Dent, C. E. (1947). *Biochem. J.* **41**, 240.
Dent, C. E. (1948). *Biochem. J.* **43**, 169.
Fischer, A. & Astrup, T. (1942). *Pflüg. Arch. ges. Physiol.* **245**, 41.
Fischer, A., Astrup, T., Ehrensvärd, G. & Øhlenschläger, V. (1948). *Proc. Soc. exp. Biol., N.Y.*, **67**, 40.
Fuller, A. T., Neuberger, A. & Webster, T. A. (1947). *Biochem. J.* **41**, 11.
Gordon, A. H. (1948). *Nature, Lond.*, **162**, 778.
Greenstein, J. P. & Leuthardt, F. M. (1946). *J. nat. Cancer Inst.* **6**, 203.
Kuttner, T. & Lichtenstein, L. (1932). *J. biol. Chem.* **95**, 661.
Needham, J. (1931). *Chemical Embryology*, 1st ed. Cambridge University Press.
Outhouse, E. L. (1937). *Biochem. J.* **31**, 1459.
Pratt, J. J. & Auclair, J. L. (1948). *Science*, **108**, 213.
Ussing, H. H. (1943). *Acta physiol. Scand.* **6**, 222.
Van Slyke, D. D. (1913). *J. biol. Chem.* **16**, 187.
Van Slyke, D. D., Hiller, A., MacFadyen, D. A., Hastings, A. B. & Klemperer, F. W. (1940). *J. biol. Chem.* **133**, 287.

The Effect of Dietary Oleic and Palmitic Acids on the Composition and Turnover Rates of Liver Phospholipins

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Ozaki (1927) found that rats fed on diets containing 20% oleic acid gained weight, whereas rats on diets containing similar amounts of palmitic or stearic acids lost weight. These weight changes indicate differences in the metabolism of oleic acid as compared with that of the saturated fatty acids. Channon & Wilkinson (1936) have shown that on low-protein, high-fat diets, lipid accumulation in the liver of the rat varies directly with the proportion of saturated fatty acids in the dietary fat; their fatty liver-producing diets were deficient in choline and accumulation of fat was prevented by choline. It was suggested that choline was involved in the desaturation of fatty acids. The differences in accumulation of fat may have been due to different phospholipin turnover rates determined by the varying supply of saturated fatty acids.

In the experiments reported in this paper low-protein diets containing pure palmitic and oleic acids were fed to rats in such a way as to eliminate effects due to different food intakes or absorption coefficients of the fatty acids. An attempt was made to correlate the different lipid accumulations with differences in liver phospholipin turnover rates.

METHODS

Male albino rats of the Wistar strain were used. All animals were bred by sister-brother matings from a single pair. The starting weight chosen was 150 g. as younger animals showed excessive weight loss, and some died on the palmitic acid diet described in Table 1. The complication of an additional requirement for lipotropic factors for growth was also more likely to be avoided at this weight.

In a preliminary experiment to determine the percentage absorption of pure oleic and palmitic acids when fed at a level of 27%, it was found that 70% of the ingested palmitic acid, and 90% of the oleic acid were absorbed. The proportions of fatty acid and cellulose powder in the two diets were therefore adjusted so that for both groups of animals the amounts of fat absorbed and the values of the diets as sources of energy were the same.

Table 1. *Composition of fatty acid diets*

	Oleic acid diet	Palmitic acid diet
Casein (Labco) (%)*	8	8
Fatty acid (%)	21	27
Glycerol (%)	3	3
Sucrose (%)	50	50
Yeast (%)	5	5
Salts (%)†	5	5
Cellulose powder (%)‡	8	2

* When choline supplements were given the casein was sprayed with a solution of choline chloride to give 30 mg. choline/10 g. diet.

