Biochemical Pharmacology, Vol. 17, pp. 2205–2213. Pergamon Press. 1968. Printed in Great Britain

DISSOCIATION OF EPINEPHRINE-INDUCED FREE FATTY ACID AND GLYCEROL RELEASE BY ADRENERGIC BLOCKING DRUGS*

JUDY A. SPITZER

Department of Physiology, Hahnemann Medical College and Hospital, Philadelphia, Pa., U.S.A.

(*Received* 12 *January* 1968; *accepted* 5 *April* 1968)

Abstract—Metabolic effects of epinephrine and adrenergic blocking agents were studied in unanesthetized, intact male albino rabbits. Changes in plasma concentration of free fatty acids (FFA), blood glycerol, glucose, lactate and pyruvate were measured. Epinephrine infusion (0·1 μg/kg/min for 40 min) caused the levels of these metabolites to rise and the ratio of plasma FFA/glycerol to decrease. Ergotamine base infusion (0·25 mg/kg and 0·75 mg/kg) inhibited the epinephrine-induced plasma FFA rise 82 per cent at the lower and 91 per cent at the higher dosage level. At the same time, blood glycerol levels were not reduced below those obtained in animals not previously treated with the blocker. β-Adrenergic blockade (propranolol, 3·5 mg/kg) inhibited the epinephrine-induced rise in blood glycerol much more (91 per cent) than the rise in FFA (22 per cent); it blocked the marked lactacidemia (73 per cent) and the rise in pyruvate (86 per cent) and reduced the elevated blood glucose level by 42 per cent. The marked decrease in FFA/glycerol ratio due to epinephrine was also eliminated. It is suggested that the use of adrenergic blocking agents promotes differential lipase activation in the adipose tissue, mediated through shifts in the adenine nucleotide cycle.

SYMPATHETIC regulation of metabolism extends to such areas as lipid metabolism, carbohydrate metabolism, oxygen consumption and electrolyte metabolism. Evidence has been accumulating that the metabolic action of the hormones of the sympathetic nervous system, epinephrine and norepinephrine, is mediated through the formation of cyclic adenosine-3′, 5′-monophosphate (3,5-AMP) by direct activation of adenyl cyclase.[1–4] Although muscle, heart and liver are also directly affected, it is the change in adipose tissue metabolism[5–7] which has the most far-reaching consequences on the whole body in terms of modifying the supply of oxidizable substrates to tissues.

The lipolytic effects of catecholamines and ACTH can be inhibited by both α- and β-adrenergic blocking agents, although the latter group has been shown to be more potent in dogs and man.[8, 9] Recent investigations suggest that α- and β-adrenolytics may have different sites of action in the lipolytic systems.[10, 11] The apparent differences in metabolic consequences of actions of α-receptors as contrasted with β-receptors have been explored recently by Ellis and Eusebi,[12] emphasizing the effects of adrenergic blocking agents rather than the relative potencies of catecholamines. These investigators reported that, in cats, hyperglycemic doses of cyclic 3′,5′-AMP do not cause hyperkalemia. They also showed that the hyperglycemic response to epinephrine could be prevented by β-adrenergic blocking agents without modifying the hyperkalemic

* Supported by Grant HE 03130 from the National Institutes of Health, Bethesda, Md.

response, while the hyperkalemia could be prevented by α-blocking agents without affecting the hyperglycemic response.

We have examined the effects of epinephrine in the presence and absence of α- and β-adrenergic blocking agents on several blood metabolites in intact rabbits and were able to show a dissociation of FFA and glycerol release by the use of these agents.

METHODS

Unanesthetized, male, albino rabbits (body wt. 7·5–9 lb) were used after an 18-hr fast in all experiments. Epinephrine and blocking agents were infused at a rate of 1 ml/min through polyethylene cannulae (PE 50) placed through an 18 gauge needle in the marginal vein of the ear. Ergotamine* was used as the α-adrenergic blocking agent at 0·25 and 0·75 mg/kg dosage levels. Although ergot alkaloids have some anomalous effects not in line with the predictable pattern of either α- or β-adrenergic blockade, e.g. inhibition of adrenaline-induced relaxation of intestinal smooth muscle *in vitro* or inhibition of FFA mobilization under certain conditions, they are considered to be most effective α-adrenergic blocking agents in other test systems.[13] Inhibition of metabolic responses does not follow expected patterns of adrenergic blockade. It was felt that the use of an agent such as ergotamine might provide important clues to the mechanism of adrenergic effects on fat and carbohydrate metabolism. β-adrenergic receptors were blocked by propranolol.† The dosage of propranolol was 3·5 mg/kg.

