Biochem. J. (1968) **106**, 677
Printed in Great Britain

The Significance of Changes in Tissue Clearing-Factor Lipase Activity in Relation to the Lipaemia of Pregnancy

By SHEILA OTWAY and D. S. ROBINSON
Medical Research Council Cell Metabolism Research Unit, Department of Biochemistry, University of Oxford

(*Received* 10 *July* 1967)

1. The concentration of triglyceride fatty acid in the plasma of the pregnant rat rises to a maximum 2–4 days before parturition. Thereafter there is a rapid decline in the concentration to near normal values at parturition. 2. A similar increase occurs in animals fed on a diet low in fat. There is no increase in food consumption at the time when the triglyceride fatty acid concentration in the plasma is at its peak. 3. Rates of entry of triglyceride fatty acid into the blood during pregnancy have been estimated from the rate of accumulation of triglyceride in the plasma of animals injected with a non-ionic detergent, Triton. A progressive increase occurs in the entry rate as the body weight increases throughout pregnancy. Expressed per constant body weight, the entry rate does not change significantly. 4. Adipose-tissue clearing-factor lipase activity is low at the time when the plasma triglyceride fatty acid concentration is raised. Activity of the enzyme in heart, lung and diaphragm is unchanged. 5. It is suggested that the 'lipaemia of pregnancy' may be due to diminished uptake of triglyceride fatty acids by adipose tissue, and, further, that the disappearance of the lipaemia may be due to increased uptake of triglyceride fatty acids by the mammary gland.

Several animal species display a 'lipaemia of pregnancy', which has been shown to be due to a rise in the concentration of triglycerides in the plasma (Scow, Chernick & Brinley, 1964). The rise occurs only during the few days before parturition, and at parturition the plasma triglyceride concentration has returned to normal.

A rise in plasma triglyceride concentration must be due either to an increase in the rate of entry of TGFA* into the circulation or to a fall in their rate of removal from the blood. A rise in the rate of TGFA input into the blood can result from an increase in the amount of dietary fat ingested, or from an increase in the extent of hepatic lipogenesis leading to a rise in the rate of release of TGFA from the liver. A decrease in the rate of removal, on the other hand, can probably be attributed to a fall in the activity of the tissue enzyme clearing-factor lipase or lipoprotein lipase. This enzyme is widely distributed in the extrahepatic tissues and is believed to regulate the uptake of TGFA by such tissues (Robinson, 1963*a*, 1964).

In the present study, clearing-factor lipase activity has been determined in a number of tissues at various stages of pregnancy in the rat. In addition, the rate of TGFA accumulation in the plasma after the injection of the non-ionic detergent Triton WR 1339 has been measured. This substance inhibits the action of clearing-factor lipase on plasma triglycerides and prevents their uptake by the tissues (Otway & Robinson, 1967*a*). From the rate of TGFA accumulation in the plasma after its injection, a value for the rate of TGFA influx into the circulation can therefore be calculated (Otway & Robinson, 1967*b*). A preliminary report of this work has been given (Otway & Robinson, 1966).

* Abbreviations: TGFA, triglyceride fatty acid(s); FFA, free fatty acid(s).

EXPERIMENTAL

Female albino rats of the Wistar strain weighing 200–230g. were used. They were normally fed on a laboratory stock diet (Oxoid pasteurized breeding diet) containing approx. 3% of digestible fat, but sometimes a special diet that contained approx. 33% of starch, 33% of sucrose and 33% of casein and only 0·3% of fat as corn oil was given. The starch and casein were extracted with ethanol and ether before they were incorporated into the diet. Vitamin supplements and salts were added.

Nine days after they had been mated, the rats were housed in individual cages in which they were kept for the remainder of the gestation period. Feeding on the low-fat high-carbohydrate diet was started at this stage in the experiments described in Figs. 2 and 3. Animals fed on this diet gained weight at a normal rate throughout their

pregnancy, though parturition was sometimes delayed by a day or two. When rats were studied after parturition, suckling and lactation were noted to be proceeding normally.

