The Lipids of Mammalian Pancreas

By C. PROTTEY AND J. N. HAWTHORNE

Department of Medical Biochemistry and Pharmacology, University of Birmingham

(Received 17 February 1966)

1. The total lipids of ox and guinea-pig pancreas were fractionated on silicic acid columns. 2. The high lipid content of both tissues was due to triglyceride. The major fatty acids of this triglyceride were stearic acid, palmitic acid and oleic acid. 3. The pattern of individual phospholipids resembled that of liver, though pancreas contained more plasmalogen.

Hokin & Hokin (1955) have shown that when protein secretion is stimulated in pancreas slices there is an increased incorporation of isotopic precursors into phosphatidylinositol and phosphatidic acid. The underlying biochemical changes are not well understood, nor is it known whether there is a direct connexion between the metabolism of these lipids and protein secretion. As an approach to these problems we have studied the biosynthesis of phosphatidylinositol in pancreas. Since little detailed information was available on the lipids of pancreas, the first part of our work, now reported, has been the examination of these lipids.

Pancreas is rich in lipids, the total lipid constituting at least 9% of the fresh weight (Müting & Wortmann, 1954), and this lipid is unusual in containing a high proportion of neutral fat (Bloor, 1928). Jones, Kock, Heath & Munson (1949) and Müting & Wortmann (1954) reported a high monopalmitin content in hog pancreas, but this seems to have been produced by the action of lipase on tissue triglyceride during homogenization (Borgström, 1961). Few results are available for the individual phospholipids of pancreas. Hokin & Hokin (1955) reported the following analysis (as percentages of total lipid P) of pigeon pancreas: phosphatidylethanolamine, 54; phosphatidylethanolamine, 26; phosphatidylserine, 10; phosphatidylinositol, 8; phosphatidic acid, 2.

MATERIALS AND METHODS

Animals. Ox pancreas was obtained from the Birmingham City Meat Market. The organs were chilled on ice and the lymph nodes dissected out and discarded. These were dark spherical masses 1–2 cm. in diameter. The tissue was then homogenized in acetone within 15 min. of the death of the animal. Adult albino guinea pigs weighing about 800 g., which had been fed normally, were also used. The pancreas was removed immediately after the death of

the animal and homogenized in chloroform-methanol (2:1, v/v).

Preparation of lipid extracts. Two separate batches of ox tissue were extracted. In each case about 800 g. fresh wt. of pancreas, the pooled organs from three bullocks, was chopped with scissors and then homogenized in a high-speed mixer for 1 min. with a total of 2 l. of acetone. After 2 hr. at room temperature the mixture was filtered through glass-fibre paper and the residue extracted for 30 min. with a further 2 l. of acetone. This was followed by two extractions for 3 hr. with 2 l. of chloroform-methanol (2:1, v/v) and a final overnight extraction with the same solvent (2 l.).

Each of the five lipid extracts was dried in a rotary evaporator at 40° and redissolved in 600 ml. of chloroform-methanol (2:1, v/v). The solution so obtained was washed by the method of Folch, Lees & Sloane-Stanley (1957) with 120 ml. of 0.01 M-CaCl₂. The upper phase was extracted with 'synthetic' lower phase and the resulting chloroform layer combined with the washed lipid solution. Washing removed considerable quantities of water-soluble phosphate, particularly from the second and third chloroform-methanol extracts. The solvent was removed in a rotary evaporator at 40° and the remaining lipid dried by the addition and evaporation of several 50 ml. portions of acetone. The five lipid extracts thus prepared were redissolved in 500 ml. of chloroform and stored at 4°. Samples of these extracts were taken for phospholipid analysis (see the text below and Table 4) and the remainders combined for total lipid analysis by chromatography on silicic acid columns as described below.

For the guinea-pig work, the pancreases from five large female animals were homogenized for 1 min. in a high-speed mixer with 250 ml. of chloroform-methanol (2:1, v/v) and allowed to extract for 2 hr. at room temperature. This extraction was repeated and a third extraction made with 200 ml. of chloroform-methanol-conc. HCl (200:100:1, by vol.). Each of the extracts was washed with 0.2 vol. of 0.01 M-CaCl₂ as described above and analysed for phosphorus. The combined extracts were then dried *in vacuo*, redissolved in 100 ml. of chloroform and stored at 4° for chromatographic analysis.

