Biochemical Pharmacology, 1963, Vol. 12, pp. 193–201. Pergamon Press Ltd., Printed in Great Britain.

ACTION OF EPINEPHRINE ON GLUCOSE UPTAKE AND GLUCOSE-6-PHOSPHATE IN THE DOG HEART *IN SITU**

STEVEN E. MAYER†

Department of Pharmacology, Division of Basic Health Sciences,
Emory University, Atlanta, Ga., U.S.A.

(*Received* 23 *August* 1962; *accepted* 17 *October* 1962)

Abstract—These experiments were designed to test the hypotheses: (1) that epinephrine inhibits glucose utilization as a result of the inhibition of phosphorylation by glucose-6-phosphate (glucose-6-P); (2) that the increase in the concentration of this ester is correlated with the positive inotropic action of the amine. The investigations were conducted on anesthetized dogs to which saline or epinephrine (1 or 10 μg/kg min^{-1}) were infused for 15 min. The chloride and glucose space of the right ventricle and its content of glucose-6-P and the activity of glycogen phosphorylase were measured. In the control series 6% of the total phosphorylase was in the *a* form, the glucose space was 14% and glucose-6-P was 0·4 mmole/kg. After 1 μg epinephrine, left ventricular-contractile force increased 76% and the enzyme was 19% in the *a* form, but the glucose space and glucose-6-P remained unchanged. After 10 μg of the drug, contractile force did not increase further and phosphorylase was 93% active, the glucose space rose to 67% and glucose-6-P concentration was 0·74 mmole/kg. Similar results were found in fed and fasted dogs and after pancreatectomy. These results indicate that inhibition of glucose utilization was associated with glucose-6-P accumulation, but this was not correlated with augmentation of contractile force of the heart.

IT HAS been known for many years that the administration of epinephrine to animals increased the glucose concentration[1] and hexose monophosphate content[2] of muscle and decreased the rate of utilization of glucose.[3, 4] Kipnis and co-workers[5] subsequently suggested that these observations could be explained on the following basis. The elevation of glucose-6-P which occurred in muscle was thought to cause a decrease in glucose phosphorylation, consequent to which free glucose accumulated in the cell. The inhibition of glucose phosphorylation was attributed to the noncompetitive inhibition of hexokinase by glucose-6-P.[6–8] The increase in hexose monophosphate was given additional significance by the proposal of Ellis that this metabolite was a factor in the inotropic action of the catecholamines.[4, 9]

The experiments reported in this paper were designed to determine the correlations between the action of epinephrine on cardiac contractile force, the uptake of glucose, and the production of glucose-6-P in the myocardium of the anesthetized dog. It will be seen that these biochemical events are elicitable by a large dose of the amine, but that they do not correlate with the increase in contractile force.

* Publication no. 528, Division of Basic Health Sciences, Emory University. A preliminary report of this work appeared in *Fed. Proc.* **21**, 177 (1962). This investigation was supported by United States Public Health Service Grant H-4626.

† Public Health Service Research Career Development Award GM-K3-6257; with the technical assistance of Betty Clark, Pauline Hoch, and Nancy Waters.

METHODS

Preparation of animals. Adult male and female mongrel dogs were fasted for 36 hr prior to use except in one set of experiments where no such restriction was imposed. The animals were anesthetized by the i.v. injection of 15 mg of sodium pentobarbital/kg and 220 mg of sodium barbital/kg. Carotid or femoral arterial blood pressure was measured with a Statham 23A transducer and recorded on a Grass oscillograph. The chest was opened in early experiments through a midsternal incision and in later ones in the right fifth intercostal space. The latter method resulted in a smaller rise in plasma lactate and a physiologically more stable preparation, especially after pancreatectomy. Cardiac contractile force was measured from the left ventricle with a strain gauge arch.[10] Bilateral cervical vagotomies were performed on all animals. Respiration was maintained with a Harvard respirator adjusted to maintain arterial blood pH between 7·35 and 7·40 in each dog. The pH was measured in a Radiometer blood pH assembly at 37°. Adequate oxygenation of the open-chest preparation, but without hyperventilation, was a requirement for stable plasma glucose levels.