Measurement of plasma TGFA concentration. Samples of blood (0·4ml.) were taken from the tail veins between 9 a.m. and 10 a.m., the animals being under light ether anaesthesia. The duration of the anaesthesia was never greater than 3min. Measurement of the plasma TGFA concentration was carried out as described by Otway & Robinson (1967*b*).

Measurement of tissue clearing-factor lipase activity. Acetone–ether-dried powders were prepared from heart, lung, diaphragm muscle and adipose tissue (Robinson, 1963*a*). The tissues were removed while the animals were under light ether anaesthesia, rinsed in 0·9% NaCl, blotted and weighed. They were stored in ice for up to 30min. before they were minced coarsely and homogenized in acetone at 4°. Between 3 and 7g. of adipose tissue was taken from each rat from the posterior abdominal wall and from around the kidneys. The heart, adipose tissue and lungs were homogenized in a Waring Blendor, and the diaphragm in the micro head of a Silverson homogenizer (Silverson Machines Ltd., London, S.E. 1).

Enzyme assays were carried out after the powders had been stored *in vacuo* overnight at 4°. Samples of the powders were homogenized in cold 25mN-NH_3 (adjusted to pH 8·1 with N-HCl) containing heparin (1i.u./ml.). Portions (3ml.) of the homogenates were added to a mixture containing 1ml. of M-tris buffer, pH 8·1, 2ml. of albumin (20%, w/v) in 0·9% NaCl, pH 8·1, and 2ml. of triglyceride substrate. The triglyceride substrate (Salaman & Robinson, 1966) was prepared from 3vol. of serum, obtained from platelet-free citrated plasma by recalcification, and 1vol. of chyle, collected from the thoracic ducts of rats that had been given olive oil (French, Robinson & Florey, 1953). The chyle contained 150–200μequiv. of TGFA/ml. The concentrations of powder in the assay medium were: adipose tissue and heart, 1mg./ml.; diaphragm, 2mg./ml.; lung, 4mg./ml.

FFA determinations were carried out by a modification of the technique of Dole & Meinertz (1960), similar to that described by Salaman & Robinson (1961). Samples (1ml.) were taken in triplicate from the assay mixture before and after incubation for either 1 or 2hr. Not more than 20% of the chyle triglyceride was hydrolysed during the assay and release of FFA occurred at a linear rate throughout. Serial samples of the powders, and of the aqueous homogenates prepared from the powders, gave activities in good agreement with each other.

Clearing-factor lipase activities were expressed in terms of the amounts of FFA released from the triglyceride substrate/hr./g. fresh wt. of tissue. No change in the dry weight of the powder expressed as a percentage of the fresh weight was detected at any stage of pregnancy in any of the tissues.

Measurement of rates of TGFA entry into the blood. The method described by Otway & Robinson (1967*b*) was used. Plasma TGFA concentrations were measured in blood samples taken before, and 90min. after, the intravenous injection of 100mg. of Triton WR 1339 (polymeric *p*-iso-octyl polyoxyethylene phenol; Winthrop Laboratories, Newcastle upon Tyne, Northumberland), all injections being carried out between 9 a.m. and 10 a.m. After the 90min. blood sample had been taken, 0·5ml. of 0·4% Evans Blue dye in 0·85% NaCl was injected and, 5min. later, a further sample of blood was taken. From the rise in the plasma TGFA concentration and the calculated value for the plasma volume derived from the dye injection, the increase in total plasma TGFA concentration during the 90min. period and hence the rate of TGFA entry into the blood (μequiv. of TGFA/hr.) were calculated. The calculated values given in Table 2 are uncorrected for any 'leak' of TGFA from the plasma that may occur in animals given Triton. However, this 'leak' is probably extremely small (Otway & Robinson, 1967*a*,*b*).

