3. The results are discussed with respect to the mechanism by which copper delays the induction of liver tumours by the azo-dye.

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REFERENCES

- Fare, G. & Woodhouse, D. L. (1963*a*). *Brit. J. Cancer*, **17**, 512.
 Fare, G. & Woodhouse, D. L. (1963*b*). *Brit. J. Cancer*, **17**, 775.
 Gelboin, H. V., Miller, J. A. & Miller, E. C. (1958). *Cancer Res.* **18**, 608.
 Howell, J. S. (1958). *Brit. J. Cancer*, **12**, 594.
 Howell, J. S. (1959). *J. Path. Bact.* **77**, 473.
 Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R. & Rhoads, C. P. (1941). *Science*, **93**, 308.
 Miller, E. C. & Miller, J. A. (1947). *Cancer Res.* **7**, 468.
 Miller, E. C., Miller, J. A., Sapp, R. W. & Weber, G. M. (1949). *Cancer Res.* **9**, 336.
 Miller, J. A. & Miller, E. C. (1953). *Advanc. Cancer Res.* **1**, 339.
 Nilsson, G. (1950). *Acta chem. scand.* **4**, 205.
 Riddett, N. J. (1955). In *Organic Reagents for Metals*, 5th ed., vol. 1, p. 31. Chadwell Heath, Essex: Hopkin and Williams Ltd.

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The Antiketogenic Action of Sorbitol

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Glycerol and sorbitol diminish the amount of acetoacetate that accumulates during the incubation of slices prepared from the liver of starved rats (Edson, 1936). In the presence of sorbitol the formation of β -hydroxybutyrate is also diminished (Blakley, 1951). Thus sorbitol is antiketogenic in excised liver but the mechanism of its action has remained obscure.

To explain the antiketogenic action of glycerol in liver slices Fritz (1961) has suggested that glycerol 1-phosphate formed from glycerol may promote the synthesis of triglycerides (Kennedy, 1957) and thereby decrease the amount of free fatty acids available for oxidation to ketone bodies. Recent observations support this view by showing that the antiketogenic effect of glycerol in perfused rat liver is accompanied by an increased esterification of [14 C]palmitate (Wieland & Matschinsky, 1962).

The first step in the metabolism of sorbitol is its oxidation to fructose (Embden & Griesbach, 1914; Blakley, 1951). Since there is evidence that fructose is a precursor of glycerol 1-phosphate (Hers, 1957; Muntz & Vanko, 1962; Wieland & Matschinsky, 1962), a single hypothesis could account for the antiketogenic effects of both glycerol and sorbitol. By using [G- 14 C]palmitate as a substrate, we have investigated the possibility that sorbitol enhances the esterification of fatty acid in rat-liver slices.

MATERIALS AND METHODS

Animals. Albino rats (about 250 g.) of a Wistar strain were obtained from the Animal Department, University of Otago Medical School. They were starved for 24 hr. before use in experiments.

Chemicals. Sorbitol (C.P.) was obtained from the Pfanstiehl Chemical Co., Waukegan, Ill., U.S.A., and [G- 14 C]palmitate from The Radiochemical Centre, Amersham, Bucks.

[G- 14 C]Palmitate-serum albumin complex was prepared by the method of Bragdon & Gordon (1958).

Liver slices. The slices were cut by hand, blotted free from adherent saline and weighed into Warburg cups containing phosphate-saline, pH 7.4 (Krebs, 1933). In isotopic experiments slices cut from two livers were pooled before transfer to the cups.

Experimental procedure. In isotopic experiments equal weights of slices were suspended in 18.0 ml. of phosphate-saline in each of two Warburg cups (200 ml. capacity), and 0.2 ml. of 1.0M-sorbitol or of 1.0M-fructose solution was added to one of the cups and 0.2 ml. of water to the other. The centre wells contained 1.0 ml. of carbonate-free 2N-NaOH, and 2.0 ml. of 2N-HCl was placed in the side arm of each flask. Both vessels received 2.0 ml. of [G- 14 C]palmitate-albumin complex. The vessels were filled with oxygen and equilibrated in the thermostat at 37° for 10 min. Oxygen uptake was measured for 2 hr. before the acid was tipped into the main compartment to stop the metabolism and liberate bound CO₂. The total CO₂ production was measured according to the method of Exton (1964). The cups were transferred to ice-water, and the slices removed

and washed with water. The lipids of the slices were extracted, fractionated into free fatty acids, neutral lipids and phospholipids, and the fatty acids of each fraction determined by the methods of Olivecrona (1962).

