showed that an average recovery of 80% was obtained under these conditions of analysis. Determinations were also performed of the squalene content of human sebum, obtained from the forearm by the method of MacKenna *et al.* (1950) and from the back by the swab method (cf. Rothman, 1950; carbon tetrachloride was used in place of ether), ear wax and ovarian dermoid cyst lipid. The results are shown in Table 2.

Table 2. *Squalene content of sebum and other materials*

Substance	No. of samples	Squalene (%)	
		Range	Average
Sebum (forearm)	8	3.3-8.7	5.5
Sebum (back)	18	5.4-11.7	8.4
Ear wax	7	1.1-12.0	3.9
Dermoid cyst lipid	2	3.7, 10.2	—

The method therefore appears to be satisfactory when applied to human sebum and similar materials known to contain squalene. It will doubtless find application to other tissue lipids, but it must be stressed that the presence of squalene in the material must first be established by chemical isolation

before the method is applied, and that other unsaturated hydrocarbons must be shown to be absent.

SUMMARY

1. A method of estimating unsaturated hydrocarbons is described which is applicable to small amounts (5 mg.) of sebum and similar materials, and which gives an average recovery of 80% of added squalene. After saponification, a hydrocarbon fraction is isolated chromatographically and the unsaturated material therein is determined iodometrically.

2. The method may be used for the determination of squalene in sebum and other lipids provided that the presence of squalene is first established and that no other unsaturated hydrocarbons are present.

3. Data on the Liebermann-Burchard reaction of squalene are recorded.

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A Note on the Influence of Energy Intake on Phospholipid Metabolism

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In a previous series of experiments (Munro, Naismith & Wikramanayake, 1953), it was observed that the amount of ribonucleic acid in the liver was largely determined by the amount of liver protein, but that the rate of synthesis of ribonucleic acid, as judged by ³²P uptake, was determined by

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energy intake. Campbell & Kosterlitz (1948, 1952) have demonstrated certain similarities between the metabolism of ribonucleic acid and phospholipid when rats are transferred from a protein-containing diet to a protein-free diet. In view of our findings with ribonucleic acid it was accordingly thought of interest to examine the influence of energy intake on phospholipid metabolism in the liver.

METHODS

Energy intake and the quantity of phospholipid in the liver. Young adult rats were studied at different energy levels on diets either containing protein or free from protein. These were the same animals employed in investigating the relationship of protein metabolism and ribonucleic acid metabolism to energy intake (Munro & Naismith, 1953; Munro *et al.* 1953). Full details of the selection of animals, the diets used and the methods of liver analysis employed are given in the first of these papers.

Energy intake and the uptake of ^{32}P by liver phospholipids. Rats having an initial body weight close to 180 g. were used. Variations in energy intake were produced by altering the amount of carbohydrate in protein-containing or protein-free diets. The rats were killed under ether anaesthesia at 2, 5 or 8 hr. after injection of ^{32}P ($10\text{ }\mu\text{C}$ inorganic phosphate/100 g. body weight). The livers were briefly perfused with 0.9% (w/v) NaCl, disintegrated in 9 vol. ice-cold water, and trichloroacetic acid added to give a final concentration of 10% (w/v). After two washings with 10% trichloroacetic acid the precipitate was successively extracted with 20 ml. portions of cold absolute ethanol, ethanol: CHCl_3 (3:1) and twice with ethanol:ether (3:1), extraction with the last two solvents being carried out at 65–70° in a water bath for 10 min. Finally, the precipitate was extracted with ether. The phosphorus content of the combined extracts was measured by the Allen (1940) method, and the radioactivity of the final blue solution estimated in a liquid counter (Yeall, 1948). The method of extracting phospholipids is essentially that used by Campbell & Kosterlitz (1952), with the addition of ethanol: CHCl_3 as one of the solvents. The efficiency of our procedure is indicated by the finding that further treatment of the precipitate with a mixture of CHCl_3 , ethanol and ether (1:3:1) for 10 min. at 60° removed only traces of phosphorus.