Chromatography of lipids on silicic acid. The different classes of lipids in the extracts were separated on columns of silicic acid (60 g.) by the method of Barron & Hanahan (1958) with slight modifications. After elution of the free

sterol fraction (20% diethyl ether in hexane), mono- and di-glycerides, if present, were eluted together in diethyl ether, the elution step with 50% diethyl ether in hexane being omitted. Fractions of volume 25 ml. were collected. After elution with ether, phospholipids were removed from the columns by elution with 500 ml. of chloroform-methanol (1:4, v/v), followed by 500 ml. or 1 l. of chloroform-methanol (1:9, v/v) and 500 ml. of methanol, and these three extracts were pooled. This procedure gave quantitative recovery of the phospholipids.

Fractions from the column were examined by thin-layer chromatography by the method of Morgan, Tinker & Hanahan (1963). For more detailed examination of the glyceride fractions the method of Barrett, Dallas & Padley (1963) was used with the following solvents: (a) carbon tetrachloride-chloroform-acetic acid-ethanol (60:140:1:3, by vol.); (b) chloroform-acetic acid (199:1, v/v). Spots were located by heating with 10% (v/v) H_2SO_4 or by spraying with 0.2% (w/v) dichlorofluorescein in ethanol, and examining in ultraviolet light.

Gas-liquid chromatography. Samples of the triglyceride fractions from the silicic acid columns containing roughly 300 mg. of lipid in 2 ml. of diethyl ether were incubated at 37° with 2 ml. of *N*-KOH (methanolic) for 4 hr. Then 3.6 ml. of *N*-HCl and 1 ml. of water were added and the ether phase was separated. The aqueous phase was extracted

twice with 2 ml. of ether, the washings were combined with the original upper layer and the ether was removed *in vacuo*. Methylation with boron trifluoride followed the method of Metcalfe & Schmitz (1961).

The methyl esters of the fatty acids were separated by gas-liquid chromatography with a Panchromatograph (Pye Ltd., Cambridge) with flame ionization detector. The stationary phase was 10% polyethylene glycol adipate on 80-100-mesh Chromosorb W that had previously been deactivated with hexamethyldisilazane. The column was 5 ft. long $\times$ 4 mm. internal diam., the flow rate 60 ml./min. and the operating temperature 197°.

Phospholipid analysis. The phospholipids in each of the lipid extracts of ox pancreas were deacylated under mild alkaline conditions and the resulting phosphate esters separated by paper chromatography (Dawson, 1960) or ion-exchange chromatography (Hübscher, Hawthorne & Kemp, 1960). The total lipid extracts were used for these analyses rather than the phospholipid fractions from silicic acid chromatography to obviate any slight changes in pattern due to losses on the columns. The following method of deacylation was used. The phospholipid sample, containing approx. 1 mg. of P, was dissolved in 1.5 ml. of chloroform. After the addition of 0.5 ml. of methanol and 0.25 ml. of 0.5 *N*-NaOH in methanol, hydrolysis was allowed to proceed for 15 min. at room temperature. The reaction

Table 1. *Lipid composition of ox pancreas*

The silicic acid column (60 g.; 15 cm. $\times$ 3.5 cm.) was activated before use by the method of Barron & Hanahan (1958). The total lipid applied was 860 mg. and the recovery was 98%. Values in parentheses are percentages of total recovered lipid. TLC (I) and TLC (II) refer to thin-layer chromatography as described by Morgan *et al.* (1963) and Barrett *et al.* (1963) respectively.

Eluting solvent	Vol. (ml.)	Fraction	Wt. (mg.)	Analysis
Hexane	160	'Pigments and hydrocarbons'	5 (0.57%)	—
15% Benzene in hexane	600	'Sterol esters'	16 (1.86%)	No cholesterol detected; ester determination negative. TLC (I), one faint spot at solvent front
5% Ether in hexane	1800	'Triglycerides and free fatty acids'	436 (51.7%)	No cholesterol detected; ester/glycerol molar ratio, 2.8:1. TLC (II), major spots corresponded to markers of tristearin, tripalmitin and triolein, and five minor spots (see Table 3)
20% Ether in hexane	1000	'Free sterol'	108 (12.8%)	Cholesterol present; ester, traces. TLC (II), major cholesterol spot and three faint triglyceride spots
Ether	1000	'Mono- and di-glycerides'	75 (8.85%)	Cholesterol, traces; ester/glycerol molar ratio, 1.18:1. TLC (II), major monoglyceride spot and six very faint spots corresponding to mono- or di-glycerides
Chloroform-methanol				
1:4 (v/v)	500	'Phospholipids'	205 (24.3%)	Total P applied, 4.66 mg.; recovery, 97% (see Table 5)
1:9 (v/v)	500			
Methanol	500			