The medium was neutralized with a measured volume of 2N-NaOH. Portions (0.5–1.0 ml.) were reserved for the determination of acetoacetate (Walker, 1954) and β -hydroxybutyrate (Berry, 1963). Another portion (2.0 ml.) was evaporated to dryness *in vacuo* and saponified with methanolic KOH, and the fatty acids were determined (Olivecrona, 1962). A further sample of the medium (1.0 ml.) was deproteinized with $\text{Ba}(\text{OH})_2\text{-ZnSO}_4$ reagent (Somogyi, 1945) and used for the estimation of fructose (Kulka, 1956) and reducing sugars (Nelson, 1944). The rest of the medium (about 18 ml.) was used for the separation of the ^{14}C -labelled ketone bodies as mercury-acetone complexes according to the method of Exton (1964).

The radioactivities of the CO_2 , the fatty acids and the ketone bodies were determined by liquid-scintillation counting (Exton, 1964).

In non-isotopic experiments conventional Warburg vessels (20 ml. capacity) were employed for measurement of oxygen uptake and the ketone bodies were determined as described above.

Detection of glucose, fructose and sorbitol. Deproteinized (Somogyi, 1945) samples of the medium were subjected to paper electrophoresis (Whatman no. 1 paper; 12 cm. $\times$ 60 cm.) in 0.05M-sodium borate for 20 min. at 5 kv and about 50 ma in a high-voltage apparatus (Shandon Scientific Co., London). The spots due to metabolites and to residual sorbitol were developed with either periodate-benzidine reagent (Gordon, Thornburg & Werum, 1956) or *p*-anisidine reagent (Hough, Jones & Wadman, 1950), and identified by comparison with those produced by authentic glucose, fructose and sorbitol.

RESULTS

Antiketogenic effects of carbohydrates. Previous work demonstrated that glycerol, fructose, sorbitol, L-iditol, xylitol and ribitol increase oxygen consumption and diminish endogenous aceto-

acetate formation in the liver slices of starved rats, but measurements of total ketone bodies were omitted (Edson, 1936; Blakley, 1951; Haydon, 1961). The results of Table 1 confirm the previous observations and show that the production of β -hydroxybutyrate is either diminished or unaltered in the presence of these substrates. The acetoacetate: β -hydroxybutyrate ratio is always much greater than 1, and, although the values of the ratio are decreased slightly in the presence of the antiketogenic substrates, this is due to suppression of acetoacetate formation.

All the antiketogenic polyols except glycerol are substrates of the hepatic enzyme L-iditol dehydrogenase (L-iditol-NAD oxidoreductase, EC 1.1.1.14), and their antiketogenic potencies are roughly parallel to their rates of oxidation by this enzyme operating under conditions of pH and substrate concentration similar to those used in the experiments of Table 1 (McCorkindale & Edson, 1954; Smith, 1962). L-Arabitol, which is not oxidized significantly under these conditions, is not antiketogenic. Glucose is less antiketogenic than fructose or sorbitol.

Effect of sorbitol on ketogenesis from $[\text{G-}^{14}\text{C}]$ -palmitate. Table 2 gives the results of an experiment in which liver slices prepared from starved rats were incubated with $[\text{G-}^{14}\text{C}]$ palmitate-albumin complex in the presence and absence of sorbitol. Three other experiments gave essentially the same results.

The presence of sorbitol diminishes the total ketone bodies formed and increases both oxygen consumption and carbon dioxide production but does not alter the value of the respiratory quotient. In the four experiments the metabolic effects lay within the following ranges: the increase in oxygen uptake was 12–23 %, the increase in carbon dioxide

Table 1. *Antiketogenic effects of polyols, glucose and fructose in liver slices of starved rats*

The rats were starved for 24 hr. Warburg vessels contained liver slices (127–173 mg. wet wt.) suspended in 2.0 ml. of phosphate-saline, pH 7.4. The gas phase was O_2 , the temperature 37°, and the incubation time 2 hr. The results are given as $\mu\text{moles}/100 \text{ mg. wet wt. of slices}$.