In order to purify the extract further for radioactivity determinations, the crude lipid extract was first evaporated to dryness at room temperature and then the residue was taken up in light petroleum. This was washed once with 0.05N-HCl and three times with water (Popják & Muir, 1950). After it had been found that this purification procedure altered the specific activity of the lipid phosphorus by only 0.5%, it was abandoned as a routine procedure.

Presumably the initial washings with trichloroacetic acid had successfully removed radioactive contaminants which might otherwise have found their way into the lipid extracts.

The specific activity of the inorganic phosphate of the liver was obtained from the trichloroacetic acid-soluble fraction by precipitation as $\text{Mg}(\text{NH}_4)\text{PO}_4$ (Davidson, Frazer & Hutchison, 1951), and also by immediate extraction with isobutanol (Ennor & Stocken, 1950). In contrast to the findings of Ennor & Rosenberg (1952), the $\text{Mg}(\text{NH}_4)\text{PO}_4$ procedure did not give higher specific activities, presumably because no carrier phosphate was used.

The results have been expressed as relative specific activities (specific activity of lipid P divided by specific activity of liver inorganic phosphate and multiplied by 100), and as 'total relative activities' (the amount of phospholipid P per liver multiplied by its relative specific activity, Campbell, Olley & Blewett, 1949).

RESULTS

Energy intake and the quantity of phospholipid in the liver

When the diet provided adequate amounts of protein, the quantity of phospholipid in the liver varied considerably with energy intake (Fig. 1). The regression coefficients were +2.51 mg. phospholipid phosphorus/1000 kcal. in the case of additions of carbohydrate and +2.57 mg./1000 kcal. in the case of fat, both being statistically highly significant ($P < 0.01$). On the other hand, neither the addition of carbohydrate nor of fat significantly increased the amount of phospholipid per liver when the rats were receiving the protein-free diet (regression coefficients of +0.01 and +0.02 mg. P/1000 kcal. respectively were obtained). Since these first four experiments were not carried out at the same time, a further study was performed in which groups of rats on protein-containing and on protein-free diets were studied simultaneously. These confirmed that increments in energy intake from carbohydrate only raise liver phospholipid content when the diet contains protein (Table 1).

Table 1. *The influence of variations in energy intake (carbohydrate) on the amount of phospholipid in the liver at different levels of protein intake, and the relationship of these changes to alterations in the amount of protein and ribonucleic acid in the liver*

(Expt. 5. Seven rats per group.)

Diet	Daily energy intake (kcal./sq.m.)	Phospholipid P/liver (mg.)	Phospholipid P/100 mg. protein N (mg.)	Ribonucleic acid P/100 mg. protein N (mg.)	Ribonucleic acid P/100 mg. phospholipid P (mg.)
Protein-containing	820	8.29	4.29	3.32	77.5
	1570	9.91	4.59	3.51	76.7
Protein-free	880	6.80	4.76	3.64	76.7
	1650	6.45	4.86	4.06	83.7
Statistical analysis*					
Protein level		+	+	+	+
Energy level		+	+	+	+
Interaction†		+	–	+	+

* A '+' sign indicates a probability of less than 0.01, and a '–' sign indicates a probability greater than 0.05.

† Significant interaction implies that a change in energy intake had a different effect at the two protein levels.

Although the response of liver phospholipid to changes in energy intake is influenced by the protein content of the diet in much the same way as the responses of liver protein and ribonucleic acid are affected (Munro & Naismith, 1953; Munro *et al.* 1953) there are small differences, notably in the case of addition of carbohydrate to the protein-free diet, which tends to raise the amount of ribonucleic acid per liver slightly, whereas protein and phospholipid are unaffected or even slightly reduced in amount. This is confirmed by computing the relationships between the amounts of protein, ribonucleic acid and phospholipid (Table 1).