† NaCl, 22 g.; $CaHPO_4.H_2O$, 130 g.; potassium citrate, 125 g.; $MgSO_4.7H_2O$, 30 g.; ferric citrate, 5 g.; 0·7 g. of trace mixture (KI, 12 g.; NaF, 10 g.; $MnSO_4$, 2 g. Cu_2I_2, 1 g.; $KAl(SO_4)_2$, 1 g.; $ZnSO_4.7H_2O$, 1 g. de Loureiro, 1931).

‡ Grade A, 100 mesh 'solka floc'; a highly purified cellulose powder obtainable from Johnsen, Jorgensen and Wettre Ltd., London.

Animals were starved for 24 hr. and then placed on the diets for 12 days. The palmitic acid diet was not eaten so readily as the oleic acid diet and therefore approximate pair feeding between litter mates was adopted. Controls were fed *ad lib.* on a stock diet consisting of skimmed milk powder, 20%; ground yellow maize, 18%; rolled oats, 15%; barley meal, 15%; biscuit meal, 15%; wheat germ, 5%; palm kernel oil, 5%; dried yeast, 5% and bran, 2%. Each animal received 10 μc. of ^{32}P as $Na_2H^{32}PO_4$ in distilled water by intraperitoneal injection, and was killed by decapitation 4 hr. later. In order to reduce the effect of recently ingested fat on the specific activities of liver phospholipins, food and water were removed from the animals 4 hr. before the injection of ^{32}P. (Specific activity (SA) = % injected ^{32}P/mg. phosphorus.)

Livers were homogenized with anhydrous Na_2SO_4 and extracted twice with ethanol and three times with 2:1 ethanol-ether in a small blender and re-extracted with light petroleum. Phospholipins were precipitated with $MgCl_2$ and acetone. Amounts of phospholipins were estimated by multiplication of the amounts of phosphorus by 22·7 (Artom & Fishman, 1943*a*). Inorganic phosphate was extracted from the livers by the method of Kaplan & Greenberg (1944*a*, *b*). Choline-containing phospholipins were separated by the method of Taurog, Entenman, Fries & Chaikoff (1944) and phosphorus was estimated by reduction of phosphomolybdate with sulphite-quinol. The percentage choline-containing phospholipin was obtained from the ratio of eluted to total phosphorus. Iodine numbers were estimated by Yasuda's (1931) method and cholesterol and cholesterol esters by the method of Popják (1943).

Radioactivities were determined with a G.T. 11 type G.M. tube arranged as an immersion counter, with Neher-Harper extinction and feeding into a Type 200 scaling unit.

All analyses were carried out in duplicate.

RESULTS

Weight loss and lipid accumulation

Animals pair-fed on the oleic and palmitic acid diets lost weight in both groups. The animals fed on oleic acid lost weight in inverse proportion to the amount of food consumed ($r = -0·791$, $n^1 = 16$, $p = 0·0001$, all statistical symbols are those used by Fisher, 1944), whereas the weight loss in the animals fed palmitic acid was not related to food consumption ($r = -0·012$, $n^1 = 20$). All animals fed palmitic acid had similar weight losses, irrespective of the amount of liver lipid accumulation. Litter mates, fed the diets with and without choline supplementation, lost the same weight.

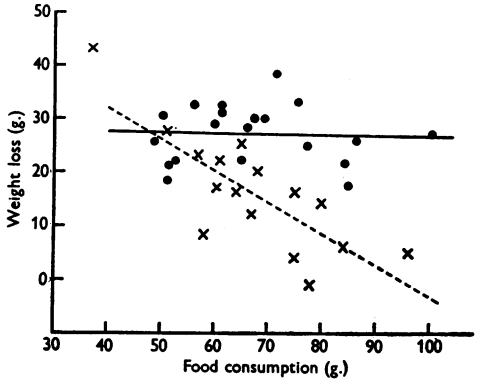

Fig. 1. Relation between total food consumption and loss of body weight for rats fed diets containing oleic and palmitic acids. The lines of regression have significantly different regression coefficients; z (palmitic) $= -0·012$, z (oleic) $= -1·074$; $n^1 = 36$, $p = 0·005$; ● = palmitic acid-fed animals; × = oleic acid-fed animals.

