Brit. J. Pharmacol. (1958), 13, 125.

STUDIES OF THE ACTION OF HYPOGLYCIN-A, AN HYPOGLYCAEMIC SUBSTANCE

BY

P. C. FENG AND S. J. PATRICK

From the Divisions of Pharmacology and Biochemistry, Department of Physiology, University College of the West Indies, Jamaica

(RECEIVED DECEMBER 2, 1957)

Some biological effects of hypoglycin-A, a compound isolated from the fruit of *Blighia sapida*, have been investigated. Administration of this compound to animals caused drowsiness progressing to coma, and when large doses were given the animals died. For the rat, the oral and intraperitoneal LD50 values were 98 and 97 mg./kg. respectively. Fasting increased the toxicity considerably. The most outstanding biochemical change produced by hypoglycin-A was a delayed hypoglycaemia, the depth of which was related to the dose. The hypoglycaemia was preceded by exhaustion of liver glycogen. There were also smaller decreases in the glycogen stores of the heart, skeletal muscle and kidney, without any increase in blood pyruvate or lactate. Hypoglycin-A lessened the effect of adrenaline on blood glucose and decreased both glucose tolerance and insulin sensitivity. Hypoglycin-A also decreased the oxygen consumption and carbon dioxide production of the intact rat. All these effects are consistent with the hypothesis that the primary action of hypoglycin-A is the interference with glycogen production by the liver.

Hypoglycin-A is a compound which may be isolated from the fruit of *Blighia sapida*, a tree growing commonly in the West Indies, where it was introduced from West Africa in 1776 (Broughton, 1794). In Jamaica it is known as the "ackee." The fleshy part (aurillus) of the fruit of the ackee is eaten in Jamaica and West Africa, and at times may constitute an important item in the diet.

There is, nevertheless, a native tradition that the ackee may be poisonous. Bowrey (1892) attributed the cause of 5 fatal cases of poisoning to eating ackee. Since then, it has been suggested that the ingestion of this fruit may be a cause of an acute and often fatal disease known in Jamaica as the "vomiting sickness" (Scott, 1916; Hill, 1952). Jordan and Burrows (1937) and Evans and Arnold (1938) obtained extracts of ackee which caused vomiting and death in experimental animals. Although the relationship between vomiting sickness and the ingestion of ackee fruit was not accepted by all (Williams, 1952; Barnett, 1954), there was no doubt as to the presence of toxic substances in the ackee. Two such substances, hypoglycin-A and B, were isolated in 1954 (Hassall, Reyle, and Feng) and were shown to cause severe hypoglycaemia, depletion of liver glycogen and death. These findings agree well with those found in vomiting sickness (Hill, Bras, and Clearkin, 1955; Patrick, 1954).

Preliminary work suggested that hypoglycin-A and B were polypeptides (Hassall *et al.*, 1954; Hassall and Reyle, 1955). It now appears, however, that hypoglycin-A is probably an amino acid of novel structure (Hassall, personal communication) with the empirical formula of $C_7H_{11}NO_2$ (Holt, Leppla, Kroner and Holt, 1956). Earlier suggestions that the active principle of the ackee was a glycoside (Jordan and Burrows, 1937) or a saponin (Evans and Arnold, 1938) must be abandoned.

The present study was undertaken to provide a more complete account of the pharmacological and biochemical effects of hypoglycin-A on animals. Hypoglycin-A was studied because it is much more active biologically than hypoglycin-B and occurs in both the seeds and the edible aurillus of the ackee. Hypoglycin-B is less active and occurs in the seeds only.

MATERIAL AND METHODS

The hypoglycin-A used in the present study was isolated from ackee seed according to the method previously described by Hassall and Reyle (1955). The method involves the preparation of an aqueous extract of the seeds, precipitation of starchy material

by the addition of alcohol, and fractionation of the material in the supernatant fluid by means of ion exchange resins. The fractionation process was guided by toxicity tests and by the hypoglycaemic action of the fractions. The purity of the final product was checked by paper chromatography. In all cases there were ninhydrin-positive contaminants estimated to constitute not more than 2% of the total sample.