Expt. no.	Substrate added	Concn. (mm)	O_2 uptake	Acetoacetate	β -Hydroxybutyrate	Total ketone bodies
1	None	—	10.6	1.79	0.55	2.34
	Sorbitol	20	12.4	0.96	0.33	1.29
	Xylitol	20	12.6	0.72	0.28	1.00
	Sorbitol	8	11.7	1.08	0.45	1.53
	Xylitol	8	11.1	1.17	0.47	1.64
	Fructose	20	14.6	1.25	0.58	1.83
2	None	—	11.1	1.87	0.25	2.12
	Sorbitol	20	12.2	0.82	0.18	1.00
	Xylitol	20	13.6	1.03	0.20	1.23
	Ribitol	20	12.2	1.59	0.27	1.86
	L-Arabitol	20	11.6	1.99	0.31	2.30
	Glycerol	20	11.5	1.10	0.20	1.30
	Fructose	20	12.2	0.87	0.13	1.00
	Glucose	20	11.8	1.35	0.23	1.58

formation 7–27% and the suppression of ketone-body formation 33–41%. In each experiment ketone-body formation from [^{14}C]palmitate was diminished by approximately the same amount as the total ketone-body production (38% in Table 2), and in consequence the addition of sorbitol had no significant effect on the specific radioactivity of acetoacetate or β -hydroxybutyrate.

Since the addition of sorbitol increased the incorporation of ^{14}C into carbon dioxide by only 15100 counts/min., not more than one-seventh of the diminution in ketone-body production (112000 counts/min.) from [^{14}C]palmitate can be attributed to enhanced oxidation of [^{14}C]acetyl-CoA in the citric acid cycle. Likewise the increase in total carbon dioxide production is too small to account for the diminution in ketogenesis from endogenous fatty acids.

Table 2 also shows the incorporation of ^{14}C into fatty acid fractions of the slices and the amount of ^{14}C remaining as fatty acid in the medium. The large amount of radioactivity in the fatty acids of the residual medium is mainly due to unused [^{14}C]palmitate but it is possible that a small part of

this radioactivity may be due to labelled lipids that have escaped from the slices (Hamosh & Shapiro, 1961).

In the presence of sorbitol there is a substantial increase (137%) in the amount of ^{14}C incorporated into the esterified fatty acids of the slices. No special significance is attached to the change in the ^{14}C content of the non-esterified fatty acid fraction of the slices because this determination may be subject to experimental error. It is possible that the procedure for washing the slices does not remove traces of [^{14}C]palmitate (specific radioactivity, 278×10^3 counts/min./ μmole) that have been adsorbed on to the surface of the slices.

The recovery of ^{14}C was 83% in the absence of sorbitol and 79% in its presence. The medium was not analysed for ^{14}C -containing substances other than ketone bodies and fatty acids.

Table 3 illustrates the distribution of ^{14}C in the carbon dioxide, the ketone bodies and the esterified fatty acids. In the control 22.4 μmoles of carbon derived from the added [$\text{G-}^{14}\text{C}$]palmitate were found in the metabolites under consideration, and when sorbitol was added the incorporation was

Table 2. *Effects of sorbitol on fatty acid metabolism in liver slices of starved rats*

The rats were starved for 24 hr. The slices (2.64 g. wet wt.) were suspended in 20 ml. of phosphate-saline, pH 7.4, in each Warburg flask. The gas phase was O_2 , the temperature 37° , and the incubation time 2 hr. [$\text{G-}^{14}\text{C}$]Palmitate (6 μmoles ; specific radioactivity, 17.4×10^3 counts/min./ $\mu\text{g. atom of C}$) was added as a serum albumin complex to each flask. The ^{14}C added was 167×10^4 counts/min. Esterified fatty acids are those of the neutral-lipid and phospholipid fractions (Olivecrona, 1962). The respiratory quotient in the absence of sorbitol was 0.65, and in the presence of sorbitol was 0.64.

	Sorbitol absent			Sorbitol (200 μmoles) present		
	Amount (μmoles)	$10^{-3} \times$ Radioactivity (counts/ min.)	$10^{-3} \times$ Specific radioactivity (counts/min./ $\mu\text{g. atom of C}$)	Amount (μmoles)	$10^{-3} \times$ Radioactivity (counts/ min.)	$10^{-3} \times$ Specific radioactivity (counts/min./ $\mu\text{g. atom of C}$)
O_2 uptake	304	—	—	351	—	—
CO_2 production	197	39.5	0.201	222	54.6	0.246
Acetoacetate	35.7	186	1.30	21.4	121	1.42
β -Hydroxybutyrate	11.8	115	2.45	8.1	69.3	2.14
	($\mu\text{equiv.}$)			($\mu\text{equiv.}$)		
Non-esterified fatty acids (slices)	17.5	130	—	17.2	119	—
Esterified fatty acids (slices)	90.4	48.4	—	104	115	—
Total fatty acids in residual medium	86.2	867	—	87	845	—