The animals fed palmitic acid drank on an average 25 % less water than those fed oleic acid, but excreted less urine. The difference between water intake and urinary excretion was the same in both groups, and therefore could not have contributed to the weight loss.

Analyses of water and fat contents of eviscerated carcasses and skins were done on six animals from each dietary group. It can be seen from Fig. 2 that the palmitic acid-fed animals lost approximately 10 g. of fat over the 12-day feeding period, independently of the food eaten, whereas the oleic acid-fed animals lost from 0 to 10 g. of fat, depending on the food consumption. The ratio of non-fat solids to water was the same for all groups.

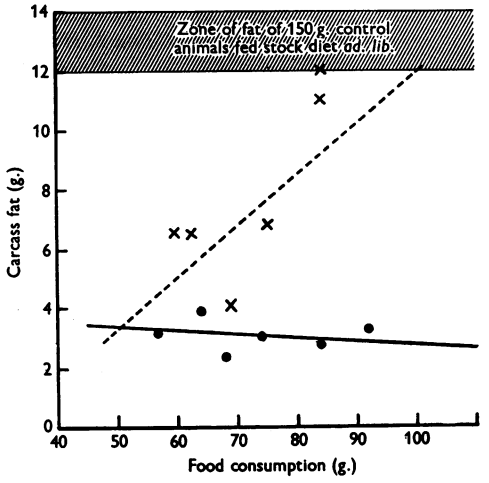

Fig. 2. Relation between total food consumption and the fat content of eviscerated carcasses of rats fed diets containing oleic and palmitic acids. The lines of regression have significantly different regression coefficients; z (palmitic) $= -0·295$; z (oleic) $= +1·83$; $n^1 = 12$, $p = 0·01$; ● = palmitic acid-fed animals; × = oleic acid-fed animals.

Table 2 shows that in the earlier experiments, a large and remarkably constant accumulation of fat in the liver was obtained with palmitic acid feeding. In the oleic acid-fed animals a smaller and more variable amount of fat accumulated. A sudden change occurred in the fifth generation of rats; there was no accumulation of fat in the livers of litter mates of the fifth generation fed diets containing oleic or palmitic acid with or without choline supplements. A single litter among the earlier rats had already shown signs of this tendency.

A choline supplement was only fed to animals of the fifth generation. There is, therefore, no evidence to show that choline would have cleared the lipid accumulations of the animals in the earlier experiments. However, feeding palmitic acid and choline did reduce the percentage of fat in the liver to a level

lower than that of control animals on the stock diet ($t = 4{\cdot}26$, $n^1 = 19$, $p = 0{\cdot}001$). This effect was not found in the animals fed oleic acid and choline.

There was no correlation between the percentage of fat in the liver and weight loss or food consumption in any group.

Average values of iodine numbers of liver triglyceride after 12 days feeding were 75 for palmitic acid-fed animals (irrespective of the extent of lipid accumulation), 110 for oleic acid-fed animals and 130 for control animals.

Fat accumulation was accompanied by proportional increases in ester cholesterol, irrespective of the nature of the dietary fat. A constant relationship between ester cholesterol and the deposition of liver triglyceride has been found by Best, Lucas, Patterson & Ridout (1946).

Table 2. *Litter variations in total liver lipids*

(Values expressed as % fresh weight.)

	No. of animals	Palmitic acid-fed animals	No. of animals	Oleic acid-fed animals
Litters of third and fourth generation	8	20·70 S.E. = 0·590	10	8·72 S.E. = 0·910
Isolated litter of fourth generation	3	11·70 S.E. = 1·23	2	5·95 S.E. = 0·350
Litters of fifth generation	9	6·84 S.E. = 0·530	2	6·20 S.E. = 0·360

S.E. = standard error of mean.