White rats were used for all *in vivo* experiments, except where otherwise stated. Hypoglycin-A was administered to the animals in the form of freshly-prepared aqueous solutions containing 10 to 30 mg. of hypoglycin-A/ml. Studies of the blood chemistry of rats were conducted with blood obtained from the tail. Blood glucose was determined by the colorimetric method of Nelson (1944). Blood lactate was determined by a micro-modification of the method of Barker and Summerson (1941) and pyruvate was determined by a micro-modification of the method of Friedemann and Haugen (1943). Glycogen was determined by the technique of Good, Kramer and Somogyi (1933). The assay for glucose-6-phosphatase was carried out by the procedure of Langdon and Weakley (1955) and the assay for phosphorylase by the method of Sutherland (1955). Plasma-urea, acid-soluble phosphate, chloride and cholesterol levels were determined by the procedures outlined by King (1946) with minor modifications. Estimation of the glucose uptake by the isolated rat diaphragm was carried out using the method described by Randle (1956) with minor modifications.

Results

Toxic Effects.—Observations on the toxic effects of lethal doses of hypoglycin-A (100 to 500 mg./kg., intraperitoneally) were made in kittens, guinea-pigs and white rats. Drowsiness, lachrymation and secretion from the nose and mouth occurred within 30 min. to 1 hr. after administration. Vomiting, starting 30 min. to 1 hr. after administration and lasting 2 to 4 hr., was observed frequently in kittens. The drowsiness progressed with time and the animals eventually became comatose. At this time, respiration was slow and cyanosis was usually evident at the terminal stage. Convulsions were not seen. Time of death varied with the dose administered.

Autopsies performed immediately after death revealed few gross pathological changes. The visceral veins and the right side of the heart were distended and filled with dark-coloured blood. The left ventricle was usually contracted. There was slight congestion in the liver, lungs, spleen, and kidneys, but otherwise these organs appeared normal. Histological sections of the liver, spleen, lungs, heart, kidneys, and suprarenal glands stained with haematoxylin and eosin revealed no significant pathological changes. Liver sections stained with Best's carmine showed a marked reduction in glycogen granules. Sections of the pancreas stained with Gomori's chrome-alum haematoxylin and phloxine, and Gomori's aldehyde fuchsin and phloxine, revealed a reduction of the granules of the alpha cells of the islets. This result has been described elsewhere (Feng, 1957).

Effects on Respiration and Blood Pressure.—Respiration and carotid arterial pressure were recorded from 5 kittens (400 to 800 g.) anaesthetized with pentobarbitone. Repeated intravenous injections (20 to 150 mg./kg.) of hypoglycin-A caused no immediate changes in the rate and depth of respiration or on the blood pressure. Just before death, which occurred after 4 to 5 hr., there was a slowing of the rate of respiration and a sudden drop in blood pressure.

Determination of Acute Toxicity.—The toxicity of hypoglycin-A on fed rats of either sex was determined by administering graded doses of the compound orally or intraperitoneally to groups of six rats (body weight 100 to 200 g.) and noting the mortality 24 hr. later. The results are shown in Table I. The LD50 values of hypoglycin-A administered orally and intraperitoneally were calculated by the probit transformation described by Burn, Finney, and Goodwin (1950), and were found to be almost identical, being 98 and 97 mg./kg. respectively with fiducial limits of 85 to 111 mg./kg. body weight.

TABLE I

ACUTE TOXICITY OF HYPOGLYCIN-A TO RATS

Mortality was observed for 48 hr.