Epinephrine was given in a continuous infusion (0·1 μg/kg/min) for 40 min. In experiments evaluating the effect of blocking agents, the agent was infused in a 20-min period, the animal was allowed to rest for 1 hr and then the epinephrine infusion followed.

Blood samples were obtained by cardiac puncture, chilled and processed immediately for determination of the various metabolites studied. Plasma FFA was determined according to the method of Dole and Meinertz.[14] Enzymatic methods were used for the determination of blood glycerol, lactate and pyruvate.[15–17] Glucose concentrations were estimated on the Technicon Auto-analyzer.

TABLE 1. METABOLIC EFFECTS OF EPINEPHRINE IN RABBITS*

Experimental conditions	FFA (μequiv./ml)	Glycerol (μmoles/ml)	FFA/ glycerol	Lacetate pyruvate (μmoles/ml)		Glucose (mg/100 ml)
Control	0·489 ± 0·057 (5)	0·091 ± 0·020 (6)	7·8 ± 2·3	3·1 ± −0·05	0·064 ± 0·019	98 ± 4·9
Epinephrine	0·828 ± 0·084	0·387 ± 0·066	2·6 ± 0·58	11 7 ± 2·1	0·159 ± 0·048	246 ± 15·7

* Control samples were taken before starting the infusion. Epinephrine, 0·1 μg/kg/min, was infused i.v. for 40 min and a blood sample obtained immediately after termination of the infusion. Each value represents the mean ± S.E. of the mean of 7 experiments, with the exception of control FFA and glycerol values, where the numbers in parentheses refer to the number of experiments.

RESULTS AND DISCUSSION

Epinephrine infusion. Intravenous infusion of epinephrine (0·1 μg/kg/min) for 40 min caused the levels of all metabolites studied to rise. The ratio of plasma FFA/ glycerol decreased (Table 1). According to Rudman *et al.*,[18] epinephrine had no FFA

* Kindly supplied by Dr. Paul S. Arcese of Sandoz Pharmaceutical, Hanover, N.J.

† Propranolol (Inderal) was generously donated by Dr. Alex Sahagian-Edwards of Ayerst Laboratories, Inc., New York, N.Y.

mobilizing effect *in vivo* in the rabbit. However, they determined the effect 150 min after the injection of epinephrine. By this time, as is pointed out by Svedmyr,[19] the transitory effect of epinephrine had disappeared.

Ergotamine base results. Administration of ergotamine base alone at the 0·25 mg/kg level leaves the serum FFA, glycerol, pyruvate and glucose concentrations unchanged. Lactate levels decrease somewhat.

Epinephrine infusion of these rabbits treated with ergotamine base raises the FFA level very little, but more than triples the blood glycerol concentrations. The degree (in per cent) to which the rise in FFA level elicited by epinephrine was reduced by administration of a "blocking" drug was calculated according to the following formula:[20]

$$\frac{[\text{FFA}]\ \text{epinephrine} - [\text{FFA}]\ \text{epinephrine} + \text{blocker}}{[\text{FFA}]\ \text{epinephrine} - [\text{FFA}]\ \text{blocker}} \times 100.$$

The same calculation was also applied to determine the extent of inhibition in the rise of other metabolites.

The epinephrine-induced FFA rise was reduced 82 per cent by this lower dose of the drug; the rise in glycerol was reduced by 35 per cent. Compared to untreated, epinephrine-infused animals, the pyruvate level increases, the rise in blood glucose is 25 per cent less and the rise in blood lactate is 57 per cent less (Fig. 1).