In these experiments, the livers were removed from the animals after the final sample of blood had been taken, and their TGFA contents were measured as described by Otway & Robinson (1967*b*).

RESULTS

Rise in plasma TGFA concentration during pregnancy. The results in Fig. 1 show that in pregnant rats on normal laboratory diets the plasma TGFA concentration rises to a peak value, which is three to five times the mean concentration of about 3μequiv./ml. in the plasma of the non-pregnant animal (see Table 2 and Otway & Robinson, 1967*b*). The peak value occurs 2–4 days before parturition, and thereafter there is a rapid decline so that at parturition the concentration has returned to near normal values.

These results are in good agreement with those reported by Scow *et al.* (1964). However, these authors found that the increase in plasma triglyceride concentrations did not occur in pregnant animals that were fed on a diet free of fat. In the present study, when rats were fed on such a diet for

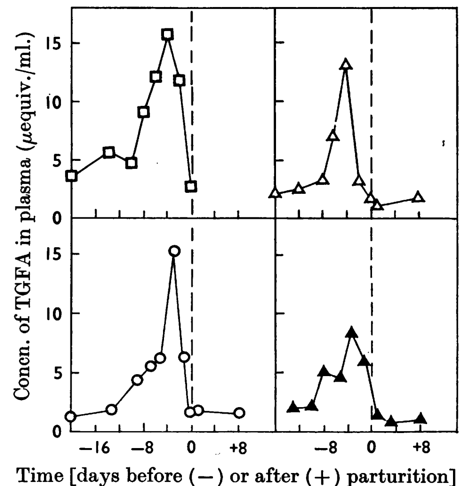

Fig. 1. Changes in plasma TGFA concentration that occurred during pregnancy in each of four pregnant rats fed on a normal laboratory diet. Blood samples were taken from the tail vein of each rat between 9 a.m. and 10 a.m.

10–12 days before parturition, the plasma TGFA concentration still rose to a peak value and then declined again (Fig. 2). On such a diet the time of the peak TGFA concentration in relation to the time of parturition appeared somewhat more variable than in rats on their normal diet.

No satisfactory explanation for the disagreement with the findings of Scow *et al.* (1964) can be given at present, although it may be relevant that they gave their fat-free diet only during the last 6 days of pregnancy whereas it was given for about 2 weeks before parturition in the present work. Our observations appear to exclude the possibility that the rise in TGFA concentration is necessarily dependent on an influx of dietary fatty acids into the blood.

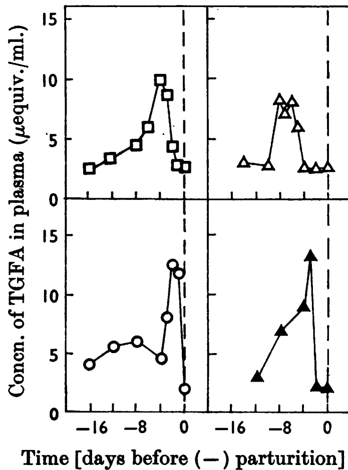

Fig. 2. Changes in plasma TGFA concentration during pregnancy in four rats fed on a low-fat high-carbohydrate diet from 9 days after mating.

Tissue clearing-factor lipase activity during pregnancy. The results in Table 1 show that, at the time when the plasma TGFA concentration in the pregnant rat is elevated, the clearing-factor lipase activity in the adipose tissue of the animal is markedly decreased. A fall in activity from 32·1 to 17·2 μmoles of FFA released/hr./g. fresh wt. of adipose tissue, which is significant ($P < 0{\cdot}001$), occurs as the mean plasma TGFA concentration rises from 4·3 to 13·7 μequiv./ml. between the estimated sixteenth and twentieth days of gestation. Clearing-factor lipase activity in adipose tissue continues to fall from the twentieth day onwards and after parturition it is significantly less (P 0·01–0·02) than it is at the twentieth day.