was stopped by the addition of 3.0 ml. of water, 2.05 ml. of ethanol and 2.7 ml. of chloroform, in that order, with shaking between each addition (Brockerhoff, 1963). The upper aqueous layer containing the phosphate esters was separated by centrifuging in a bench centrifuge. For ion-exchange chromatography boric acid was added (Hübscher *et al.* 1960), and for paper chromatography by the method of Dawson (1960) the aqueous layer was passed through a small column of Dowex 50W (pyridinium form) as described by Brockerhoff (1963).

The large quantity of triglyceride in some of the extracts presented problems in that excessive amounts of methyl esters were formed on deacylation. On application of the hydrolysate to the papers a greasy spot was obtained, presumably due to methyl esters or salts of fatty acids that were not completely extracted into the chloroform phase after stopping the reaction. These esters caused poor separations and were removed by rolling up the paper and extracting it in a measuring cylinder with 250 ml. of ether. When the hydrolysate was diluted for application to the Dowex column a white precipitate of methyl esters formed and was removed by filtration.

Fractions from the ion-exchange columns (Hübscher *et al.* 1960) were analysed for total P and the peak fractions further examined as follows. Cations were removed by treatment with Dowex 50 and the resulting solution was concentrated *in vacuo* to a volume of approx. 1 ml. Then conc. HCl was added to give a final concentration of 6N and the mixture was heated at 110° for 12 hr. in a sealed tube. The resulting hydrolysate was examined for inositol, glycerol, choline, ethanolamine and serine by paper

chromatography in propan-2-ol-acetic acid-water (3:1:1, by vol.) (Hübscher & Hawthorne, 1957).

Other analytical methods. Total plasmalogen was estimated by the method of Ansell & Spanner (1963) and glycerol by that of Burton (1957). Carboxylic ester was determined by the hydroxamate method of Stern & Shapiro (1953). Cholesterol was detected by the procedure of Rodnight (1956). Analysis of phosphate was by the method of King (1932).

RESULTS

Total lipid. In two experiments with ox pancreas, the total lipid represented 13.8 and 16.1% respectively of the tissue fresh weight. For each experiment organs from three animals were extracted together.

The composition of the total lipid fraction is summarized in Table 1 for ox pancreas and in Table 2 for guinea-pig pancreas. In both cases there was a good recovery from the silicic acid column of both total lipid (by weight) and phospholipid (by P analysis). The major lipid was triglyceride and only the ox pancreas contained appreciable quantities of monoglyceride.

Table 3 gives the fatty acid composition of the triglyceride fractions prepared from the silicic acid column. Under the experimental conditions used methyl stearate and methyl oleate were only

Table 2. *Lipid composition of guinea-pig pancreas*

The silicic acid column (60 g.; 15 cm. × 3.5 cm.) was activated before use by the method of Barron & Hanahan (1958). The total lipid applied was 1.125 g. and the recovery was 94.3%. Values in parentheses are percentages of total recovered lipid. TLC (I) and TLC (II) refer to thin-layer chromatography as described by Morgan *et al.* (1963) and Barrett *et al.* (1963) respectively.