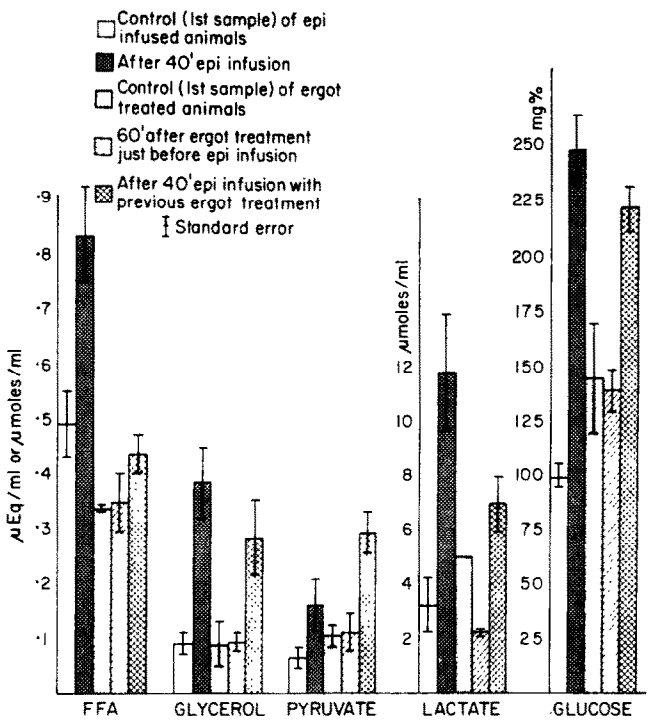

FIG. 1. Effect of ergotamine base (0·25 mg/kg) upon epinephrine-induced (0·1 μg/kg/min) metabolic changes in rabbits. Bars representing values after 40 min of epinephrine infusion and their controls are mean values of 7 animals. Each of the rest of the bars represents mean values of 3 animals serving as their own controls. Abbreviations used in this figure and in Fig. 2 are; epi, epinephrine; ergot, ergotamine base.

Infusion of 0·75 mg/kg of ergotamine base causes the plasma FFA, glycerol and glucose levels to rise; pyruvate and lactate concentrations are unaffected. Epinephrine infusion in these animals elicits practically no further detectable increase in plasma FFA (there is 91 per cent blocking of the epinephrine effect), whereas glycerol and pyruvate levels rise, although the levels attained are not statistically different from those in animals not treated with ergotamine. Lactate and glucose concentrations reach about the same levels in both treated and untreated rabbits after epinephrine infusion (Fig. 2). Assuming that the blood lactate level reflects the extent of muscle

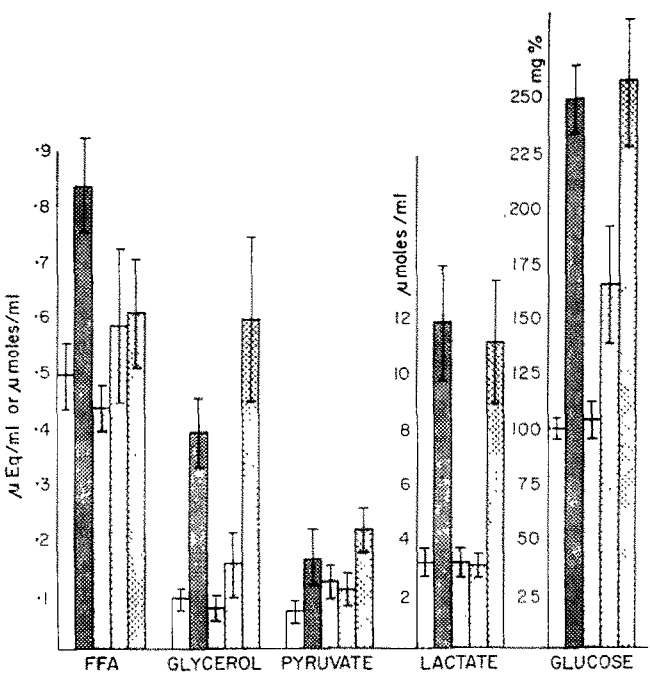

FIG. 2. Effect of ergotamine base (0·75 mg/kg) upon epinephrine-induced (0·1 μg/kg/min) metabolic changes in rabbits. Bars representing values after 40 min of epinephrine infusion and their controls are mean values of 7 animals. Each of the rest of the bars represents mean values of 5 animals serving as their own controls. The key for the cross-hatching of the bars is shown in Fig. 1.

glycogenolysis, it may be said that this dosage of ergotamine in the rabbit does not affect the epinephrine-induced muscle glycogenolytic response, since the rise in blood lactate is comparable to the rise seen in animals treated with epinephrine alone.