Table 3. *Effects of sorbitol and fructose on the conversion of [^{14}C]palmitate into ^{14}C -labelled carbon dioxide, ketone bodies and esterified fatty acids in liver slices of starved rats*

The values are calculated from Tables 2 and 4 as $\mu\text{g. atoms of C}$ having the specific radioactivity of the carbon atoms of the added [$\text{G-}^{14}\text{C}$]palmitate (17.4×10^3 counts/min./ $\mu\text{g. atom of C}$).

	Control	Change in presence of sorbitol	Control	Change in presence of fructose
Ketone bodies	17.3	-6.4	20.6	-5.9
Carbon dioxide	2.3	+0.8	2.1	+1.7
Esterified fatty acids	2.8	+3.8	5.1	+2.7
Total	22.4	-1.8	27.8	-1.5

Table 4. *Effects of fructose on fatty acid metabolism in liver slices of starved rats*

Conditions were the same as in Table 2 except for the following: fructose replaced sorbitol; 2.88 g. wet wt. of slices/flask was used; 4 μ moles (specific radioactivity 17.4×10^3 counts/min./ μ g. atom of C) of [G- 14 C]palmitate was added; the 14 C added was 111×10^4 counts/min. The respiratory quotient in the absence of fructose was 0.71, and in the presence of fructose was 0.88.

	Fructose absent			Fructose (200 μ moles) present		
	Amount (μ moles)	$10^{-3} \times$ Radioactivity (counts/ min.)	$10^{-3} \times$ Specific radioactivity (counts/min./ μ g. atom of C)	Amount (μ moles)	$10^{-3} \times$ Radioactivity (counts/ min.)	$10^{-3} \times$ Specific radioactivity (counts/min./ μ g. atom of C)
O ₂ uptake	335	—	—	366	—	—
CO ₂ production	239	36.9	0.154	323	65.5	0.203
Acetoacetate	38.5	224	1.46	23.9	164	1.72
β -Hydroxybutyrate	14.1	135	2.40	10.7	92.1	2.15
	(μ equiv.)			(μ equiv.)		
Non-esterified fatty acids (slices)	20	129	—	24	133	—
Esterified fatty acids (slices)	66	88.2	—	63	135	—
Total fatty acids in residual medium	133	147	—	123	126	—

20.6 μ moles. The difference could be due to experimental errors, but it is possible that part of the deficit may result from enhanced incorporation of 14 C into citric acid-cycle intermediates when [14 C]-palmitate is oxidized in the presence of sorbitol.

Table 3 also shows that in the presence of sorbitol 72 % of the decrease in the formation of 14 C-labelled ketone bodies is accounted for by the sum of the increase in 14 CO₂ production and esterification of 14 C-labelled fatty acids. The results suggest that the main antiketogenic effect of sorbitol is to promote the esterification of fatty acids.

Effect of fructose on ketogenesis from [G- 14 C]-palmitate. Since fructose is the primary product of the oxidation of sorbitol in mammalian liver (Embden & Griesbach, 1914; Blakley, 1951; Todd, 1954), fructose could be expected to exert its antiketogenic action in a similar manner. Table 4 gives the results of an experiment in which fructose was substituted for sorbitol. The effects on the metabolism of added [14 C]palmitate and of endogenous fatty acids are essentially in the same directions as those observed in the presence of sorbitol. An analysis of the 14 C distribution appears in Table 3 and the balance shows a deficit of 1.5 μ moles of carbon. Fructose increased 14 CO₂ production to a greater extent than sorbitol and raised the respiratory quotient, but it was somewhat less effective in promoting the esterification of 14 C-labelled fatty acids. Together these two processes account for 75 % of the antiketogenic action.

The observations of several authors show fructose to be less antiketogenic than sorbitol (Edson, 1936; Blakley, 1951; Haydon, 1961). A comparison of the experiments in Tables 2 and 4 appears to confirm this impression. On the other hand the two

Table 5. *Production of reducing sugars by rat-liver slices incubated with sorbitol*

The conditions were as given in Table 2.