Estimation of rate of turnover of phospholipin phosphorus

The ratio, specific activity of phospholipin phosphorus/specific activity of inorganic phosphate at 4 hr. which is a function of the percentage rate of turnover of the phospholipin molecules, is referred to subsequently as 'relative specific activity' (Hevesy, 1938). The total amount of any metabolite turned over in a given time depends on the number of molecules present and their percentage turnover rate. As a measure of this total turnover rate, the product of the amount of metabolite and its relative specific activity may be employed and is referred to in this paper as 'total relative activity'.

A preliminary experiment was done to assess the true percentage molecular turnover rate of total and choline-containing phospholipin phosphorus in rats of the weight and strain used in this experiment. In attempting to estimate the true rate of renewal of phospholipin phosphorus, other workers have employed a method of constant intravenous perfusion with a solution of radioactive phosphate, with the intention of keeping the specific activity of the inorganic phosphate constant throughout the experiment. Estimates of the turnover rate of phospholipin phosphorus have been based on the relationship between the specific activity time curves of ^{32}P in phospholipins and that of ^{32}P in inorganic phosphate. This method is inconvenient in practice and therefore a method using a single injection of ^{32}P was devised. While the present work was in progress a similar procedure was described by Bollman, Flock & Berkson (1948). Male rats (150 g.) were maintained for 8 days on a diet of 50 % sucrose, 22 % casein, 12 % lard, 5 % yeast, 5 % salt mixture, 5 % cellulose powder and adequate supplements of concentrated vitamins A and D. Vitamin E was not given. These animals received injections of $Na_2H^{32}PO_4$ and were killed at 0·5, 1, 2, 4, 6 and 8 hr. intervals after injection. Food and water were removed from all animals 4 hr. prior to the injection of the first animal. From the measured time curve of specific activity of liver inorganic phosphate, theoretical curves for specific activities of phospholipin phosphorus were obtained for different selected percentage molecular turnover rates by the following procedure. The mean value of specific activity of inorganic phosphate over the first half-hour period, $[SA\ PO_4]_0^{0.5\ hr.}$, was measured from the phosphate activity curve. The assumption was made that freshly synthesized phospholipin molecules containing ^{32}P are as available for degradation as the original non-active phospholipin (Chaikoff, 1942). If the immediate precursor of phospholipin is taken to be in rapid equilibrium with either the inorganic phosphate or the phospholipin, an estimate of the specific activity of the latter at the end of the first half hour $[SA\ PL]_{0.5\ hr.}$ can be obtained from the following equation

$$[SA\ PL]_{0.5\ hr.} = \frac{\{[100 - \Delta PL] \times 0\} + \{\Delta PL \times [SA\ PO_4]_0^{0.5\ hr.}\}}{100},$$

where ΔPL = the percentage of phospholipin phosphorus atoms renewed in 0·5 hr. Then the specific activity at the end of the nth 0·5 hr.

$$[SA\ PL]_n = \frac{\{[100 - \Delta PL] \times [SA\ PL]_{n-1}\} + \{\Delta PL \times [SA\ PO_4]_{n-1}^{n}\}}{100}.$$

A theoretical curve of liver phospholipin specific activity for a selected percentage molecular turnover of 25 % in 4 hr. was found to be the best fit to the practical curve, and is shown together with the latter in Fig. 3. The measured specific activity-time curves for total and choline-containing phospholipins were in close agreement.

If phosphate enters and leaves the molecules at the same rate as the fatty acid components, then a renewal of 25 % of the phosphate in 4 hr. means that 100 mg. of phospholipin is concerned in the metabolism of 97·5 mg. of fatty acid or 108 mg. of

triglyceride/day. Allowing for the normal variation in percentage molecular turnover rates, differences of 30 % in fatty acid metabolism by the phospholipins, i.e. about 30 mg./day, should be detectable.