The results indicate that in intact rabbits ergotamine base at a 0·75 mg/kg dosage blocks FFA release in response to epinephrine by 91 per cent and it does not reduce the concentration of glycerol below the epinephrine response. This may be due to some difference in the mechanism of release, the FFA release being subject to regulation by α-type receptors. In favor of this hypothesis, one may cite the finding by Hagen and Hagen[21] that during intravenous infusion of norepinephrine in rabbits the increase of serum glycerol concentration is more rapid than that of FFA. Although glycerol is distributed in a space approximating 65 per cent of body weight,[22] whereas plasma FFA levels are curtailed by the available albumin space, upon epinephrine infusion,

the rise in blood glycerol is proportionately much greater than the concurrent rise in FFA. It has been reported previously in dogs that norepinephrine infusion does not always cause proportionate increases in blood glycerol concentration.[23] Since it is assumed that triglyceride breakdown in adipose tissue is the normal source of blood glycerol,[24] one would expect the opposite, i.e. a proportionately greater elevation of plasma FFA. For several reasons, however, this is not so.

After hydrolysis, part of the fatty acids will be reesterified and returned to the "triglyceride pool" without ever leaving the adipose tissue cell. In contrast, the liberated glycerol can diffuse rapidly into the blood, since it is not utilized again for trigylceride synthesis in white adipose tissue. Epinephrine stimulates esterification, as has been shown quite dramatically with rat adipose tissue;[25] this effect is species dependent. In pigeon adipose tissue, the esterification of FFA is not increased by epinephrine or norepinephrine, despite a large increase in FFA production.[26] The metabolic disappearance of FFA and glycerol from the plasma is also very different. The half-life of FFA in the plasma is very rapid, about 1·5 to 2·5 min,[27] whereas that of glycerol is estimated to be about 30 min.[28] Finally, epinephrine increases FFA oxidation by increasing the proportion of FFA within the total amount of substrates available to tissues. This may also contribute to the disproportionate increase in glycerol observed.

Prevention by ergotamine of epinephrine-induced FFA rises (in 2 of 8 dogs), of hepatic glucose responses[29] and of blood glucose responses[8] has been reported. Ergotamine, however, does not block the hyperglycemic response to glucagon in rabbits[30] or in homogenates of cat and dog liver.[31, 32] As our present data indicate, ergotamine reduces the epinephrine-induced hyperglycemia considerably, due mainly to the markedly elevated blood glucose level after ergotamine infusion. However, the final blood glucose concentrations in the ergotamine-treated and untreated animals after epinephrine infusion are very similar. The differences observed underscore the species variation in metabolic receptors for a particular response.

Since our results were obtained in a system *in vivo*, a number of different factors have to be considered as possible influences. Changes in insulin secretion may have occurred as a result of the experimental manipulations. Reciprocity between blood sugar level and insulin secretion has been known for a long time. Not only does insulin regulate blood sugar concentrations, but high blood sugar levels also stimulate insulin secretion.[33] Epinephrine-induced hyperglycemia in the ergotamine-treated rabbits in conjunction with possibly increased insulin secretion may well have augmented reesterification and thus contributed to the observed significant reduction in plasma FFA.

The relationship between plasma FFA and pyruvate levels brings to mind another possible mechanism at play. Randle *et al.* have demonstrated[34] that fatty acids can diminish the rate of oxidation of pyruvic acid in the isolated perfused heart and in the diaphragm. Epinephrine-induced plasma FFA rise in the ergotamine-treated (0·75 mg/kg) rabbits is negligible. Yet, from the amount of glycerol released, one might assume that considerable lipolysis had taken place. If a large portion of the FFA had been retained by the tissues, this would diminish the oxidation of pyruvate and contribute to the observed tendency to raised blood pyruvate concentration. One must remember, however, that changes in the blood lactate to pyruvate ratio also contribute to any alterations in pyruvate oxidation.

In rabbits we find the α- and β-adrenergic blocking drugs quite useful in selectively accentuating the two components of the lipolytic response, FFA and glycerol release. It may be that different cells in the adipose tissue are involved rather than fundamentally different receptors. More plausible, perhaps, is the explanation that we are dealing with a manifestation of differential lipase activation—monoglyceride lipase being activated or inhibited preferentially over triglyceride lipase under certain conditions[35]—due to intracellular compartmentalization and shifts in contact with cyclic AMP and ATP.