	Sorbitol absent	Sorbitol (200 μ moles) present
Ketose (calculated as fructose) (μ moles)	0.31	1.77
Reducing sugar (calculated as glucose) (μ moles)	63	183

substances are equally effective in some liver slices (Table 1), but in no experiment has fructose shown a greater antiketogenic action than sorbitol.

Fate of sorbitol. The small increase in oxygen uptake found in the presence of sorbitol (Table 2) is insufficient to account for the oxidation of all the added hexitol to fructose, but the oxygen available for sorbitol metabolism is probably greater than the amount indicated by the difference because the hexitol spares the oxidation of fatty acids to ketone bodies. Table 5 shows that there is very little increase in the fructose content of the medium after incubation of the slices for 2 hr. whereas there is a large increase in glucose. The reducing sugars in the medium account for 60 % of the sorbitol added.

When a sample of the medium was subjected to high-voltage paper electrophoresis, the presence of much glucose and a trace of fructose was confirmed. Some residual sorbitol was also present.

DISCUSSION

Since the effects of sorbitol and fructose on the oxidation of [14 C]palmitate are quantitatively similar to those exerted on the endogenous keto-

genic substrate, it is probable that the mechanism of their antiketogenic action is the same for both added and endogenous fatty acids. Both sorbitol and fructose decrease the amount of ^{14}C -labelled fatty acid oxidized to ketone bodies and cause a small increase in the amount oxidized to carbon dioxide (Table 3). The enhancement of the oxidation of fatty acids to carbon dioxide could be due to an increase in the concentration of oxaloacetate. It is well known that pyruvate is an important source of oxaloacetate in liver (see Haslam & Krebs, 1963) and that fructose increases the concentration of pyruvate in this organ (Helmreich, Goldschmidt, Lamprecht & Ritzl, 1953; Lamprecht & Trautschold, 1958; Kattermann, Dold & Holzer, 1961; Wieland & Matschinsky, 1962).

Tables 2–4 make it clear that stimulation of the oxidation of [^{14}C]palmitate to carbon dioxide by sorbitol and fructose can account for only a part of the antiketogenic action and that the greater effect is increased esterification of ^{14}C -labelled fatty acid. A reasonable interpretation of the antiketogenic action is the assumption that acyl-CoA has been partly diverted from the process of β -oxidation to the esterification of glycerol 1-phosphate.

The present findings closely resemble those of Wieland & Matschinsky (1962), who perfused excised rat liver with a suspension of oleate in albumin solution and studied the effects of adding glycerol or fructose to the perfusion fluid. Both substrates diminished the rate of ketone-body formation and increased the concentration of glycerol 1-phosphate in the liver. When [$1\text{-}^{14}\text{C}$]palmitate was present in the perfusion medium, glycerol diminished the ^{14}C content of acetoacetate and increased that of carbon dioxide and the liver lipids. The experiments of Wieland & Matschinsky (1962) and those reported in the present paper are consistent with the hypothesis that the antiketogenic effects are due largely to the development of a higher concentration of glycerol 1-phosphate derived from glycerol, sorbitol or fructose. This suggestion is supported by the fact that glycerol 1-phosphate is a major product of fructose metabolism in liver (Muntz & Vanko, 1962). The same hypothesis will account for the antiketogenic effects of L-iditol, xylitol and ribitol, which, like sorbitol, are substrates of L-iditol dehydrogenase. The pathways by which they may form glycerol 1-phosphate in liver are outlined in Scheme 1.

Scheme 1. Outline of pathways by which glycerol, pentitols and hexitols may be converted into glycerol 1-phosphate in rat liver. The enzymes involved are: (1) L-iditol dehydrogenase; (2) ketohexokinase; (3) aldolase; (4) xylulokinase; (5) ribulokinase; (6) ribulose phosphate epimerase; (7) pentose phosphate pathway; (8) triokinase; (9) 'aldehyde' reductase (Hers, 1957); (10) glycerol kinase; (11) glycerol 1-phosphate dehydrogenase. There are possible alternative routes for D- and L-glyceraldehyde (see Landau & Merlevede, 1963).

Additional factors may operate in the regulation of ketogenesis. It has been suggested that the NAD-linked dehydrogenation of sorbitol plays a part in the antiketogenic action (Blakley, 1952; Touster & Shaw, 1962). Since the concentration of glycerol 1-phosphate is believed to be greatly dependent on the NADH:NAD⁺ ratio in the extra-mitochondrial compartment of the liver cell (Hohorst, Kreutz & Bücher, 1959), any superiority of sorbitol or xylitol over fructose as an antiketogenic agent could be related to the initial dehydrogenation.