The decline in clearing-factor lipase activity noted above appears to be restricted to the enzyme in adipose tissue. Thus the mean (± S.D.) clearing-factor lipase activities (μmoles of FFA released/hr./g. fresh wt. of tissue) of heart, lung and diaphragm muscle, in a group of six pregnant rats with an elevated mean (± S.D.) plasma TGFA concentration of 11·2 ± 3·4 μequiv./ml., were respectively 171 ± 25, 18·9 ± 3·8 and 48·2 ± 7·3, as compared with activities of 172 ± 23, 15·8 ± 0·5 and 49·0 ± 4·8 in a control group of six non-pregnant animals.

Table 1. *Adipose-tissue clearing-factor lipase activity during pregnancy and after parturition*

The stage of gestation in each group of pregnant animals is estimated from the mean weight of the foetuses by using the Tables given by Stotsenburg (1915). The range of the weights of the foetuses in each group is given in parentheses. The mean (± S.D.) plasma TGFA concentration is given for certain of the groups of rats. Measurements were made on plasma samples obtained immediately before the adipose tissue was taken for clearing-factor lipase assay. Values for clearing-factor lipase activity are expressed as means, ± S.D. where appropriate.

Group	No. of animals	State	Mean wt. of foetuses (g.)	Estimated stage of gestation (days)	Mean no. of foetuses/rat	Concn. of TGFA in plasma (μequiv./ml.)	Clearing-factor lipase activity (μmoles of FFA released/hr./g. fresh wt.)
A	7	Pregnant	0·07 (0·04–0·09)	13–14	11	—	28·8 ± 6·8
B	8	Pregnant	0·22 (0·12–0·48)	15–17	10	4·3 ± 2·6	32·1 ± 7·0
C	13	Pregnant	1·1 (0·7–1·4)	18–19	10	9·4 ± 4·0	27·2 ± 6·9
D	14	Pregnant	2·7 (1·9–3·8)	20	10	13·7 ± 5·8	17·2 ± 5·8
E	3	Pregnant	4·7 (3·9–5·1)	21–22	9	5·0	12·4
F	6	2–3 days post-parturition	—	—	—	—	10·1 ± 2·1
G	6	Non-pregnant	—	—	—	—	30·0 ± 8·4

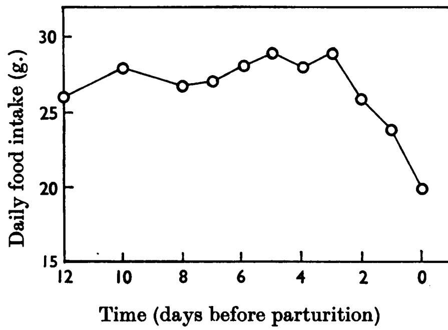

Fig. 3. Food intake during pregnancy in the rat; mean dietary intake of 10 rats fed on the low-fat high-carbohydrate diet.

Food consumption during pregnancy. The results in Fig. 3 show that the daily food intake during pregnancy is not significantly increased in rats on a low-fat diet at the time when the plasma TGFA concentration rises to a maximum, 2–4 days before parturition. Similar findings were reported by Scow *et al.* (1964) for animals on a normal laboratory diet.

The decline in food consumption before parturition was also reported by Scow *et al.* (1964) for animals on their normal diet. It clearly could be a factor concerned in the fall in the clearing-factor lipase activity of adipose tissue that occurs at this time, since the activity of the enzyme in this tissue is known to be influenced by the nutritional state (see the Discussion section). The rate of TFGA accumulation in the plasma after the injection of Triton (μequiv. of TGFA/ml. of plasma/hr.) and the estimated rate of TGFA entry into the plasma, expressed as μequiv./hr./200g. body wt., which can be calculated from this information, do not change significantly during pregnancy, though, as the plasma volume rises with the increase in body weight, the rate of TGFA entry expressed as μequiv./hr./rat does increase progressively (Table 2).