Energy intake and uptake of ^{32}P

Table 2 shows the amounts of ^{32}P incorporated into liver phospholipid at 2, 5 and 8 hr. after injection of labelled phosphorus. The relative specific activity rose with increasing energy intake, and there was no significant difference in this respect between the protein-containing and protein-free diets. However, relative specific activity only expresses the amount of radioactivity/100 μg . P. When the relative specific activity is multiplied by the amount of phospholipid in the liver, we obtain a measure of the total number of phosphorus atoms incorporated into phospholipid per liver in a given time ('total relative activity'). In the case of the protein-containing diet, the total relative activity showed a 34% increase per 1000 kcal. added to the diet, whereas there was no significant increment in the case of the protein-free diet. The large change in the protein-containing diet was due to a considerable increase in the amount of phospholipid per liver brought about by the augmented energy intake, as well as by the rise in relative specific activity. In the case of the protein-free diet, the increase in relative specific activity was offset by a diminution in the total amount of phospholipid as energy intake was raised.

DISCUSSION

In agreement with previous authors (Kosterlitz, 1947; Thomson, Heagy, Hutchison & Davidson, 1953), the administration of a protein-free diet was observed to reduce the amount of phospholipid in the liver. In addition, the amount rose in response to increments in energy intake when the diet provided an adequate amount of protein, but failed to do so when the diet was devoid of protein (Fig. 1; Tables 1 and 2). The dietary level of protein also affects the response of liver protein and ribonucleic acid to changes in energy intake (Munro & Naismith, 1953; Munro *et al.* 1953). There are, however, small differences in the responses of these liver constituents to dietary conditions which result in changes in the ratio of one constituent to another as the

dietary protein and energy levels are varied (Table 1). The change observed in the ribonucleic acid/protein ratio is in agreement with the findings of Campbell & Kosterlitz (1950), but these authors found no change in the ratio of phospholipid to protein when protein intake was varied.

Fig. 1. Changes in the amount of phospholipid in the liver produced by varying energy intake from carbohydrate (●—●) or from fat (○---○). The upper two regression lines were obtained with diets containing protein, the lower two with protein-free diets. Each point is the mean of observations on four or five rats.

The influence of energy intake on the total amount of ^{32}P incorporated into liver phospholipid in a given time (total relative activity) was also found to depend on the level of protein in the diet (Table 2). In the case of the protein-containing diet there was an increase in the total amount incorporated per liver as energy intake rose, but in the case of the protein-free diet increments in energy intake had no significant action. In this respect the incorporation of ^{32}P into liver phospholipids differs from its incorporation into liver ribonucleic acid, the latter being dependent on level of energy intake irrespective of the amount of protein in the diet

Table 2. *The effect of variations in energy intake on the incorporation of ^{32}P into the phospholipids of the liver*

(Three rats per observation. The changes in relative specific activity and in total relative activity brought about by increasing the intake of energy have been given as the change per 1000 kcal. added to the diet. In each case, the change has been expressed as a percentage of the activity corresponding to 1200 kcal./sq.m. body surface area.)

Diet	Time after ^{32}P injection (hr.)	Daily energy intake (kcal./sq.m.)	Phospholipid P/liver (mg.)	Relative specific activity	Total relative activity
Protein-containing	2	789	6.61	14.4	0.95
		1656	8.23	15.6	1.28
	5	781	7.60	32.0	2.41
		1671	7.92	40.2	3.18
	8	779	5.88	56.5	3.31
		1659	7.49	60.4	4.52
	Mean	783	6.70	+ 15 %	+ 34 %
		1662	7.88		
Protein-free	2	802	4.86	15.0	0.74
		1767	4.07	17.2	0.70
	5	827	4.74	45.0	2.10
		1831	4.01	56.7	2.26
	8	819	4.06	67.9	2.79
		1803	4.05	80.7	3.27
	Mean	816	4.55	+ 18 %	+ 6 %
		1800	4.04		

Statistical analysis shows that increments in energy intake produced highly significant increases in relative specific activity ($P < 0.01$) on both the protein-containing and protein-free diets; the effect was similar on both diets ($P > 0.05$). A highly significant change in total relative activity occurred with addition of energy to the protein-containing diet ($P < 0.01$) but not in the case of the protein-free diet ($P > 0.05$). This difference in the effect of energy intake on the two diets was highly significant ($P < 0.01$).