Pancreatectomies were performed on four animals prior to opening the chest;* 1 to 2 hr elapsed between the completion of the surgery and the beginning of the following experimental procedures. At this time blood pressure was stable, but control plasma glucose values were more variable.

Control blood samples (1 ml each) for glucose, lactate, and chloride determinations were taken from a cannulated artery at 15-min intervals. The glucose concentration did not vary more than 10% and chloride more than 3% during the 45-min control period. At the end of this time a 15-min i.v. infusion of 0·9% NaCl, 1 μg/kg min^{-1} or 10 μg/kg min^{-1} of *l*-epinephrine bitartrate (calculated as the base) in a volume of 0·494 ml/min, was begun. The solution of the amine was prepared by dilution of a stable stock solution with 0·9% NaCl just prior to use. At the same time the infusion was started, 75 to 100 μc of ^{131}I-serum albumin (RISA, Abbott; or Albumotope, Squibb) was rapidly injected i.v. During the infusion 1-ml samples of arterial blood were taken at 5-min intervals. After 15 min a sample of right ventricle was cut from the exposed heart, immediately frozen in dichlorodifluoromethane (Freon-12) at −150°,[11] and stored in a liquid nitrogen refrigerator (−180°).

Chemical methods. Heparinized blood samples were placed in ice immediately after being withdrawn, then centrifuged at 2,500 × *g* for 10 min at 0°, and the plasmas removed and frozen. Glucose was measured by the glucose oxidase procedure of Fales *et al.*[12] and later by the more convenient modification of Washko and Rice[13] after precipitation of protein with $Ba(OH)_2$ — $ZnSO_4$. Volumes were reduced 10-fold from those described by these authors. Chloride was determined directly on plasma with the Aminco-Cotlove titrator.[14]

Blood content of the heart samples was determined from the ratio of ^{131}I-albumin radioactivity in an aliquot of heart to that of plasma.[15] Determinations were made directly on plasma and on the perchloric acid (PCA) precipitate of heart homogenate (see below) in a well scintillation counter. The assumption that the label remained on the albumin and did not escape from the circulation during the 15 min between injection and termination of the experiment was verified by the fact that 97 to 100% of the radioactivity in plasma and heart was precipitated by PCA.

* The author is indebted to Dr. Pierre Galletti, Department of Physiology, Emory University, for performing these operations.

Heart chloride was determined with the Cotlove method. Twenty mg of tissue was ground in a Kontes Duall homogenizer with 0·25 ml of 1 N HNO_3. The homogenate was kept at 0° for 15 min, then centrifuged, and chloride was determined on the supernate. Drying and defatting of the sample was not necessary to achieve consistent results.[16]

Glucose, glucose-6-P, and lactate were determined on a 50- to 100-mg sample of myocardium. This was ground with a Kontes Duall homogenizer with 10 volumes of 0·33 N PCA. Extraction with trichloroacetic acid, ethanol, or PCA after powdering the sample in a mortar at −180° led to the same results. After 15 min at 0° the homogenate was centrifuged for 10 min at 2,500 × *g*, the supernate then removed, and the precipitate re-extracted with PCA. The two supernates were analyzed separately. The second contained 5 to 15% as much of the metabolites as did the first extract. The precipitate was used for the radioiodine determinations described above. The supernates were brought to pH 6 to 8 (pH paper) with 5 M K_2CO_3 as soon as possible, the $KClO_4$ then removed by centrifugation at 0°, and the supernates stored at −20°.

Glucose and glucose-6-P were determined in triplicate 50-μl aliquots of the above supernates in 6 × 50 mm borosilicate tubes containing 10 μl of trihydroxymethylaminomethane (recrystallized), 0·25 M, pH 8·0 to 8·8; 2 μl of 10 mM nicotinamide adenine dinucleotide phosphate (NADP) (Sigma)*; and 1 μl of glucose-6-P dehydrogenase (Boehringer) diluted to give a final concentration of 1 μg/ml. After incubation for 10 min at room temperature the solutions were transferred to 1·5 × 25 mm microcuvets with 1·0 cm light paths, and glucose-6-P was determined by the absorption of $NADH_2$ at 340 mμ in a Beckman DU spectrophotometer. Fifty-μl or smaller aliquots were then removed from the cuvets and were added to 6 × 50 mm tubes containing: 4 μl of a solution containing 75 mM adenosine triphosphate (Pabst) and 40 mM $MgCl_2$, pH 7; and 1 to 5 μl of yeast hexokinase, sufficient to complete the reaction in 5 to 10 min. The change in absorbance at 340 mμ was used to calculate the glucose concentration. The recovery of added glucose-6-P (Sigma) was 95 to 100% and of glucose, 95 to 102%. No corrections for incomplete recovery were made.