Eluting solvent	Vol. (ml.)	Fraction	Wt. (mg.)	Analysis
Hexane	150	'Pigments and hydrocarbons'	0	—
15% Benzene in hexane	500	'Sterol esters'	12 (1.13%)	No cholesterol detected. TLC (I), very faint spot at solvent front
5% Ether in hexane	1500	'Triglycerides and free fatty acids'	938 (88.3%)	No cholesterol; ester/glycerol molar ratio, 2.7:1. TLC (II), three major spots corresponding with tristearin, tripalmitin and triolein, and five minor spots (see Table 3)
20% Ether in hexane	1000	'Free sterol'	17 (1.6%)	Cholesterol present; no ester detected. TLC (II), one spot corresponding to cholesterol
Ether	1000	'Mono- and di-glycerides'	8 (0.75%)	No cholesterol; traces of ester. TLC (II), three faint spots with mobilities of mono- or di-glycerides
Chloroform-methanol				
1:4 (v/v)	1000	'Phospholipids'	86 (8.1%)	Total P applied, 3.01 mg.; recovery, 103% (see Table 5)
1:9 (v/v)	1000			
Methanol	500			

partially separated. The double peak was symmetrical and so its composition was considered to be 50% stearate and 50% oleate. In both ox and guinea pig, stearic acid, palmitic acid and oleic acid accounted for about 90% of the total fatty acids. They were present in roughly equimolar amounts in ox pancreas. The guinea-pig tissue had considerably more palmitic acid and less of the other two acids.

Phospholipid. Though phospholipids are traditionally considered to be relatively insoluble in acetone, extraction of tissues with this solvent can remove considerable amounts (for references see Ansell & Hawthorne, 1964). With ox pancreas,

two extractions with acetone removed almost half the total phospholipid (Table 4). This effect is probably due to the high content of triglyceride. As the extraction proceeded to the second and third chloroform-methanol stages, proportionally less lecithin and more phosphatidylinositol and phosphatidylserine were extracted. The Dawson (1960) method was used for the phospholipid analyses in Table 4.

Table 5 gives a more detailed analysis of the total phospholipid fraction of ox and guinea-pig pancreas. The two tissues have a very similar phospholipid pattern. Phosphatidic acid was never detected by the Dawson (1960) method since the

Table 3. *Fatty acids of pancreatic triglyceride*

The results are expressed as moles/100 moles of the recovered methyl esters.

Fatty acid...	Fatty acid composition (moles/100 moles)					
	C _{14:0}	C _{14:1}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:2}
Ox	3.6	1.8	31.5	3.3	29.4	1.0
Guinea pig	2.7	0	43.0	0	23.8	6.7

Table 4. *Extraction of phospholipids from ox pancreas*

About 800g. fresh wt. of tissue was extracted (see the Materials and Methods section). Phospholipid values were obtained by the method of Dawson (1960). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol.

Extract	Lipid P extracted (mg.)	% of total lipid P	% of component phospholipid in each extract		Component as % of total phospholipid
Acetone (first): 2l., 2 hr.	223	32.2	PC	73.2	23.6
			PE	13.6	4.4
			PS	0.9	0.3
			PI	2.3	0.7
			Alkali-stable	10.0	3.2
Acetone (second): 2l., 30 min.	103	14.8	PC	74.0	10.9
			PE	16.0	2.4
			Alkali-stable	10.0	1.5
Chloroform-methanol (first): 2l., 3 hr.	271	39.1	PC	38.2	14.9
			PE	29.9	11.7
			PS	5.9	2.3
			PI	6.5	2.5
			Alkali-stable	19.5	7.7
Chloroform-methanol (second): 2l., 3 hr.	69.5	10.0	PC	22.2	2.2
			PE	19.4	1.9
			PS	13.0	1.3
			PI	25.6	2.6
			Unidentified	1.8	0.2
			Alkali-stable	18.0	1.8
Chloroform-methanol (third): 2l., overnight	27.2	3.9	PC	34.7	1.4
			PE	26.1	1.0
			PS	10.5	0.4
			PI	12.6	0.5
			Alkali-stable	16.1	0.6
Total	693.7				

Table 5. *Phospholipid composition of pancreas*

The results are expressed as percentages of total lipid P and are averages of two determinations by the paper or resin methods on the same lipid sample.