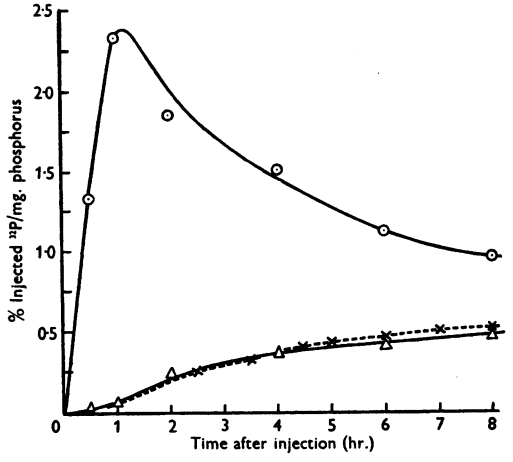

Fig. 3. Specific activity-time curve for liver inorganic phosphate and total phospholipin phosphorus; —⊙— = inorganic phosphate; —△— = total phospholipin phosphorus; -- × -- = theoretical curve for a turnover of 25 % of phospholipin phosphorus in 4 hr. The curve for choline-containing phospholipin phosphorus is not shown as it closely approximated to the curve for total phospholipin.

The effects of diet on phospholipin turnover rates

A significant correlation between the specific activity of liver inorganic phosphate and the amount of lipid/unit of dry defatted liver weight has been found ($r = 0{\cdot}309$, $n^1 = 42$, $p = 0{\cdot}05$). Table 3 shows that this relationship was independent of the type or amount of fat ingested. Specific activities of the liver inorganic phosphate were not correlated with the final body weight, and therefore the differences in specific activity are unlikely to be related to changes in plasma volume.

There were no significant differences in relative specific activities or total relative activities of the phospholipins on an absolute or body weight basis at 4 hr. between any of the dietary groups with or without choline supplements (Table 4). There was less variability in the phospholipin relative specific activities of the animals on low-protein, high-fat diets than was shown by animals on the more mixed stock diet.

The values of specific activities at 4 hr. of choline-containing phospholipins were on the whole close to those for total phospholipin in all groups of animals, suggesting that different fat accumulations were not related to differences in the percentage molecular turnover rates of the phosphorus of the choline and non-choline-containing phospholipin fractions. This is confirmed by the close agreement of the specific activity-time curves of the choline-containing and total phospholipins.

Changes in amounts and composition of phospholipin

From Table 4 it can be seen that the amount of liver phospholipin per unit of final body weight is significantly lower in the animals from the third and fourth generations fed palmitic acid for 12 days than in animals of the same generation fed oleic acid ($t = 2{\cdot}34$, $n^1 = 22$, $p = 0{\cdot}035$) or the control diet ($t = 2{\cdot}65$, $n^1 = 22$, $p = 0{\cdot}017$). There was no significant difference between the third and fourth generation oleic acid-fed animals and the controls. The amount of phospholipin per unit of body weight was not related to the liver lipid accumulation in third and fourth generation animals fed palmitic acid as these did not differ significantly from the fifth generation animals which failed to accumulate liver fat. Supplementation of the diet with choline did not alter this quantity either in palmitic or oleic acid-fed animals of the fifth generation. The ratio of dry defatted liver weight to body weight was the same in all groups. Therefore, there were similar differences in amounts of phospholipin on a dry defatted liver weight basis.

The composition of the liver lipids of those rats in which the phospholipins were separated into choline-containing and non-choline-containing fractions is shown in Table 5.

Table 3. *Liver lipids and specific activity of liver inorganic phosphate*

(4 hr. after injection of ^{32}P.)

Description	No. of animals	Final body wt. (g.)	Fat absorbed in 12 days (g.)	Liver lipid (% dry defatted liver wt.)	Specific activity liver inorganic phosphate
Third and fourth generation palmitic acid-fed animals (with fatty livers)	10	115	12·6	98·4 S.E. = 9·43	2·56 S.E. = 0·179
Oleic acid-fed animals	13	125	12·5	36·1 S.E. = 3·98	2·27 S.E. = 0·103
Fifth generation palmitic acid-fed animals	9	114	11·8	30·2 S.E. = 3·05	2·08 S.E. = 0·113
Controls	10	140	—	27·4 S.E. = 1·54	1·88 S.E. = 0·191

Table 4. *Liver lipids and phospholipin radioactivities*

(4 hr. after injection of ^{32}P.)