The validity of the glucose-6-P dehydrogenase and hexokinase method was based on an examination for interfering enzymes.[18] The Boehringer preparation of the dehydrogenase was found to be free of contamination, including hexokinase and glutathione reductase,[19] at the dilution in which it was used. The hexokinase preparation was that of Darrow and Colowick carried through the first crystallization.[20] It still contained 2% phosphoglucoisomerase, but this resulted in a negligible error in the glucose determination. Other potentially interfering enzymes were absent.

Glycogen was determined on 10-mg samples of heart by the anthrone method[21] with a four fold reduction in volumes throughout the procedure.

Glycogen phosphorylase in myocardium was determined by modification of the procedure used previously,[11] based on the method of Cori and Illingworth.[22] The changes in the procedure will be presented in detail elsewhere. Briefly, the method consisted of rapidly homogenizing a 10- to 15-mg heart sample in 100 volumes of a 20 mM NaF — 2 mM EDTA solution, in such a manner that the sample was well dispersed before it thawed, the tissue having been cooled to −180° before being dropped into the Kontes Duall homogenizer. After removal of insoluble tissue

* Nucleotide solutions were stored at −180° for maximum stability.[17]

components by centrifugation, an aliquot of the supernate was added to an equal volume of a solution containing 2% glycogen and 33 mM glucose-1-phosphate, while another aliquot was added to a solution containing glycogen, glucose-1-P, and 2 mM adenylic acid. The pH was 6·1 to 6·2. Incubation was carried out for 10 min at 30°. The reaction was stopped by the addition of 10 volumes of the phosphate reagent of Buell *et al.*[20] which had been adjusted to a pH of 2·3 to 2·5 by the addition of perchloric acid. Phosphorylase activity was measured in terms of the increase in inorganic phosphate which occured as the result of the incorporation of glucose from glucose-1-phosphate into glycogen.

RESULTS

Pharmacologic responses to epinephrine

The effects of epinephrine on the contractile force of the left ventricle and the systolic blood pressure of the dogs are summarized in Table 1. Maximum augmentation of

TABLE 1. CARDIAC CONTRACTILE FORCE AND BLOOD PRESSURE RESPONSES TO EPINEPHRINE IN NORMAL AND PANCREATECTOMIZED DOGS

The data were obtained by measuring force of contraction of the left ventricle with a strain gauge arch and arterial pressure with a pressure transducer, and determining the changes which occurred in each animal between the control period and 15 min after the infusions were started. The number of experiments is given in parentheses. Italicized values differ from their controls ($P \leq 0{\cdot}01$; t test).

Experimental procedure	Change in contractile force (g)	Change in systolic pressure (mm Hg)
Fasted controls (5)	*	†
Fasted, E‡ 1 μg/kg min^{-1} (5)	*96·6* ± 12·8	*+88·2* ± 13·8
Fasted, E 10 μg/kg min^{-1} (5)	*114* ± 10·2	*+162* ± 9·0
Fed, E 10 μg/kg min^{-1} (5)	*93·2* ± 8·2	*+152* ± 6·5
Pancreatect. controls (2)		
Pancreatect. E, 10 μg/kg min^{-1}	93; 111	+149; 172

* Control contractile force = 131 ± 17 g.
† Control systolic pressure = 105 ± 4·4 mm Hg.
‡ Epinephrine.

contractile force was obtained with the 1-μg infusion of the amine, whereas 10 μg produced only a further increase in blood pressure. The latter dose had toxic actions in that cardiac arrhythmias were often noted during the infusions and, in separate experiments in which the animals were not sacrificed at the end of the infusion period, blood pressure and contractile force fell below preinfusion levels and remained depressed for at least half an hour.