Scow *et al.* (1964) showed that, though the liver weight increased during pregnancy, there was no change in the total liver TGFA content. The results in Table 3 show that the total liver TGFA content in animals that were injected with Triton 90min. previously is also not significantly changed during pregnancy, though the concentration of TGFA falls as the liver weight increases. Plasma TGFA may be expected to have contributed to the liver TGFA in these experiments, but, on the basis of the results reported by Otway & Robinson (1967*b*), this contribution is unlikely to have been more than 10% of the total liver TGFA.

Table 2. *Triglyceride accumulation in the plasma after Triton injection in pregnant rats*

The rates of TGFA accumulation are derived from the plasma TGFA concentrations before and 90min. after Triton was injected, assuming linear rates of TGFA increase (Otway & Robinson, 1967*b*). Plasma volumes are determined by the dilution of an injected dye. The stage of gestation in each group of pregnant animals is estimated from the mean weight of the foetuses by using the Tables given by Stotsenburg (1915). The number of foetuses/rat varied between 8 and 13. Values are expressed as the mean, ±S.D. where appropriate.

Estimated stage of gestation (days)	Body wt. (g.) (no. of rats in parentheses)	Mean wt. of foetuses (g.)	Plasma volume (ml.)	Concn. of TGFA in plasma (μequiv./ml.)		Rate of accumulation of TGFA (μequiv./ml. of plasma/hr.)	Estimated rate of entry of TGFA into plasma	
				Initial	90min. after Triton injection		(μequiv./hr./rat)	(μequiv./hr./200g. body wt.)
Non-pregnant	211±4 (7)	—	8·97±1·04	2·90±0·81	28·49±3·25	16·9±1·8	152	145
10–15	244±15 (14)	<0·15	10·86±2·93	3·71±1·28	27·80±4·29	15·9±2·3	174	144
16–18	271±6 (8)	0·45±0·22	12·11±1·21	8·41±4·39	31·10±6·29	15·1±3·5	182	134
19–20	295±7 (8)	1·96±0·54	14·75±0·41	11·21±3·30	34·28±6·01	15·3±2·5	225	152
21–22	306±9 (4)	4·26±0·77	13·94±0·32	4·15±1·02	26·71±2·76	15·1±1·7	209	136

Table 3. *Liver triglyceride concentrations in pregnant rats injected with Triton*

Experimental groups were as in Table 2. Results are expressed as the means ± S.D. The concn. of TGFA (μequiv./g. of liver) at 19–20 days gestation is significantly lower than those in non-pregnant animals ($P<0.05$) and in animals at 10–15 days gestation ($P<0.01$).

Estimated stage of gestation (see Table 2) (days)	No. of rats	Liver wt. (g.)	Concn. of TGFA (μequiv./liver)	Concn. of TGFA (μequiv./g. of liver)
Non-pregnant	7	8·5±1·2	340±10	40±11
10–15	14	11·0±0·7	400±48	37±6
16–18	8	11·8±0·5	354±77	31±7
19–20	8	13·3±1·4	395±70	30±3
21–22	4	12·2±1·2	340±40	28±1

DISCUSSION

The clearing-factor lipase activity of rat adipose tissue alters markedly with changes in physiological or pathological state. Thus it is high in the fed animal but low in the starved one (Hollenberg, 1959; Cherkes & Gordon, 1959; Robinson, 1960; Páv & Wenkeová, 1960; Salaman & Robinson, 1961; Wing & Robinson, 1968), and it is also low in the diabetic animal (Páv & Wenkeová, 1960; Schnatz & Williams, 1963; Brown, 1967). The activity of the enzyme in adipose tissue in such conditions can be directly correlated with TGFA uptake by the tissue (Bragdon & Gordon, 1958; Havel, Felts & Van Duyne, 1962; Bezman, Felts & Havel, 1962; Brown & Olivecrona, 1966; Brown, 1967). This finding is consistent with the view that the removal of TGFA from the blood by particular extrahepatic tissues depends on their hydrolysis to FFA by the clearing-factor lipase in these tissues (see Robinson, 1963*a*).