Propranolol results. The metabolic effects of propranolol, by itself, are shown in Table 2. The drug induced a moderate hyperglycemic response and lowered the lactate level significantly. The other metabolites measured were unaffected.

TABLE 2. METABOLIC EFFECTS OF PROPRANOLOL IN RABBITS*

	Before infusion	60 Min after infusion
FFA (μEquiv./ml)	0·579 ± 0·093†	0·600 ± 0·137
Glycerol, (μmoles/ml)	0·106 ± 0·020	0·087 ± 0·029
Lactate (μmoles/ml)	6·0 ± 1·02	2·9 ± 0·54
Pyruvate (μmoles/ml)	0·062 ± 0·013	0·061 ± 0·011
Glucose (mg/100 ml)	103 ± 6·8	136 ± 4·2

* 3·5 mg/kg of the drug was infused in 20 min.

† Mean ± S.E.M. These data were obtained from 13 animals.

Propranolol at the 3·5 mg/kg level inhibited the epinephrine-induced (0·1 μg/kg/min) rise in blood glycerol much more (91 per cent) than the rise in FFA (22 per cent); it blocked the marked lactacidemia (73 per cent) and the rise in pyruvate (86 per cent) and reduced the elevated blood glucose level by 42 per cent (Fig. 3). The marked decrease in the FFA/glycerol ratio caused by epinephrine administration was also eliminated. The marked reduction in lactacidemia indicates that in the rabbit muscle, glycogen depletion is a β-receptor function. The data also support the suggestion that rabbit livers have receptors which have characteristics different from those of the typical β-receptors.[36]

Of special interest is the lipolytic response evoked by epinephrine in the propranolol-treated animals. FFA release is blocked only to a moderate extent (22 per cent), whereas glycerol release is almost completely obliterated. In this instance also, a number of processes contribute to the observed net lipolytic response. Epinephrine-induced hyperglycemia in the propranolol-treated animals is modified by the elevated blood glucose level due to the administration of the blocker itself. Therefore, it seems reasonable to assume that stimulation of insulin secretion via plasma glucose is overshadowed by inhibition of insulin release by epinephrine.

The reduced availability of insulin can lessen re-esterification and thus promote the high plasma FFA level noted (Fig. 3). Under these conditions there is probably a relative absence of high tissue FFA levels, which may explain the lack of interference with pyruvate oxidation[34] as evidenced by no increase in plasma pyruvate levels after epinephrine infusion in propranolol-treated rabbits.

Epinephrine can quickly inhibit insulin release from the β-cell as well as stimulate release of FFA from the fat cell. In the intact animal, both decreased insulin availability and increased concentration of FFA are essential to an integrated metabolical response. Since insulin and adrenergic blocking drugs both inhibit the activation of adipose tissue lipase, it has been proposed that there may be separate but adjacent

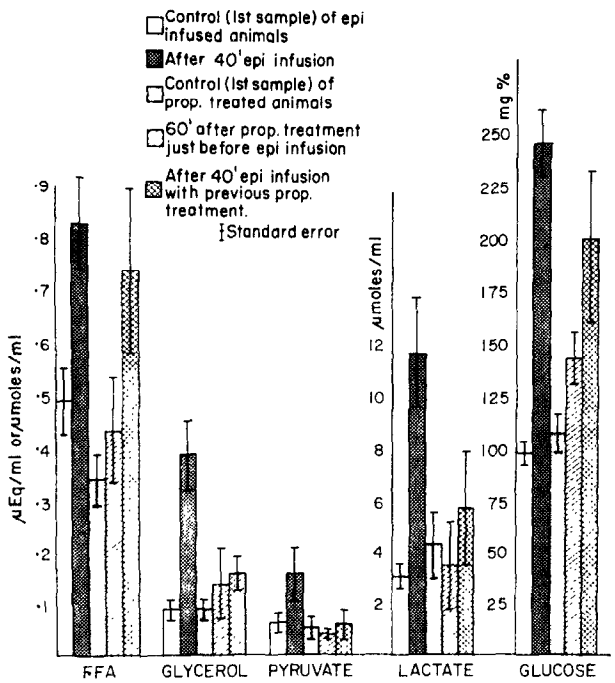

FIG. 3. Effect of propranolol (3·5 mg/kg) upon epinephrine-induced (0·1 μg/kg/min) metabolic changes in rabbits. Bars representing values after 40 min of epinephrine infusion and their controls are mean values of 7 animals. Each of the rest of the bars represents mean values of 4 animals serving as their own controls. Abbreviations used are: epi, epinephrine; prop., propranolol.