Measurement of chloride and glucose spaces of the heart

The calculations of chloride and glucose concentrations in dog myocardium are corrected for the amounts of these substances present in the plasma remaining in the heart samples. The importance of such a correction in heart, as opposed to skeletal muscle, has been pointed out.[16] In control experiments, 3·5 to 5% of the wet weight of the dog myocardium was plasma on the basis of the ^{131}I-albumin determinations. After administration of epinephrine this varied between 5 and 9 per cent.

As a standard for comparison to the glucose space it is useful to determine the extracellular space (ECF). Danforth and his colleagues found a raffinose space equal to 17·5% of the wet weight of dog heart *in situ*.[24] The chloride space of rat heart *in situ* has been estimated as 25 to 27%,[25] and in the present investigation it was 22 to 24% of the wet weight of dog heart samples. The chloride space is thus larger than the ECF but was a constant value in the present experiments with one exception. The administration of 10 μg of epinephrine/kg min^{-1} to fed dogs resulted in a small but significant rise of the chloride space (Table 2; note that the data are expressed in terms of heart water). Such an action of a large dose of the amine has been noted by others,[26] but could also be ascribed to feeding and hydration rather than to epinephrine.

TABLE 2. GLUCOSE AND CHLORIDE DISTRIBUTION BETWEEN PLASMA AND MYOCARDIUM OF NORMAL AND PANCREATECTOMIZED DOGS GIVEN EPINEPHRINE

The data were obtained from blood and right ventricle samples removed 15 min after the infusions were started in the same animals described in Table 1. Italicised values differ from their controls with $P \leqq 0{\cdot}01$. The values for plasma and heart glucose in the fed animals receiving 10 μg epinephrine differ from the corresponding fasted group, with $P < 0{\cdot}01$

Experimental procedure	Plasma*		Heart†		$C_H/C_P \times 100$‡	
	Glucose (mmole/l water)	Chloride (mEq/l water)	Glucose (mmole/l water)	Chloride (mEq/l water)	Glucose	Chloride
Fasted controls	8·00±0·54§	122±1·0	1·12±0·10	34·6±0·9	14·1±0·7	28·4±0·8
Fasted, epinephrine 1 μg/kg min^{-1}	*14·3* ±1·5	119±0·7	*2·22*±0·25	34·7±1·4	15·5±0·4	29·2±1·3
Fasted, epinephrine 10 μg/kg min^{-1}	*13·8* ±0·7	119±1·1	*9·25*±1·05	35·0±1·3	*67·0*±9·2	29·4±1·3
Fed, epinephrine 10 μg/kg min^{-1}	*22·6* ±1·9	117±3·2	*13·1* ±2·5	37·8±1·2	*58·0*±7·2	*32·3*±0·9
Pancreatectomized fasted controls	6·28; 11·1	117; 116	0·559; 1·59	30·0; 31·8	8·9; 14·3	25·6; 27·4
Pancreat., epineph. 10 μg/kg min^{-1}	16·8; 21·9	112; 114	8·4; 12·0	32·8; 33·2	50; 55	29·3; 29·1

* Water content assumed as 94%.
† Water content assumed as 76%.
‡ Ratio of concentration in heart to that in plasma ×100.
§ Standard error.

Effect of epinephrine on glucose space, glucose-6-phosphate content, and phosphorylase activity

The distribution of glucose in control experiments was approximately that of a conservative estimation of the ECF (Table 2). A value of 14% was observed with a plasma level of 137 mg/100 ml. The infusion of 1 μg of epinephrine/kg min^{-1} did not increase the heart glucose space, despite an 80% increase in plasma glucose; *i.e.* this dose of the amine did not appear to inhibit glucose utilization. A 10-fold increase in the dose of the amine did not produce any further rise in plasma glucose of fasted animals, but marked intracellular accumulation of the hexose was observed (glucose space of 67%). Ten μg of epinephrine/kg min^{-1} administered to fed dogs had a similar effect, accompanied by a rise of glucose to 415 mg/100 ml plasma water.

The action of epinephrine on phosphorylase activity and glucose-6-P is established from the data in Table 3. One μg of the amine produced a small conversion of phosphorylase *b* to the *a* (active) form. Glucose-6-P seemed to decrease, but this was not statistically significant. Ten μg elicited almost total activation of the enzyme, and this

was accompanied by an 85% increase in the concentration of the hexose ester. Compared with controls, essentially the same results were obtained in fed as in fasted dogs.

