muscle to a series of catechol amines (combining with α-receptors, in descending order of potency: adrenaline—noradrenaline—isoprenaline; with β-receptors: isoprenaline—adrenaline—noradrenaline). However, our results are consistent with the hypothesis suggested by others (Vrij, Gho, Groot & Weber, 1956; Pol, 1956; Hynie, Wenke & Mühlbachová, 1961; Roy & Schulhof, 1961) that in the rat liver glycogenolysis is mediated by an α-receptor and that muscle glycogenolysis is mediated by a β-receptor.

There seems to be little controversy concerning the belief that α-receptors predominate in the liver of the rat (Roy & Schulhof, 1961; Kennedy & Ellis, 1963). The results of our study are consistent with this idea. The fact that isoprenaline exhibited no hyperglycaemic activity in fed rats (Fig. 1) and the observation that fasting, which reduces the glycogen content of the liver to 1% of its normal value (Long, Katzin & Fry, 1940), greatly decreased the hyperglycaemic response to noradrenaline (Fig. 2) may be cited in support of the concept.

The concept that β-receptors predominate in the glycogenolytic response in muscle has not been consistently supported in the literature. Ellis, Davis & Anderson

(1955), studying the *in vitro* effects of catechol amines on glycogenolysis in rat diaphragm muscle, found that the descending order of potency was isoprenaline—adrenaline—noradrenaline. This work, elegantly documented with dose/response curves, supports the concept, as does a later report of an *in vivo* study from the same laboratory (Kennedy & Ellis, 1963). However, Vrij *et al.* (1956) measured the depletion *in vivo* of glycogen in the tibialis anterior muscle of the fasted rat. They found that the descending order of potency, appraised from the effects seen with only single doses of each drug, was adrenaline—isoprenaline—noradrenaline, a finding which conflicts with that of Ellis *et al.* (1955) and with the results that we present here (Fig. 4).

The evidence in the literature concerning the hyperglycaemic activity of isoprenaline is discordant. Whereas Ellis & Anderson (1951) reported that isoprenaline had no hyperglycaemic activity in the rat, even at doses as high as 25 mg/kg, other investigators have reported definite hyperglycaemic effects in the rat (Pol, 1956 ; Claassen & Noach, 1960). This inconsistency can now be understood if the conditions of the experiments are examined ; Ellis & Anderson (1951) used fed rats, whereas the latter investigators used fasted animals. The picture is not as clearcut in the dog. Although several investigators have found that isoprenaline causes a hyperglycaemic response in the fasted dog (Mayer, Moran & Fain, 1961 ; McClure, 1960 and personal communication ; McCutcheon, 1962), and thus confirm our findings in the rat, there is one report of a hyperglycaemic response to isoprenaline in the fed dog (Ashmore, Preston & Love, 1962). The latter observation, in conflict with our results in the rat, suggests that dog liver contains a significant number of β-receptors. We do note, however, that some of the control concentrations of blood glucose, in what were claimed to be fed dogs (Ashmore *et al.*, personal communication), were 65 to 80 mg/100 ml. The work of Murad, Chi, Rall & Sutherland (1962), in which the descending order of potency in stimulating *in vitro* the accumulation of adenosine 3′,5′-phosphate in dog liver was isoprenaline—adrenaline—noradrenaline, supports the view that in dog liver, in contrast to rat liver, β-receptors predominate.

If the hyperglycaemic response results from the interaction of the sympathomimetic amine with both α- and β-receptors it is possible to explain why complete block of this response has proved difficult. The classical α-receptor blocking drugs, such as phenoxybenzamine or dibenamine, have not been tested under the right circumstances. In most instances they have been tested in the fasted animal, a condition which tends to favour the response to drugs acting on β-receptors (Fig. 2). Under these circumstances it can be expected that these blocking agents would be ineffective, as indeed they have proved to be in rats (Tobe *et al.*, 1961), in cats and rabbits (Harvey, Wang & Nickerson, 1952), and in dogs (Mayer *et al.*, 1961 ; McCutcheon, 1962). The crucial experiment would be to test the effectiveness of these antagonists against noradrenaline, or even adrenaline, in the rat which has been fed, a condition which favours the response to drugs acting on α-receptors (Fig. 1). Schwartz (1962) tested the antagonism of phenoxybenzamine against adrenaline in fed rats ; as is to be expected, relatively good block of the hyperglycaemic response was obtained. Dichloroisoprenaline, the β-receptor blocking agent, should give