receptor sites for insulin, catecholamines and β-adrenergic blockers. The high concentrations of β-adrenergic blocking agents required to inhibit insulin action make it unlikely that these compounds have any significant effect on insulin *in vivo*.[37]

In studying the lipid-mobilizing actions of some sympathomimetic drugs, in addition to the usually assumed simple bimolecular reaction (drug + receptor), "quadratic" dose-response relations, very similar to trimolecular reaction laws (drug + two receptor sites), were also described.[38, 39] As with the lipid-mobilizing sympathomimetics, good agreement was found for β-blocking agents with mathematical models based on the existence of two different quantitative laws applicable to lipid mobilization. According to these quantitative studies, propranolol behaves like a "two-receptor" antagonist when affecting lipid mobilization in rat epididymal tissue.[40] Furthermore, the two-receptor characteristic is not due to masking of release by reesterification.

Adipose tissue contains a number of lipolytic enzymes: lipoprotein lipase, which cleaves triglycerides in the presence of lipoproteins and is presumably active in fat assimilation; a "hormone-sensitive" triglyceride lipase, activated by epinephrine through the evolution of cyclic AMP; a monoglyceride lipase; diglyceride lipase; and possibly also a trigylceride–monoglyceride fatty acyl transferase.[41]

It is suggested that active and inactive forms of the lipolytic enzymes exist in equilibrium in adipose tissue. Local concentrations of ATP and cations seem to be involved in shifts of the equilibrium and may be instrumental in hormonal effects on adipose tissue lipolysis. ATP exerts a stronger inhibitory influence on the lipolysis of monoglycerides than on that of triglycerides.[35] β-adrenergic receptor stimulation produces an increase in the cyclic 3′,5′-AMP level in isolated pancreatic islets, isolated fat cells and toad bladders.[42] By using propranolol, it is reasonable to assume that the relationship between the cyclic nucleotide and ATP is altered in favor of the latter. This increased concentration of ATP in the adipose tissue can then differentially inhibit monoglyceride lipase more than triglyceride lipase, resulting, upon epinephrine administration, in the observed high release of FFA in the face of minimal release of glycerol.

Acknowledgements—The author wishes to express her sincere thanks to Dr. Sidney Ellis for discussion of the problem and critical reading of the manuscript. The interest and cooperation given so freely by Dr. John J. Spitzer throughout completion of these investigations is greatly appreciated. It is a pleasure to acknowledge the skillful technical assistance rendered by Mr. Thomas J. Thurman and Mr. Joseph Bellesorte.