The administration of 10 μg of epinephrine/kg min^{-1} not only augmented the conversion of phosphorylase *b* to *a*, but also produced an increase in the sum of the two forms. The most likely explanation is that the adenylic acid which was used to activate phosphorylase *b* in the assay of total enzyme activity also promoted the activity of the *a* form.[28] Thus, as the proprtion of *a* to *b* was altered from 0·06:1 to 11·5:1, the apparent sum of the two also rose.

TABLE 3. MYOCARDIAL GLYCOGEN PHOSPHORYLASE AND GLUCOSE-6-PHOSPHATE CONTENT OF NORMAL AND PANCREATECTOMIZED DOGS GIVEN EPINEPHRINE

The data were obtained under the same conditions and from the same animals described in Table 2. Italicised values differ from their controls, with $P \leq 0{\cdot}01$.

Experimental procedure	Phosphorylase activity (units/g*) Active	Total	Active (%)	Glucose-6-P (mmole/kg†)
Fasted controls	51·3 ± 9·7	771 ± 35	6·5 ± 1·1	0·396 ± 0·024
Fasted, E‡ 1 μg/kg min^{-1}	*160* ± 41	946 ± 133	*18·6* ± 3·2	0·305 ± 0·080
Fasted, E 10 μg/kg min^{-1}	*1,176* ± 89	*1,279* ± 96	*94·7* ± 5·4	*0·733* ± 0·054
Fed, E 10 μg/kg min^{-1}	*1,070* ± 51	*1,152* ± 48	*92·7* ± 4·8	*0·683* ± 0·059
Pancreatect. controls	283; 88·0	1,081; 683	26·2; 12·9	0·365; 0·324
Pancreatect., E 10 μg/kg min^{-1}	1,677; 1,336	1,820; 1,448	92·1; 92·3	1·41; 1·12

* Calculated according to Cori *et al.*[27] on a wet weight basis.
† Wet weight.
‡ Epinephrine.

Epinephrine effects in pancreatectomized dogs

Insulin has been shown to increase the glucose space of frog muscle,[29] of perfused heart from normal rats,[30] and skeletal muscle and heart[31] of diabetic rats. Furthermore, Newsholme and Randle reported a 100% increase in glucose-6-P content of perfused heart from normal, fasted rats when insulin was added to the perfusate.[32] The actions of epinephrine in intact dogs could be due to stimulation of insulin release. However, acute pancreatectomy did not appear to modify the responses to epinephrine (Tables 1–3). There is actually an indication that glucose-6-P increased even more than it did in heart of normal animals, but the number of experiments is not sufficient to prove this (Table 3). Kipnis *et al.*[5] also found the same response in the change in the intracellular distribution of glucose in diabetic as in normal animals.

Changes in glycogen concentrations in dog heart

Increased glycogenolysis in the heart of intact animals as the result of epinephrine injection has been both affirmed (Ellis[3]) and denied (Bloom and Russell[33]). In the present experiments infusion of either of the doses of epinephrine which were used for 15 min did not produce changes in cardiac glycogen from the control range of 7·1 to 9·8 g/kg (fasted series). This was true in spite of the almost complete activation of phosphorylase with the larger dose of the amine. A possible explanation of these results is that glycogenesis (presumed to be primarily via the uridine diphosphate glucose pathway) was maintained at an equal level with glycogenolysis.