Dose (mg./kg.)	Mortality	
	Oral	Intraperitoneal
50	0/6	0/6
60	0/6	1/6
70	1/6	1/6
80	2/6	1/6
90	3/6	2/6
100	3/6	2/6
110	3/6	4/6
120	5/6	5/6
130	4/6	5/6
140	5/6	6/6
150	6/6	6/6

A second series of experiments was made to compare the mortalities of groups of rats which had either been fed or fasted for 48 hr. before giving hypoglycin-A intraperitoneally. The results (Table II) did not permit an accurate estimation of the LD50, but it is evident that fasting rendered the rats much more susceptible to the toxic action of hypoglycin-A.

TABLE II

INTRAPERITONEAL TOXICITY OF HYPOGLYCIN-A TO FED AND FASTED RATS

Food was withheld from the fasted rats for 48 hr. before injection. Mortality was observed for 48 hr.

Dose (mg./kg.)	Mortality	
	Fed Rats	Fasted Rats
30	—	0/6
50	0/6	6/6
70	2/6	6/6
90	3/6	—
110	2/6	—
130	6/6	—

Effects on Blood Glucose, Lactate, and Pyruvate.—Blood glucose estimations following the administration of hypoglycin-A to rats, rabbits, and guinea-pigs showed that a lethal dose was followed by profound hypoglycaemia with values for blood glucose below 30 mg./100 ml. just before the death of the animal. Fig. 1 shows the pattern of blood sugar response after the administration of graded oral doses of hypoglycin-A to rats. There was occasionally an initial slight hyperglycaemia of 10 to 30 mg./100 ml. above normal, followed by a marked hypoglycaemia to values as low as 10 mg./100 ml. The time of onset and degree of hypoglycaemia were related to the dose. It was also noted that the progress of the drowsiness and coma and the time of death were related to the degree of hypoglycaemia. The pattern of the changes in blood sugar was the same whether the hypoglycin-A was administered orally or intraperitoneally.

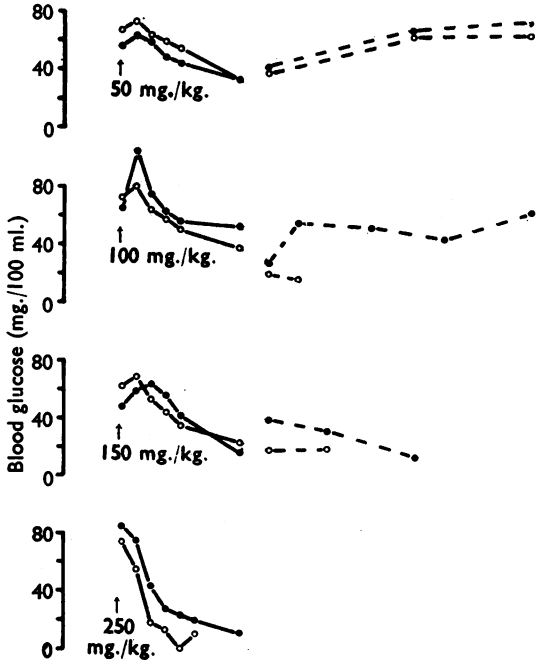

FIG. 1.—The effect of oral doses of hypoglycin-A on the blood glucose of rats. Each curve is from a single rat.

Blood lactate and pyruvate measurements were carried out on rats to which lethal doses (250 mg./kg.) had been administered by intramuscular injection. No outstanding changes were noted in the concentrations of these metabolites within the first 6 hr. after the administration of the hypoglycin-A although the blood sugar had fallen to 30 mg./100 ml. in this time.

Effects on Glycogen Stores, Liver Glucose-6-phosphatase and Phosphorylase.—A study was made of the glycogen concentrations of liver, heart, skeletal muscle and kidney in fed and fasted control rats and in fed and fasted rats to which a lethal dose (250 mg./kg.) of hypoglycin-A had been administered. Table III shows average values for the 4 groups of rats. The dose was administered intramuscularly 6 hr. before the glycogen determination. It is apparent that hypoglycin-A caused a decrease in the glycogen stores of some tissues whether the rats had been fed or fasted. The most striking effects were on the liver and the heart, and there was a small decrease in skeletal muscle glycogen. It is noteworthy that fasting in itself caused an increase in heart glycogen, whereas hypoglycin-A administration had the opposite effect.