In the fed animal, it seems likely that a considerable proportion of the circulating TGFA is taken up and stored in adipose tissue (Bragdon & Gordon, 1958; Havel *et al.* 1962; Brown & Olivecrona, 1966). In such animals therefore the clearing-factor lipase activity of this tissue may play an important part in determining the overall rate of TGFA removal from the blood. If this is so then a marked fall in the activity of the enzyme in adipose tissue, such as has been reported here, would be expected to cause a fall in the rate of TGFA removal. It could, therefore, provide an explanation for the appearance of the pregnancy lipaemia, provided that there was no compensatory increase in the activity of the enzyme in other tissues; no such increase has been found to occur in heart, lung or diaphragm.

Meng & McGanity (1958) and Sandhofer, Sailer, Braunsteiner & Braitenberg (1961) have reported that the amount of clearing-factor lipase released into the circulation after injection of heparin is decreased in pregnant women at the time when their plasma lipid concentration is raised. Although activity of the enzyme in plasma after injection of heparin may not always reflect tissue activity (Robinson, 1963*a*), these observations are consistent with the explanation that has been suggested for the pregnancy lipaemia.

Disappearance of the lipaemia shortly before parturition may also be explained by changes in tissue clearing-factor lipase activity. The activity of the enzyme rises markedly in the mammary gland of the guinea pig (McBride & Korn, 1963; Robinson, 1963*b*) and of the rat (D. S. Robinson, unpublished work) 1–2 days before parturition, and during lactation large quantities of TGFA are taken up from the blood by this organ (Barry, Bartley, Linzell & Robinson, 1963; McBride & Korn, 1964; Glascock *et al.* 1966; Annison, Linzell, Fazackerley & Nichols, 1967). Thus the increase in enzyme activity in the mammary gland, leading to a rise in the rate of removal of TGFA from the blood, may account for the disappearance of the lipaemia.

Although rates of TGFA removal can be determined from the rates of disappearance of radioactivity after the injection of labelled TGFA, such measurements have not been made in this study for two reasons. First, the results are difficult to interpret when the plasma TGFA pool is varying in size (Nestel, 1964; Brown, 1967), and, secondly, any changes in the rate of TGFA removal are likely to be small. Thus the total accumulation of TGFA in the blood over a period of several days during pregnancy is less than 200 μequiv., whereas the rates of removal of TGFA from the blood will be equivalent to the rates of influx and therefore of the order of 200 μequiv./hr. (Table 2). Clearly, only a small change in rate would be needed to produce the observed increase in TGFA concentration.

The foregoing explanation for the lipaemia of pregnancy is based on the assumption that the rates of influx of TGFA into the plasma are unchanged when the lipaemia appears and disappears. Studies to determine whether this is so have been carried out in the present work but the findings are not easily interpreted. When the influx rates are expressed per animal they increase progressively

during pregnancy until shortly before parturition (Table 2). However, the body weight, the liver weight, the food intake and the plasma volume all show similar patterns of increase (Scow *et al.* 1964 and the present study). The question then is whether the progressive increase in TGFA influx/animal is accompanied by a corresponding increase in the capacity of the extrahepatic tissues to take up the TGFA from the blood, until the time of the lipaemia when this capacity is lowered by the fall in adipose-tissue clearing-factor lipase activity. Expression of the influx rates per constant unit of body weight might be expected to eliminate the difficulty of the increase in body weight, and expressed in this way the rates are relatively constant throughout. However, in the earlier stages of pregnancy most of the increase in weight is due to proliferation of the maternal tissues, whereas in the later stages the increase in the weight of the foetuses with their associated membranes and fluids becomes increasingly significant. It is difficult to know to what extent these changes should be taken into account.