DISCUSSION

At first glance these results appear to support the hypothesis that epinephrine inhibits glucose utilization in the heart when this is measured in terms of the accumulation of free glucose within the myocardial cell. Since this effect is accompanied by an increase in the content of glucose-6-P, the action of epinephrine could be due to inhibition of heart hexokinase.[5, 32] However, several problems arise in this interpretation. One, it is possible that the intracellular accumulation of glucose is not due to decreased phosphorylation, but results from increased penetration of glucose due to the action of insulin. The experiments on pancreatectomized dogs seem to eliminate this possibility. Two, the physiological significance of these alterations in carbohydrate metabolism is difficult to establish, since a moderate dose of the amine produced a considerable hyperglycemia without alteration of heart glucose space and glucose-6-P. A toxic dose of epinephrine (10 μg/kg min^{-1}) was required to produce these changes. It should be noted that the effects observed by Kipnis and collaborators in skeletal muscle were observed after a dose of 1 to 2 mg/kg, s.c., also a very large dose. Danforth *et al.*[24] observed a rise in myocardial glucose-6-P in dog hearts *in situ* consequent to the infusion, into the coronary circulation, of 15 to 50 μg of epinephrine/min. A significant discrepancy between the present data and those of Danforth and coworkers is in the control levels of glucose-6-P which the latter investigators found to be 50 μmole/kg wet weight of dog heart. These control levels were considered maximal by the authors, because the metabolite accumulated in samples which were not immediately inactivated by freezing in liquid nitrogen and subsequently extracted with perchloric acid. Since the present data were derived from samples frozen at least as quickly, and subsequently treated so as to prevent thawing until inactivation had occurred (see Methods), the discrepancy is not resolved.

Although glucose-6-P concentration and glucose space changes were parallel, the relation between the two is not necessarily one of cause and effect.[24, 29] The concentration of the phosphate ester is most likely a consequence of several processes: glycogenolysis (which appeared to have been increased by epinephrine on the evidence of phosphorylase activation), glucose phosphorylation, and the degree to which phosphofructokinase limits glycolysis.[34] Furthermore, the intracellular accumulation of glucose after epinephrine could be due to saturation of the phosphorylative mechanism under conditions in which cellular permeability is not a limiting factor. The apparent K_m for glucose phosphorylation in the isolated, perfused rat heart has been calculated by Morgan *et al.*[30] to be $1{\cdot}6 \times 10^{-3}$ M. Whether or not this can be applied to the present investigation is doubtful. The plasma concentration of glucose was more than twice this value in the control experiments on dogs, but the heart glucose space was apparently that of the extracellular space, and remained so even in animals in which epinephrine raised the plasma glucose 80%. Finally, the K_i for the inhibition of calf heart hexokinase by glucose-6-P has been reported to be about 10^{-4} M.[8] If this were true of the dog heart enzyme *in vivo* one would expect it to be almost completely inhibited even without epinephrine administration.

Other metabolic pathways in the synthesis and degradation of glucose-6-P could be significant in determining its concentration. Although the pentose phosphate pathway seems to have relatively little activity in heart,[35] this tissue contains some glucose-6-P dehydrogenase and transaldolase,[36] so that inhibition of the former and stimulation of the latter could increase the level of glucose-6-P. The inhibition of

glucose phosphate isomerase would also have this effect. Insufficient information is available to allow any further evaluation of these possibilities.

The hypothesis of Ellis,[4] that the increase in glucose-6-P induced by epinephrine is an important factor in the positive inotropic action of the drug, is not supported by the data in this investigation. The 1-μg infusion of the amine augmented contractile force 76% without an increase in myocardial glucose-6-P. The latter was altered after the 10-μg infusion, but this dose did not produce an additional increase in contractile force. However, it is possible that the role of the ester does not depend on its concentration but rather on the rate of its formation. Since the more moderate dose of epinephrine did elicit a significant rise in phosphorylase *a* activity, one would expect an increase in glucose-6-P turnover, but without an increase in its concentration if phosphofructokinase is not yet a limiting step in glycolysis.

Acknowledgements—The author wishes to thank Drs. Neil C. Moran, James A. Bain, and Ernest Bueding for their advice and encouragement and Miss Marjorie Perkins for her efforts in preparing many of the animal experiments.

Note added in proof—J. Belford and M. R. Feinleib (*Biochem. Pharmacol.* **11**, 987, 1962) have recently shown that catecholamines increased glucose-6-phosphate in hearts from both ***in vivo*** and *in vitro* experiments. The authors concluded that this effect was concomitant with the inotropic action of the amines. However, they did not determine if small doses, which would augment contractile force, would necessarily produce a significant rise in the concentration of the hexose ester.