TABLE III

THE EFFECT OF HYPOGLYCIN-A ON THE GLYCOGEN CONTENT OF RAT TISSUES

Food was withdrawn from the fasted animals 48 hr. before the intramuscular injection of hypoglycin-A (250 mg./kg.). Glycogen was determined in the tissues 6 hr. after the dose. Each numeral is the average for 4 rats.

Group of Rats	Glycogen (g./100 g. Tissue, Wet Weight)			
	Liver	Skeletal Muscle	Heart	Kidney
Fed; control	3·88	0·49	0·35	0·07
Fed; hypoglycin-A ..	0·07	0·32	0·08	0·06
Fasted, control	0·24	0·32	0·60	0·12
Fasted; hypoglycin-A ..	0·07	0·23	0·07	0·07

An increase in the activities of glucose-6-phosphatase or phosphorylase could conceivably explain the reduction in liver glycogen which occurs as a result of administration of the drug. For this reason, liver homogenates were prepared from rats which had been given lethal doses, and the activities of these enzymes in the homogenates were assayed. No evidence was obtained for

significant changes in the activities of these enzymes during the first 6 hr. after administration of hypoglycin-A, whereas the liver glycogen content decreased progressively during that period.

Effects on Glucose Tolerance.—Previous results (Patrick, 1954) had indicated that there was probably a decrease in glucose tolerance. This finding has been confirmed and extended in the present study. Glucose (3 g./kg.) in aqueous solution was administered intraperitoneally to 10 rats, and the changes in blood glucose were determined. On the following day, the same rats were given first a sublethal dose of hypoglycin-A (70 mg./kg.), then 1 hr. later the glucose administration and blood sugar determinations were repeated. A typical result is shown in Fig. 2. The decreased glucose tolerance illustrated resembled that found in diabetes, which would indicate a defective uptake of glucose from the blood by tissues.

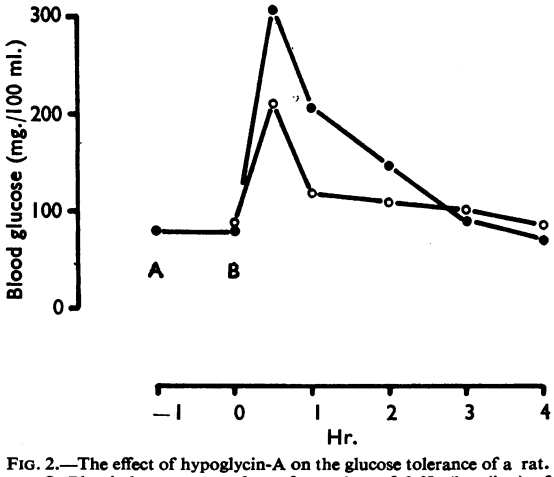

FIG. 2.—The effect of hypoglycin-A on the glucose tolerance of a rat. O, Blood glucose curve of rat after a dose of 3·0 g./kg. (i.p.) of glucose at B; ●, curve from the same rat when 70 mg./kg. (i.p.) of hypoglycin-A was given (at A) 1 hr. before the glucose (at B).

Effects on the Response of Blood Sugar to Adrenaline and Insulin.—Tests of the effects of adrenaline and insulin on blood glucose were carried out on groups of 6 rats. A predetermined dose of adrenaline (0.2 mg./kg.) or insulin (3 units/kg.) was administered to the rat and the changes in blood glucose were followed. On the following day the hormone administration and blood sugar determination were repeated using the same rats 1 hr. after they had been given a sublethal dose of hypoglycin-A. The drug was administered intraperitoneally in doses of 70 mg./kg. for the adrenaline test and 30 mg./kg. for the insulin test. There was a decrease in the effect of adrenaline on blood sugar after administration of hypoglycin-A (Fig. 3), and this may probably be ascribed to the decrease in liver glycogen. The drug also caused a slight but persistent decrease in insulin sensitivity (Fig. 4). It would thus appear that at this dose the effects of insulin and hypoglycin-A are antagonistic.