Phospholipid	Phospholipid composition (% of total lipid P)		
	Ox		Guinea pig
	Paper chromatography	Dowex 1	Paper chromatography
Phosphatidylcholine	53.0	51.5	50.3
Phosphatidylethanolamine	21.4	17.0	17.8
Phosphatidylserine	4.3	4.2	4.4
Phosphatidylinositol	6.3	8.7	7.9
Phosphatidic acid	—	2.0	—
Unidentified	0.3	0.8	2.3
Plasmalogens	5.9	15.8	8.0
Acid- and alkali-stable	8.8		9.3

total amount of hydrolysate on each chromatogram was so small. On the resin columns, however, a small peak was seen in the position expected for glycerophosphate. After allowance for contaminating inorganic phosphate, this peak accounted for 2% of the total lipid P. The resin-column method gives a high result for phosphatidylinositol. This is due to the presence of methyl glycerophosphate and cyclic glycerophosphate in the glyceryl-phosphorylinositol peak. The column gave a very small peak in the position expected for the hydrolysis product of diphosphoinositide. Hydrolysis of the compound responsible showed that it contained inositol. [Kfoury & Kerr (1964) have reported the presence of diphosphoinositide in extracts of pig pancreas by ion-exchange chromatography of the deacylated lipids.] In an attempt to follow up this observation, guinea-pig pancreas (11g. fresh wt.) was extracted twice with chloroform-methanol (2:1, v/v) (250ml. and 100ml. respectively) and once with 100ml. of chloroform-methanol-conc. hydrochloric acid (200:100:1, by vol.). The acidified solvent contained no detectable lipid P, but the first two extracts yielded 10.0mg. of P and 0.42mg. of P respectively.

DISCUSSION

Glycerides. Assuming that fresh pancreas contains 74% by weight of water [Marx (1926) gave a value of 74.2% for human pancreas], lipid accounts for about half the dry weight of this tissue. The results show that triglyceride is the major lipid component. Only the ox pancreas contained significant amounts of monoglyceride (8.8% of the total lipid, compared with 0.67% for guinea-pig pancreas) and this was almost certainly due to lipase action *post mortem* (Borgström, 1961). It

was possible to extract the guinea-pig pancreas almost immediately after the death of the animal, but some delay was inevitable with ox tissues.

The lipid content of the guinea-pig pancreas depends on the age of the animal, young animals weighing 300g. having relatively small amounts of neutral fat in comparison with the 800g. adult animals used in the present study. This became apparent during the preparation of subcellular fractions from pancreas (C. Prottey & J. N. Hawthorne, unpublished work), a lipid layer of variable size being seen at the surface of the sucrose homogenate after the first centrifugation. Palade & Siekevitz (1956) reported a similar variation with age.

The major fatty acids of the triglyceride fraction of ox pancreas were stearic acid, palmitic acid and oleic acid, as in ox depot fat (Hilditch & Williams, 1964). The guinea-pig pancreas had relatively more palmitic acid and less of the other two acids than ox pancreas. It also contained appreciably more linoleic acid. In removing the pancreas care was taken to avoid mesenteric fat. This is not difficult in the guinea pig since the pancreas is a well-defined organ and the fat is much paler in colour.

Because of its high content of saturated acids, the triglyceride of pancreas is relatively insoluble. On standing at 4°, layers of lipid formed at the surface of the initial acetone extract and the subsequent chloroform-methanol extract. A similar effect was seen by King & Aloisi (1945).

Deacylation of phospholipids. The deacylation method used in this work was based on that of Hübscher *et al.* (1960). These authors used 1–2 moles of sodium hydroxide/mole of phospholipid. In the present study 4–8 moles of sodium hydroxide/mole were needed, because of the large excess of

triglyceride present. Even though the deacylation may be considered a base-catalysed methanolysis independent of alkali concentration, smaller quantities of hydroxide decreased the degree of hydrolysis considerably. This may indicate removal of sodium hydroxide from the system by formation of soaps. This is less likely under non-aqueous conditions, though the solvents and lipids used will all have contained traces of water. If a large excess of alkali was used, the water-soluble phosphodiester were unstable and more methyl glycerophosphate was formed.

For the extraction of the phosphate esters, the method of Brockerhoff (1963) was used. This involves methanolysis in chloroform-methanol (1:4, v/v) but, since this solvent mixture did not completely dissolve the pancreatic lipids, the method of Hübscher *et al.* (1960), with chloroform-methanol (2:1, v/v), was followed. Chloroform, ethanol and water were then added to give the concentrations used by Brockerhoff (1963). Moderate centrifugation gave a complete phase separation. The extraction method of Hübscher *et al.* (1960) gave intractable emulsions.