(Munro *et al.* 1953). Campbell & Kosterlitz (1948, 1952) have also noted a difference in the effect of protein deprivation on the rates of synthesis of phospholipid and ribonucleic acid by the liver. Rats fed a protein-free diet showed an immediate loss of phospholipid and ribonucleic acid accompanied at first by a compensatory increase in turnover of the remainder, with the result that there was the same total amount synthesized per liver on the protein-free diet as on a protein-containing diet. They concluded that the total turnover of the liver phospholipids and ribonucleic acid was determined by the metabolic needs of the liver and was independent of the amount of each present. In the case of ribonucleic acid this rate of synthesis was maintained over a prolonged period of protein deficiency, but after a few days the rate of phospholipid synthesis declined. Campbell & Kosterlitz (1952) ascribe this to a reduction in metabolic rate, which does not, however, explain why the rate of incorporation into ribonucleic acid continued to be maintained at the same level throughout the period of protein deficiency. It seems more likely that the feeding of a protein-free diet eventually reduces the concentration of some essential component in phospholipid synthesis to such an extent that it becomes the limiting factor in the rate of synthesis. This would

explain why the addition of energy to a protein-free diet fails to stimulate a greater total turnover of phospholipid. The limiting factor is not likely to be choline, since the diets used in our isotope incorporation experiments provided 40 mg. of choline/rat/day.

A similar explanation has been advanced by us to account for the observation that energy intake influences the protein metabolism of rats receiving protein-containing diets but not of rats receiving protein-free diets (Munro & Naismith, 1953). There appears to be no such factor limiting the response of ribonucleic acid metabolism to changes in energy level of a protein-free diet (Munro *et al.* 1953). We have recently demonstrated that the stimulation of ribonucleic acid synthesis produced by raising energy intake can be prevented by increasing energy expenditure to a corresponding extent (Munro, Naismith & Wikramanayake, unpublished results). It is thus not the high energy intake itself but the accompanying increase in energy-yielding nutrients in the tissues which stimulates ribonucleic acid synthesis. A similar conclusion has been drawn regarding the relationship between dietary energy intake and protein metabolism (Munro, 1951).

As a hypothesis consistent with our observations on the metabolism of phospholipid, protein and

ribonucleic acid in the liver, we may picture the amount of energy available as the factor determining their rates of synthesis, provided no other component in the synthetic mechanism limits the response to a change in available energy.

Such a relationship is capable of explaining the findings of Campbell & Kosterlitz (1949) that the total turnover of ribonucleic acid and phospholipid in the liver of the rat increases during pregnancy, since, in the rat, energy intake exceeds requirements during pregnancy (Morrison, private communication), and on the above hypothesis this would be expected to lead to such a change in the rates of synthesis of ribonucleic acid and phospholipid.

SUMMARY

1. A study has been made of the effect of variations in energy intake on the amount of phospholipid in the rat liver and its uptake of labelled phosphorus.

2. When the diet provided an adequate supply of protein, the amount of phospholipid in the liver rose in response to increments in energy intake, whereas it failed to do so when the diet was devoid of protein.

3. The total turnover rate of phospholipid was influenced by energy intake only when the diet contained protein. It is suggested that the rate of phospholipid synthesis on protein-free diets is unaffected by energy intake within the range studied because some other component in synthesis is reduced to a level at which it becomes the factor determining the rate of synthesis.

4. The relationship of energy balance to the rates of synthesis of protein, phospholipid and ribonucleic acid is discussed.

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Sedimentation Coefficients of Small Molecules: Methods of Measurement Based on the Refractive-Index Gradient Curve. The Sedimentation Coefficient of Polyglucose A

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Svedberg & Pedersen (1940, p. 283) and Gutfreund & Ogston (1949) have proposed methods for measuring the sedimentation coefficient,* s , when a substance sediments too slowly for s to be measured in the usual way, from the movement of the maximum

* The term 'sedimentation coefficient' instead of 'sedimentation constant' has been introduced to indicate that it is not a constant except under specified conditions.

gradient of refractive index. In the case of slowly-sedimenting molecules, the boundary remains sufficiently close to the meniscus that the shape of the boundary curves must be predicted for the case of restricted rather than free diffusion. Boundary curves like those of Fig. 2 result, in which the maximum gradient of refractive index may remain at the meniscus throughout the centrifuge run.