Description	No. of animals	Liver fat (% fresh wt.)	Total liver phospholipin (g.) $\times 10^4$/body wt. (g.)	Relative specific activity $\times 100$	Total relative activity (g.)	Total relative activity (g.) $\times 100$/body wt.
Palmitic acid-fed animals:						
Third and fourth generation (with fatty livers)	11	18·24 S.E. = 1·37	8·38 S.E. = 0·093	29·98 S.E. = 2·04	2·95 S.E. = 0·275	2·55 S.E. = 0·222
Fifth generation	9	6·84 S.E. = 0·532	8·14 S.E. = 0·470	31·46 S.E. = 2·20	2·94 S.E. = 0·981	2·59 S.E. = 0·254
Fifth generation palmitic acid + choline-fed animals	9	4·74 S.E. = 0·250	7·79 S.E. = 0·239	35·60 S.E. = 2·25	3·18 S.E. = 0·257	2·74 S.E. = 0·218
Oleic acid-fed animals:						
Third and fourth generation	11	8·26 S.E. = 0·814	10·50 S.E. = 0·270	29·70 S.E. = 1·76	4·15 S.E. = 0·639	3·27 S.E. = 0·422
Fifth generation	2	6·20 S.E. = 0·360	9·42 S.E. = 1·89	29·10 S.E. = 6·30	3·35 S.E. = 0·370	2·68 S.E. = 0·340
Fifth generation oleic acid + choline-fed animals	5	5·30 S.E. = 0·500	9·51 S.E. = 1·03	31·50 S.E. = 1·92	3·62 S.E. = 0·621	3·00 S.E. = 0·414
Controls:						
Third and fourth generation	11	6·06 S.E. = 0·190	13·37 S.E. = 1·86	38·90 S.E. = 6·00	3·77 S.E. = 0·363	2·73 S.E. = 0·269

Table 5. *Changes in phospholipin composition and liver lipid accumulation*

Description	No. of animals	Liver fat (% fresh wt.)	Acetone-soluble lipins (mg.)	Total phospholipin (mg.)	Choline-containing total phospholipin (%)	Amount choline-containing phospholipin (mg.)	Amount non-choline-containing phospholipin (mg.)
Palmitic acid-fed animals:							
Third and fourth generation (with fatty livers)	6	19·7 S.E. = 1·59	881·8 S.E. = 120·8	105·5 S.E. = 5·17	59·1 S.E. = 4·18	63·03 S.E. = 6·43	42·87 S.E. = 3·98
Fifth generation	9	6·84 S.E. = 0·532	150·9 S.E. = 22·3	92·5 S.E. = 4·63	65·0 S.E. = 5·17	58·5 S.E. = 2·71	34·3 S.E. = 6·25
Fifth generation palmitic acid + choline-fed animals	9	4·74 S.E. = 0·250	85·2 S.E. = 7·20	88·9 S.E. = 4·04	71·5 S.E. = 2·25	63·2 S.E. = 4·06	25·5 S.E. = 3·77
Oleic acid-fed animals:							
Third and fourth generation	7	9·57 S.E. = 1·17	334·1 S.E. = 68·4	140·1 S.E. = 21·1	47·9 S.E. = 4·01	66·4 S.E. = 9·74	73·6 S.E. = 13·9
Fifth generation	2	6·20 S.E. = 0·360	155·0 S.E. = 26·0	118·0 S.E. = 13·0	64·2 S.E. = 9·25	76·9 S.E. = 19·1	41·0 S.E. = 6·20
Fifth generation oleic acid + choline-fed animals	5	5·30 S.E. = 0·500	117·4 S.E. = 19·9	114·3 S.E. = 16·2	82·0 S.E. = 1·98	93·6 S.E. = 13·1	20·6 S.E. = 4·06
Controls	5	6·18 S.E. = 1·13	175·1 S.E. = 45·7	202·0 S.E. = 29·1	57·9 S.E. = 7·47	109·6 S.E. = 9·28	92·4 S.E. = 29·6