effective block of the hyperglycaemic response when tested against isoprenaline, or even adrenaline, in the fasted animal. This was the finding reported by Mayer *et al.* (1961), Claassen & Noach (1960) and McCutcheon (1962). Conversely dichloroisoprenaline should be completely ineffective as an antagonist of the hyperglycaemic response to noradrenaline, or even to adrenaline, in the fed rat. Using adrenaline in fed mice, Mayer *et al.* (1961) failed to demonstrate any blocking action of dichloroisoprenaline. The effectiveness of dihydroergotamine as an antagonist of adrenaline (Harvey *et al.*, 1952 ; Ellis, Anderson & Collins, 1953 ; Tobe *et al.*, 1961) is not surprising in view of the fact that this antagonist blocks both α- and β-receptors (Levy & Ahlquist, 1961).

We hold that, if the hyperglycaemic response to sympathomimetic amines is discussed in terms of α- and β-receptors, it is possible to make a generalization on the basis of the discussion presented above. It may be concluded that in the fed rat the hyperglycaemic response is mediated predominantly by the α-receptors of liver and that in the fasted rat the response is mainly due to the action of the β-receptors of muscle. A special case arises in the fasted rat when a drug with activity on both α- and β-receptors, such as adrenaline, is used. Although the receptors in liver would not normally respond to a drug which acts on α-receptors, due to lack of substrate glycogen, it must be remembered that the drug, in possessing also activity on β-receptors, can cause glycogenolysis in muscle and a concomitant increase in blood lactate concentration (Cori & Buchwald, 1930). This lactate may then be converted to liver glycogen (Cori & Cori, 1929 ; Winternitz, Dintzis & Long, 1957) and supply the substrate for the drug's activity on α-receptors. Under these circumstances both α- and β-receptors will respond to the drug's action. It follows that a β-receptor blocking agent, by preventing the release of lactic acid, should antagonize adrenaline in the fasted rat. In the fed rat, the β-receptor blocking agents should be completely ineffective in blocking the hyperglycaemic response to adrenaline. On the other hand an α-receptor blocking agent should be effective in the fed rat.

Caution should be exercised in extrapolating these observations in rats fasted for 20 hr to those subjected to longer periods of fasting and to other species. The liver glycogen is greatly depleted in the rat by a 24 hr fast. With more prolonged fasting the liver glycogen begins to rise as the result of glucogenesis (Long *et al.*, 1940). Such a rise in glycogen would influence the hyperglycaemic response, particularly to noradrenaline. Animals of other species, with a different distribution of α- and β-receptors between liver and other tissues and with different relationships between the liver glycogen content and the duration of fasting, would not necessarily respond in a fashion similiar to that in rats.

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REFERENCES

AHLQUIST, R. P. (1948). A study of the adrenotropic receptors. *Amer. J. Physiol.*, **153**, 586–600.

ASHMORE, J., PRESTON, J. E. & LOVE, W. C. (1962). Effects of dichloroisoproterenol on blood sugar and plasma free fatty acids. *Proc. Soc. exp. Biol. (N.Y.)*, **109**, 291–294.

CLAASSEN, V. & NOACH, E. L. (1960). Dichloro-isuprel inhibition of sympathomimetic hyperglycaemia. *Arch. int. Pharmacodyn.*, **126**, 332–340.

CORI, C. F. & BUCHWALD, K. W. (1930). Effect of continuous intravenous injection of epinephrine on the carbohydrate metabolism, basal metabolism and vascular system of normal men. *Amer. J. Physiol.*, **95**, 71–78.

CORI, C. F. & CORI, G. T. (1929). Glycogen formation in the liver from d- and l-lactic acid. *J. biol. Chem.*, **81**, 389–403.

ELLIS, S. & ANDERSON, H. L. (1951). The effect of amines on the blood sugar of the rat. *J. Pharmacol. exp. Ther.*, **101**, 92–100.