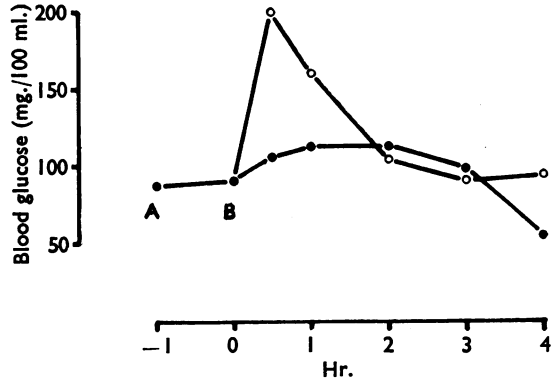

FIG. 3.—The influence of hypoglycin-A on the hyperglycaemic effect of adrenaline on a rat. O, Control blood glucosel response to adrenaline; ● response to adrenaline in the same rat after the administration of hypoglycin-A. At A, hypoglycin-A 70 mg./kg. (i.p.). At B, adrenaline 0·2 mg./kg. (i.p.).

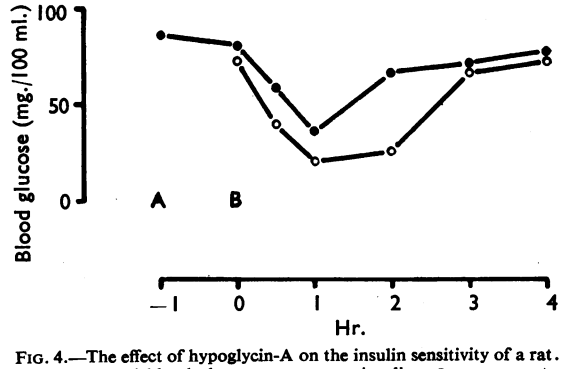

FIG. 4.—The effect of hypoglycin-A on the insulin sensitivity of a rat. O, Control blood glucose response to insulin; ●, response to insulin in the same rat after the administration of hypoglycin-A. At A, hypoglycin-A 30 mg./kg. (i.p.). At B, insulin 3 i.u./kg. (i.p.).

Effects on Oxygen Consumption and Carbon Dioxide Production of Rats.—Experiments were carried out on the oxygen consumption and carbon dioxide production of normal rats, rats which had been given hypoglycin-A and rats which had been given insulin. After administration of the drug, the rats were placed in a metabolism chamber of the type described by Robbie (1948). Pressure readings for the calculation of the oxygen consumption and carbon dioxide production were obtained hourly for 6 hr.

Figs. 5 and 6 illustrate the average results for groups of 4 rats subjected to each treatment. The dose of hypoglycin-A was 150 mg./kg., and the dose of insulin was 8 units/kg., both given intramuscularly. The actions of insulin and hypoglycin-A on metabolism were completely different. Insulin caused marked increases in both oxygen consumption (Fig. 5) and carbon dioxide (Fig. 6) production during the hypoglycaemic stage, when the respiratory quotient rose to 1.0; hypoglycin-A caused decreases in both oxygen consumption and carbon dioxide production during the stage of its hypoglycaemic action.

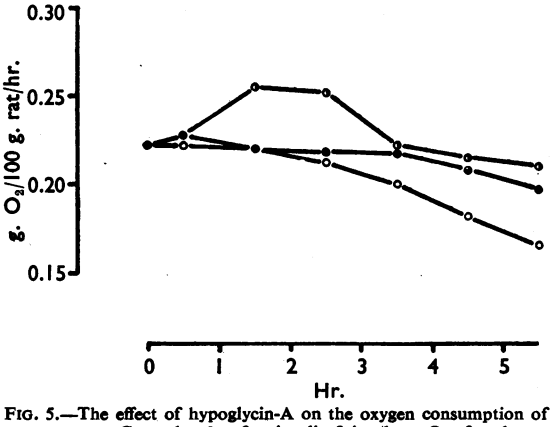

FIG. 5.—The effect of hypoglycin-A on the oxygen consumption of rats. ●, Control; ◑, after insulin 8 i.u./kg.; O, after hypoglycin-A 150 mg./kg. Average results of 4 animals.