In summary, though an explanation for the lipaemia of pregnancy in terms of changes in the pattern of clearing-factor lipase activity in adipose tissue and mammary gland remains attractive, the possibility that changes in the rates of TGFA entry into the plasma may also be involved cannot be excluded.

REFERENCES

Annison, E. F., Linzell, J. L., Fazackerley, S. & Nichols, B. W. (1967). *Biochem. J.* **102**, 637.

Barry, J. M., Bartley, W., Linzell, J. L. & Robinson, D. S. (1963). *Biochem. J.* **89**, 6.

Bezman, A., Felts, J. M. & Havel, R. J. (1962). *J. Lipid Res.* **3**, 427.

Bragdon, J. H. & Gordon, R. S. (1958). *J. clin. Invest.* **37**, 574.

Brown, D. F. (1967). *Diabetes*, **16**, 90.

Brown, D. P. & Olivecrona, T. (1966). *Acta physiol. scand.* **66**, 9.

Cherkes, A. & Gordon, R. S. (1959). *J. Lipid. Res.* **1**, 97.

Dole, V. P. & Meinertz, H. (1960). *J. biol. Chem.* **235**, 2595.

French, J. E., Robinson, D. S. & Florey, H. W. (1953). *Quart. J. exp. Physiol.* **38**, 101.

Glascock, R. F., Welch, V. A., Bishop, C., Davies, T., Wright, E. W. & Noble, R. C. (1966). *Biochem. J.* **98**, 149.

Havel, R. J., Felts, J. M. & Van Duyne, C. M. (1962). *J. Lipid Res.* **3**, 297.

Hollenberg, C. H. (1959). *Amer. J. Physiol.* **197**, 667.

McBride, O. W. & Korn, E. D. (1963). *J. Lipid Res.* **4**, 17.

McBride, O. W. & Korn, E. D. (1964). *J. Lipid Res.* **5**, 453.

Meng, H. C. & McGanity, W. J. (1958). *Fed. Proc.* **17**, 110.

Nestel, P. J. (1964). *J. clin. Invest.* **43**, 943.

Otway, S. & Robinson, D. S. (1966). *Biochem. J.* **100**, 50P.

Otway, S. & Robinson, D. S. (1967*a*). *J. Physiol.* **190**, 309.

Otway, S. & Robinson, D. S. (1967*b*). *J. Physiol.* **190**, 321.

Páv. J. & Wenkeová, J. (1960). *Nature, Lond.*, **185**, 926.

Robinson, D. S. (1960). *J. Lipid Res.* **1**, 332.

Robinson, D. S. (1963*a*). *Advanc. Lipid Res.* **1**, 133.

Robinson, D. S. (1963*b*). *J. Lipid Res.* **4**, 21.

Robinson, D. S. (1964). In *Metabolism and Physiological Significance of Lipids*, p. 275. Ed. by Dawson, R. M. C. & Rhodes, D. N. London: J. Wiley and Sons (Inc.) Ltd.

Salaman, M. R. & Robinson, D. S. (1961). In *Enzymes of Lipid Metabolism*, p. 218. Ed. by Desnuelle, P. Oxford: Pergamon Press Ltd.

Salaman, M. R. & Robinson, D. S. (1966). *Biochem. J.* **99**, 640.

Sandhofer, F., Sailer, S., Braunsteiner, H. & Braitenberg, H. (1961). *Wien. klin. Wschr.* **73**, 392.

Schnatz, J. D. & Williams, R. H. (1963). *Diabetes*, **12**, 174.

Scow, R. O., Chernick, S. S. & Brinley, M. S. (1964). *Amer. J. Physiol.* **206**, 796.

Stotsenburg, J. M. (1915). *Anat. Rec.* **9**, 667.

Wing, D. R. & Robinson, D. S. (1968). *Biochem. J.* **106**, 667.