REFERENCES

1. E. W. SUTHERLAND and T. W. RALL, *Pharmac. Rev.* **12**, 265 (1960).
2. S. THYMIE, G. KRISHNA and B. B. BRODIE, *J. Pharmac. exp. Ther.* **153**, 90 (1966).
3. B. WEISS, J. I. DAVI and B. B. BRODIE, *Biochem. Pharmac.* **15**, 1553 (1966).
4. R. W. BUTCHER, J. G. T. SNEYD, C. R. PARK and E. W. SUTHERLAND, *J. biol. Chem.* **241**, 1652 (1966).
5. L. M. KLAINER, Y. M. CHI, S. L. FREIDBERG, T. W. RALL and E. W. SUTHERLAND, *J. biol. Chem.* **237**, 1239 (1962).
6. M. A. RIZACK, *J. biol. Chem.* **236**, 657 (1961).
7. M. VAUGHAN, J. E. BERHER and D. STEINBERG, *J. biol. Chem.* **239**, 401 (1961).
8. S. MAYER, N. C. MORAN and J. FAIN, *J. Pharmac. exp. Ther.* **134**, 18 (1961).
9. T. R. E. PILKINGTON, D. LOWE, B. F. ROBINSON and E. TITTERINGTON, *Lancet* **ii**, 316 (1962).
10. K. STOCK and E. WESTERMANN, *Life Sci.* **4**, 1115 (1965).
11. R. W. BUTCHER, R. J. FLO, H. C. MENG and E. W. SUTHERLAND, *J. biol. Chem.* **240**, 4515 (1965).
12. S. ELLIS and A. J. EUSEBI, *Fedn Proc.* **24**, 151 (1965).
13. S. C. HARVEY, C. Y. WANG and M. NICKERSON, *J. Pharmac. exp. Ther.* **104**, 363 (1952).
14. V. P. DOLE and H. MEINERTZ, *J. biol. Chem.* **235**, 2595 (1960).
15. O. WIELAND, *Methods of Enzymatic Analysis* (Ed. H. V. BERGMEYER), p. 211. Academic Press, New York (1963).
16. H. J. HOHORST, *Methods of Enzymatic Analysis* (Ed. H. V. BERGMEYER), p. 266. Academic Press, New York (1963).
17. T. BUCHER, R. CZOK, W. LAMPRECHT and E. LATZKO, *Methods of Enzymatic Analysis* (Ed. H. V. BERGMEYER), p. 253. Academic Press, New York (1963).
18. D. RUDMAN, S. J. BROWN and M. F. MALKIN, *Endocrinology* **72**, 527 (1963).
19. N. SVEDMYR, *Acta physiol. scand.* **71**, 1 (1967).
20. C. R. BOSHART, L. WILL, A. PIRRE and I. RINGLER, *J. Pharmac. exp. Ther.* **149**, 57 (1965).
21. J. H. HAGEN and P. B. HAGEN, *Can. J. Biochem. Physiol.* **40**, 1129 (1962).
22. J. A. LARSEN, *Acta physiol. scand.* **57**, 224 (1963).
23. R. J. HAVEL and L. A. CARLSON, *Life Sci.* **3**, 651 (1963).

24. L. A. Carlson and L. Oro, *Metabolism* **12**, 132 (1963).
25. R. L. Jungas and E. G. Ball, *Biochemistry, N.Y.* **2**, 383 (1963).
26. Alan G. Goodridge and E. G. Ball, *Comp. Biochem. Physiol.* **16**, 367 (1965).
27. D. S. Frederickson, R. S. Gordon, U. Ono and A. J. Cherkes, *Clin. Invest.* **37**, 1504 (1958).
28. E. Shafrir and E. Gorin, *Metabolism* **12**, 580 (1963).
29. W. T. McElroy and J. J. Spitzer, *Am. J. Physiol.* **200**, 318 (1961).
30. S. Ellis, H. O. Anderson and M. C. Collins, *Proc. Soc. exp. Biol. Med.* **84**, 383 (1953).
31. J. Berthet, E. W. Sutherland and T. W. Rall, *J. biol. Chem.* **229**, 351 (1957).
32. M. H. Makan and E. W. Sutherland, *Endocrinology* **75**, 127 (1964).
33. V. G. Foglia and R. Fernendez, *C.r. Séanc. Soc. Biol.* **121**, 355 (1936).
34. P. J. Randle, P. B. Garland, C. N. Hales and E. A. Newsholme, *Lancet* **i**, 785 (1963).
35. E. Gorin and E. Shafrir, *Biochim. biophys. Acta* **137**, 189 (1967).
36. S. Ellis, B. L. Kennedy, A. J. Eusebi and N. H. Vincent, *Ann. N.Y. Acad. Sci.* **139**, 826 (1967).
37. P. D. Bewsher, C. C. Hillman and J. Ashmore, *Ann. N.Y. Acad. Sci.* **139**, 891 (1967).
38. M. Cernohorsky, J. Cepelik and K. Elisova, *Čslká. Fysiol.* **15**, 29 (1966).
39. M. Wenke, D. Schusterova, M. Cernohorsky and J. Cepelik, *Čslká. Fysiol.* **15**, 30 (1966).
40. M. Wenke, D. Lincova, J. Cepelik, M. Cernohorsky and S. Hybie, *Ann. N.Y. Acad. Sci.* **139**, 860 (1967).
41. M. Vaughan and D. Steinberg, in *Handbook of Physiology*, (Eds. A. E. Renold and G. F. Cahill, Jr.), p. 239. Am. Physiol. Soc. (1965).
42. J. R. Turtle and D. M. Kipnis, *Biochem. biophys. Res. Commun.* **28**, 797 (1967).