The antiketogenic action of glycerol and sorbitol has been interpreted as a sparing of fatty acid oxidation that is the outcome of competition for oxygen between fatty acids and the polyhydric alcohols (Edson, 1936). This interpretation is based on the theory of substrate competition advanced by Krebs (1935) and subsequently elaborated by the same author in the light of contemporary knowledge of electron transport (Krebs, 1960). The observations reported in the present paper do not exclude the possibility that substrate competition is responsible for diminishing the rate of oxidation of acyl-CoA to ketone bodies. If this occurs a greater amount of acyl-CoA could become available for esterification.

SUMMARY

1. The antiketogenic effects of glucose, fructose and polyols have been measured in liver slices of starved rats.

2. Sorbitol and fructose diminish the formation of ¹⁴C-labelled ketone bodies in liver slices prepared from starved rats and incubated with [G-¹⁴C]-palmitate. The production of ¹⁴CO₂ is slightly increased, but the total amount of [¹⁴C]palmitate oxidized is diminished. The diminution is partly accounted for by incorporation of ¹⁴C-labelled fatty acids into the esterified fatty acids of the slices.

3. The relevance of these results to the theory of polyol antiketogenesis is discussed.

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REFERENCES

- Berry, M. N. (1963). *Analyt. Biochem.* **6**, 352.
 Blakley, R. L. (1951). *Biochem. J.* **49**, 257.
 Blakley, R. L. (1952). *Biochem. J.* **52**, 269.
 Bragdon, J. H. & Gordon, R. S. (1958). *J. clin. Invest.* **37**, 574.
 Edson, N. L. (1936). *Biochem. J.* **30**, 1862.
 Embden, G. & Griesbach, W. (1914). *Hoppe-Seyl. Z.* **91**, 217.
 Exton, J. H. (1964). *Biochem. J.* (in the Press).
 Fritz, I. B. (1961). *Physiol. Rev.* **41**, 52.
 Gordon, H. T., Thornburg, W. & Werum, L. N. (1956). *Analyt. Chem.* **28**, 849.
 Hamosh, M. & Shapiro, B. (1961). *Amer. J. Physiol.* **201**, 1030.
 Haslam, R. J. & Krebs, H. A. (1963). *Biochem. J.* **86**, 432.
 Haydon, R. K. (1961). *Biochim. biophys. Acta*, **46**, 598.
 Helmreich, E., Goldschmidt, S., Lamprecht, W. & Ritzl, F. (1953). *Hoppe-Seyl. Z.* **292**, 184.
 Hers, H. G. (1957). *Le Métabolisme du Fructose*, pp. 33, 136. Bruxelles: Éditions Arscia.
 Hohorst, H. J., Kreutz, F. H. & Bücher, T. (1959). *Biochem. Z.* **332**, 18.
 Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
 Kattermann, R., Dold, U. & Holzer, H. (1961). *Biochem. Z.* **334**, 218.
 Kennedy, E. P. (1957). *Annu. Rev. Biochem.* **26**, 119.
 Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 191.
 Krebs, H. A. (1935). *Biochem. J.* **29**, 1635.
 Krebs, H. A. (1960). *Proc. R. Soc. Med.* **53**, 71.
 Kulka, R. G. (1956). *Biochem. J.* **63**, 542.
 Lamprecht, W. & Trautschold, I. (1958). *Hoppe-Seyl. Z.* **311**, 245.
 Landau, B. R. & Merlevede, W. (1963). *J. biol. Chem.* **238**, 861.
 McCorkindale, J. & Edson, N. L. (1954). *Biochem. J.* **57**, 518.
 Muntz, J. A. & Vanko, M. (1962). *J. biol. Chem.* **237**, 3582.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Olivecrona, T. (1962). *Acta physiol. scand.* **54**, 295.
 Smith, M. G. (1962). *Biochem. J.* **83**, 125.
 Somogyi, M. (1945). *J. biol. Chem.* **160**, 69.
 Todd, C. M. (1954). *Aust. J. exp. Biol.* **32**, 827.
 Touster, O. & Shaw, D. R. D. (1962). *Physiol. Rev.* **42**, 181.
 Walker, P. G. (1954). *Biochem. J.* **58**, 699.
 Wieland, O. & Matschinsky, F. (1962). *Life Sci.* **2**, 49.