ELLIS, S., ANDERSON, H. L. & COLLINS, M. C. (1953). Pharmacologic differentiation between epinephrine- and HGF-hyperglycemias: application in analysis of cobalt-hyperglycemia. *Proc. Soc. exp. Biol. (N.Y.)*, **84**, 383–386.

ELLIS, S., DAVIS, A. H. & ANDERSON, H. L., JR. (1955). Effects of epinephrine and related amines on the contraction and glycogenolysis of the rat's diaphragm. *J. Pharmacol. exp. Ther.*, **115**, 120–125.

FLEMING, W. W. & KENNY, A. D. (1961). Relative hyperglycemic activities of sympathomimetic drugs in intact rats. *Pharmacologist*, **3**, 50.

GOOD, C. A., KRAMER, H. & SOMOGYI, M. (1933). The determination of glycogen. *J. biol. Chem.*, **100**, 485–491.

HARVEY, S. C., WANG, C. Y. & NICKERSON, M. (1952). Blockade of epinephrine-induced hyperglycemia. *J. Pharmacol. exp. Ther.*, **104**, 363–376.

HYNIE, S. VON, WENKE, M. & MÜHLBACHOVÁ, E. (1961). Zur charackeristik der Beeinflussung des Glycid-Metabolismus durch Adrenomimetica. *Arzneimittel-Forsch.*, **11**, 858–861.

KENNEDY, B. L. & ELLIS, S. (1963). Interactions of sympathomimetic amines and adrenergic blocking agents at receptor sites mediating glycogenolysis. *Fed. Proc.*, **22**, 449.

KENNY, A. D. & MOREY, E. R. (1962). The hyperglycemic activity of 2,2'-bipyridine. *J. Pharmacol. exp. Ther.*, **135**, 317–322.

LEVY, B. & AHLQUIST, R. P. (1961). An analysis of adrenergic blocking activity. *J. Pharmacol. exp. Ther.*, **133**, 202–210.

LONG, C. N. H., KATZIN, B. & FRY, E. G. (1940). The adrenal cortex and carbohydrate metabolism. *Endocrinology*, **26**, 309–344.

MAYER, S., MORAN, N. C. & FAIN, J. (1961). The effect of adrenergic blocking agents on some metabolic actions of catecholamines. *J. Pharmacol. exp. Ther.*, **134**, 18–27.

MCCLURE, D. A. (1960). Dichloroisoproterenol (DCI) inhibition of sympathomimetic-induced hyperglycemia. *Pharmacologist*, **2**, 94.

MCCUTCHEON, R. S. (1962). Canine blood sugar and lactic acid responses to adrenergic block. *J. Pharmacol. exp. Ther.*, **136**, 209–212.

MURAD, F., CHI, Y.-M., RALL, T. W. & SUTHERLAND, E. W. (1962). Adenyl cyclase. III. The effect of catecholamines and choline esters on the formation of adenosine 3',5'-phosphate by preparations from cardiac muscle and liver. *J. biol. Chem.*, **237**, 1233–1238.

NELSON, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. biol. Chem.*, **153**, 375–380.

POL, M. C. VAN DER (1956). The effect of some sympathicomimetics in relation to the two receptor-theory. *Acta physiol. pharmacol. neerl.*, **4**, 541–546.

ROY, F. P. VAN & SCHULHOF, L. W. (1961). The effect of sympathomimetics on glycogenolysis of rat liver slices *in vitro*. *Arch. int. Pharmacodyn.*, **130**, 368–373.

SCHWARTZ, N. B. (1962). Effect of dibenzyline on the metabolic actions of epinephrine and thyroxine. *Amer. J. Physiol.*, **203**, 525–531.

TOBE, M., KURIHARA, N. & TAKEUCHI, S. (1961). Studies on intravenous glucose tolerance curve. 6. Effect of adrenergic blocking agents. *Endocr. jap.*, **8**, 77–85.

VRIJ, C. J., GHO, B. K., GROOT, C. A. DE & WEBER, J. F. (1956). The effect of isopropyl-noradrenaline and nor-adrenaline on the glycogen content of skeletal muscle and liver of the rat. *Acta physiol. pharmacol. neerl.*, **4**, 547–554.

WINTERNITZ, W. W., DINTZIS, R. & LONG, C. N. (1957). Further studies on the adrenal cortex and carbohydrate metabolism. *Endocrinology*, **61**, 724–741.