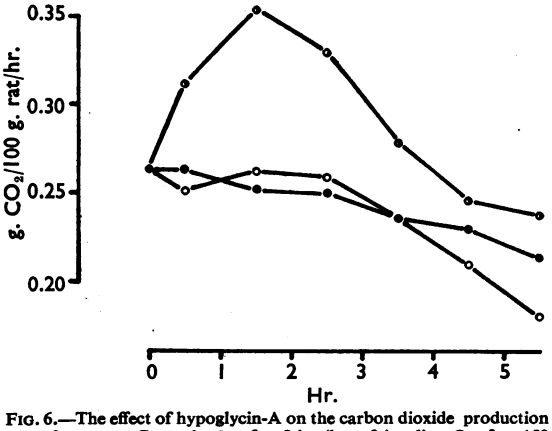

FIG. 6.—The effect of hypoglycin-A on the carbon dioxide production of rats. ●, Control; ◑, after 8 i.u./kg. of insulin; O, after 150 mg./kg. of hypoglycin-A. Average results of 4 animals.

Effect on Other Blood Constituents.—Repeated determinations of plasma urea, acid-soluble phosphate, chloride, and cholesterol levels were made on groups of 8 rats after the administration of a lethal dose of hypoglycin-A (150 to 300 mg./kg.). No significant changes were observed except some increase in plasma urea and acid-soluble phosphate just before the death of the animals.

Lack of in vitro *Effects.*—Attempts to demonstrate *in vitro* effects of hypoglycin-A on isolated tissues have not succeeded. Concentrations of 1:1,000 to 1:1,000,000 of the drug had no effect on the spontaneous contraction of intestine or uterus of the rat or guinea-pig. Also, hypoglycin-A in these concentrations did not affect the characteristic actions of adrenaline, acetylcholine, histamine or pituitary (posterior lobe) extract on smooth muscle.

The uptake of glucose by the isolated rat diaphragm appeared to be unaffected by the presence in the medium of 2 mg./ml. of hypoglycin-A.

The metabolism of isolated liver slices also did not appear to be affected by the presence of hypoglycin-A in the medium at a concentration of 1 to 2 mg./ml. The QO_2 and RQ of the liver slices, and their ability to synthesize glycogen, were unchanged.

DISCUSSION

The finding of hypoglycaemia and low liver glycogen concentrations in animals after hypoglycin-A administration and in patients suffering from "vomiting sickness" is evidence in support of the theory that the ingestion of the ackee is a cause of the disease in man. Two considerations, however, make it evident that this theory must be qualified before it can be accepted. Firstly, the fruit of the ackee is commonly eaten by Jamaicans, yet the incidence of vomiting sickness is very low. Secondly, it would appear likely from the toxicity data in this paper that a very large amount of ackee fruit would have to be ingested by normal people before toxic symptoms would appear. The demonstration that the toxicity of hypoglycin-A is much increased by fasting rats, or by feeding them a deficient diet (Feng and Kean, 1955), may be significant in view of the fact that the "vomiting sickness" occurs frequently in children with a history of malnutrition (Jelliffe and Stuart, 1954).

Several inferences regarding the metabolism of hypoglycin-A may be drawn from the present work. The fact that the effects of hypoglycin-A are similar, whether the compound is administered orally or intraperitoneally, suggests that no destruction of the drug occurs in the gastro-intestinal tract and absorption is efficient. The failure to demonstrate effects of hypoglycin-A on isolated tissues *in vitro* leads one to speculate

whether the substance must first be metabolized or activated in the body before it exerts its characteristic effects *in vivo*.