REFERENCES

1. G. T. CORI, J. O. CLOSS and C. F. CORI, *J. biol. Chem.* **103**, 13 (1933).
2. G. T. CORI and C. F. CORI, *J. biol. Chem.* **107**, 5 (1934).
3. S. ELLIS, *Pharmacol. Rev.* **8**, 485 (1956).
4. S. ELLIS, *Pharmacol. Rev.* **11**, 469 (1959).
5. D. M. KIPNIS, E. HELMREICH and C. F. CORI, *J. biol. Chem.* **234**, 165 (1959).
6. H. WEIL-MALHERBE and A. D. BONE, *Biochem. J.* **49**, 339 (1951).
7. R. K. CRANE and A. SOLS, *J. biol. Chem.* **210**, 597 (1954).
8. A. SOLS and R. K. CRANE, *Fed. Proc.* **13**, 301 (1954).
9. S. ELLIS, *J. Pharmacol.* **113**, 17 (1955).
10. K. J. BONIFACE, O. J. BRODIE and R. P. WALTON, *Proc. Soc. exp. Biol., N.Y.* **84**, 263 (1953).
11. S. E. MAYER and N. C. MORAN, *J. Pharmacol.* **129**, 271 (1960).
12. F. W. FALES, J. A. RUSSELL and J. N. FAIN, *Clin. Chem.* **7**, 289 (1961).
13. M. E. WASHKO and E. W. RICE, *Clin. Chem.* **7**, 542 (1961).
14. E. COTLOVE, H. V. TRANTHAM and R. L. BOWMAN, *J. Lab. clin. Med.* **51**, 461 (1958).
15. J. J. FRIEDMAN, *Proc. Soc. exp. Biol., N.Y.* **88**, 323 (1955).
16. O. H. LOWRY and A. B. HASTINGS, *J. biol. Chem.* **143**, 257 (1942).
17. O. H. LOWRY, J. V. PASSONNEAU and M. K. ROCK, *J. biol. Chem.* **236**, 2756 (1961).
18. H. J. HOHORTS, F. H. KREUTZ and T. BUCHER, *Biochem. Z.* **332**, 18 (1959).
19. H. T. NARAHARA and P. OZAND, *Proc. Soc. exp. Biol., N.Y.* **103**, 529 (1960).
20. R. A. DARROW and S. P. COLOWICK, *Methods in Enzymology*, S. P. COLOWICK and N. O. KAPLAN, Eds., vol. V, p. 226. Academic Press, New York (1962).
21. F. W. FALES, *J. biol. Chem.* **193**, 113 (1951).
22. G. T. CORI and B. ILLINGWORTH, *Biochim. biophys. Acta* **21**, 105 (1956).
23. M. V. BUELL, O. H. LOWRY, N. R. ROBERTS, M. W. CHANG and J. I. KAPPHAHN, *J. biol. Chem.* **232**, 979 (1958).
24. W. H. DANFORTH, J. J. MCKINSEY and J. T. STEWART, *J. Physiol., Lond.* **162**, 367 (1962).
25. O. H. LOWRY, A. B. HASTINGS, T. Z. HULL and A. N. BROWN, *J. biol. Chem.* **143**, 271 (1942).
26. W. V. B. ROBERTSON and P. PEYSER, *Amer. J. Physiol.* **166**, 277 (1951).
27. C. F. CORI, G. T. CORI and A. A. GREEN, *J. biol. Chem.* **151**, 39 (1943).
28. E. G. KREBS and E. H. FISCHER, *J. biol. Chem.* **216**, 113 (1955).
29. H. T. NARAHARA, P. OZAND and C. F. CORI, *J. biol. Chem.* **235**, 3370 (1960).

30. H. E. MORGAN, M. J. HENDERSON, D. M. REGEN and C. R. PARK, *J. biol. Chem.* **236**, 253 (1961).
31. H. E. MORGAN, E. CADENAS, D. M. REGEN and C. R. PARK, *J. biol. Chem.* **236**, 262 (1961).
32. E. A. NEWSHOLME and P. J. RANDLE, *Biochem. J.* **80**, 655 (1961).
33. W. L. BLOOM and J. A. RUSSELL, *Amer. J. Physiol.* **183**, 356 (1955).
34. T. E. MANSOUR, M. E. CLAGUE and K. D. BEERNINK, *Fed. Proc.* **21**, 238 (1962).
35. B. BLOOM, *Proc. Soc. exp. Biol., N.Y.* **88**, 317 (1955).
36. G. E. GLOCK and P. MCLEAN, *Biochem. J.* **56**, 171 (1954).