Phospholipid composition. Mütting & Wortmann (1954) give the phospholipid content of ox pancreas as 14.0–23.5 g./kg. fresh wt. of tissue. Assuming the phospholipids to have an average P content of 4%, the results in Table 4 would give about 21.7 g. of phospholipid/kg. This phospholipid content, which is equivalent to 0.87 mg. of lipid P/g. fresh wt. of tissue, resembles that of many other mammalian organs (Ansell & Hawthorne, 1964). It is the increase in triglyceride that gives pancreas its characteristic high lipid content.

The acid- and alkali-stable fraction shown in Table 5 is probably sphingomyelin, though traces of glycerol ether phospholipids could be present. McKibbin & Taylor (1950) obtained similar values to those of Table 5 for choline phospholipids and sphingomyelin in dog pancreas. Klenk & Friedrichs (1952) estimated the plasmalogens of pig pancreas to comprise 4% of the total phospholipid. For ox, our value was 5.9% (Table 5). The values obtained for individual phospholipids in the present study agree well with those obtained for pigeon pancreas by Hokin & Hokin (1955).

The phospholipid patterns of ox and guinea-pig pancreas present no unusual features. They closely resemble that of liver, for instance, though

the plasmalogen contents are appreciably higher in pancreas (Ansell & Hawthorne, 1964).

We are grateful to Mr A. Wilson, M.R.C.V.S., of the City Meat Market, Birmingham, for his help in obtaining fresh ox pancreas. C. P. is in receipt of a Science Research Council Scholarship for research training.

REFERENCES

- Ansell, G. B. & Hawthorne, J. N. (1964). *The Phospholipids*, pp. 88, 411. Amsterdam: Elsevier Publishing Co.
- Ansell, G. B. & Spanner, S. (1963). *J. Neurochem.* **10**, 941.
- Barrett, C. B., Dallas, M. S. J. & Padley, F. B. (1963). *J. Amer. Oil Chem. Soc.* **40**, 580.
- Barron, E. J. & Hanahan, D. J. (1958). *J. biol. Chem.* **231**, 493.
- Bloor, W. R. (1928). *J. biol. Chem.* **80**, 443.
- Borgström, B. (1961). *Biochim. biophys. Acta*, **51**, 373.
- Brockerhoff, H. (1963). *J. Lipid Res.* **4**, 96.
- Burton, R. M. (1957). In *Methods in Enzymology*, vol. 3, p. 246. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Dawson, R. M. C. (1960). *Biochem. J.* **75**, 45.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
- Hilditch, T. P. & Williams, P. N. (1964). *The Chemical Constitution of Natural Fats*, 4th ed., pp. 104–112. London: Chapman and Hall.
- Hokin, L. E. & Hokin, M. R. (1955). *Biochim. biophys. Acta*, **13**, 102.
- Hübscher, G. & Hawthorne, J. N. (1957). *Biochem. J.* **67**, 523.
- Hübscher, G., Hawthorne, J. N. & Kemp, P. (1960). *J. Lipid Res.* **1**, 433.
- Jones, M. E., Kock, F. C., Heath, A. E. & Munson, P. L. (1949). *J. biol. Chem.* **181**, 755.
- Kfoury, G. A. & Kerr, S. E. (1964). *Biochim. biophys. Acta*, **84**, 391.
- King, E. J. (1932). *Biochem. J.* **26**, 292.
- King, E. J. & Aloisi, M. (1945). *Biochem. J.* **39**, 470.
- Klenk, E. & Friedrichs, E. (1952). *Hoppe-Seyl. Z.* **290**, 169.
- McKibbin, J. M. & Taylor, W. E. (1950). *J. biol. Chem.* **185**, 357.
- Marx, H. (1926). *Biochem. Z.* **179**, 414.
- Metcalfe, L. D. & Schmitz, A. A. (1961). *Analyt. Chem.* **33**, 363.
- Morgan, T. E., Tinker, D. O. & Hanahan, D. J. (1963). *Arch. Biochem. Biophys.* **103**, 54.
- Mütting, D. & Wortmann, V. (1954). *Biochem. Z.* **325**, 448.
- Palade, G. E. & Siekevitz, P. (1956). *J. biophys. biochem. Cytol.* **2**, 671.
- Rodnight, R. (1956). *J. Neurochem.* **1**, 207.
- Stern, I. & Shapiro, B. (1953). *J. clin. Path.* **6**, 158.