Present evidence indicates that the pharmacological action of hypoglycin-A is confined to its effects on carbohydrate metabolism and the death of the animals may be ascribed to hypoglycaemia. Unlike insulin, the hypoglycaemic action of hypoglycin-A is delayed, and its effects on muscle glycogen, oxygen consumption, carbon dioxide production and glucose tolerance are all the reverse of insulin effects. Furthermore, hypoglycin-A in small doses actually antagonizes the usual response of blood sugar to insulin.

There is no evidence to indicate that hypoglycin-A causes increased utilization of carbohydrate; there was no increase of blood pyruvate or lactate, muscle glycogen or respiratory carbon dioxide. It must be concluded that hypoglycin-A interferes with carbohydrate production by the liver. This agrees with the previous suggestion (Patrick, 1954) that hypoglycin-A hypoglycaemia is a result of the exhaustion of liver glycogen.

The authors wish to express their gratitude to members of the Department of Pathology of the University College of the West Indies for some histological examinations, and to the Department of Chemistry for the suppy of hypoglycin-A. This study was supported by the Government of Jamaica from a grant for the "Vomiting Sickness Study."

References

Barker, S. B., and Summerson, W. H. (1941). *J. biol. Chem.*, **138**, 535.

Barnett, W. L. (1954). *Rep. Govt. Chem., Jamaica.*

Bowrey, J. J. (1892). *Jamaica Gaz.*, **15**, 99.

Broughton, A. (1794). *Hortus Eastensis*, Spanish Town, Jamaica.

Burn, J. H., Finney, D. J., and Goodwin, L. G. (1950). In *Biological Standardization*, 2nd ed. Oxford: University Press.

Evans, K. L., and Arnold, L. E. (1938). *Trans. R. Soc. trop. Med. Hyg.*, **32**, 355.

Feng, P. C., and Kean, E. A. (1955). *Brit. J. Nutr.*, **9**, 368.

—— (1957). *Nature, Lond.*, **180**, 855.

Friedemann, T. E., and Haugen, G. E. (1943). *J. biol. Chem.*, **147**, 415.

Good, C. A., Kramer, H., and Somogyi, M. (1933). Ibid., **100**, 485.

Hassall, C. H., and Reyle, K. (1955). *Biochem. J.*, **60**, 334.

—— —— and Feng, P. C. (1954). *Nature, Lond.*, **173**, 356.

Hill, K. R. (1952). *W. Ind. med. J.*, **1**, 243.

—— Bras, G., and Clearkin, K. P. (1955). Ibid., **4**, 91.

Holt, C. v., Leppla, W., Kroner, B., and Holt, L. v. (1956). *Naturwissenschaften*, **43**, 279.

Jelliffe, D. B., and Stuart, K. L. (1954). *Brit. med. J.*, **1**, 75.

Jordan, E. O., and Burrows, W. (1937). *Amer. J. Hyg.*, **25**, 520.

King, E. J. (1946). In *Micro-analysis in Medical Biochemistry*. London: Churchill.

Langdon, R. G., and Weakley, D. R. (1955). *J. biol. Chem.*, **214**, 167.

Nelson, N. (1944). Ibid., **153**, 375.

Patrick, S. J. (1954). *J. appl. Physiol.*, **7**, 140.

Randle, P. J. (1956). *J. Endocrin.*, **14**, 82.

Robbie, W. A. (1948). *Methods in Medical Research*, **1**, ed. Potter, van R., p. 276. Chicago: Year Book Publishers.

Scott, H. H. (1916). *Ann. trop. Med. Parasit.*, **10**, 1.

Sutherland, E. W. (1955). In *Methods in Enzymology*, **1**, ed. Colowick, S. P., and Kaplan, N. O., p. 215. New York: Academic Press.

Williams, C. D. (1952). *W. Ind. med. J.*, **1**, 265.