In the oleic acid-fed animals there is an indication that as the choline-containing phospholipin increased there was a reduction in the amount of non-choline-containing phospholipin with resulting constancy in the total phospholipin. The palmitic acid-fed animals, however, showed a remarkable constancy in amounts of choline-containing phospholipin, so that reductions in total amounts of phospholipin were due to reductions in the non-choline-containing fractions. There is also some indication that reductions in amounts of non-choline-containing phospholipin in both oleic and palmitic acid-fed animals were accompanied by reductions in amounts of acetone-soluble lipids.

DISCUSSION

Figs. 1 and 2 show that in both oleic and palmitic acid-fed animals depots are depleted due to a low food intake. The fact that an increased food consumption does not reduce the weight loss of the palmitic acid-fed animals indicates the presence of some factor in addition to the amount of food eaten. Hodge, MacLachlan, Bloor, Stoneburg, Oleson &

Whitehead (1941) and MacLachlan (1944) have obtained evidence that in fasting mice the depot fat which is metabolized has an iodine number of 80. If palmitic acid provides a large proportion of the energy value of the food supplied to the body, and if in the rat an iodine number of the same order is obligatory for fat combustion by the liver and possibly elsewhere in the body, then it is likely that either the palmitic acid will not be metabolized and will accumulate, or the iodine number of the fat being metabolized will either be raised by desaturation or by blending with unsaturated fatty acids. The present work indicates that the latter is the case. Hodge *et al.* (1941) found that the liver of the fasting mouse metabolizes 92 % of the depot fat on the first day; a maximum of 8 % accumulates in the liver. Animals in our experiment consumed up to 20 g. of palmitic acid, and depleted their depots of approximately 10 g. of fat; the highest accumulation of fat in the liver was only 750 mg. The fact that the dietary fat had an iodine number of zero, but that the liver triglyceride maintained an iodine number of 75 indicates that a considerable proportion of the liver fat accumulation is not dietary palmitic acid. This may be partly due to desaturation, but the depletion of the depots indicates that mobilization and blending of the fatty acids of the depots with palmitic acid are also occurring. Release of lipids from the depots may explain the diminished water intake of the palmitic acid-fed animals as being due to the operation of a depot-mobilizing pituitary factor, influencing the water balance. (Best & Campbell, 1936).

The significant reduction of percentage of liver fat below the normal level when choline is fed with palmitic acid may indicate that choline has a particularly important role in the metabolism of saturated fats. Work is in progress to determine if choline restores the iodine number of the liver triglyceride to normal levels.

Hodge *et al.* (1941) found a constant level of phospholipin in their fasting mice despite combustion of considerable amounts of depot fat by the liver, and interpreted this as indicating a relatively unimportant participation of the phospholipins in this combustion. Studies of the levels of liver phospholipins in fasting have been complemented by work with ^{32}P, by Hodge, MacLachlan, Bloor, Welch, Kornberg & Falkenheim (1947), who found that the specific activity of liver phospholipins showed a sharp increase on the second day of fasting. However, Kaplan & Greenberg (1944*c*) have shown that rats fasted for 3 days had marked increases in the specific activity of their liver inorganic phosphate, which could in turn produce a rise in the specific activity of the liver phospholipins without any real change in the turnover rate of the latter. A reinvestigation of the turnover of liver phospholipin during fasting including measurements of activities of inorganic phosphate would appear to be worth while, but at present it seems that evidence for increased turnover rates produced by release of depot fats to liver is ambiguous.

The correlation of specific activity of liver inorganic phosphate with amounts of liver lipid shows the importance of measuring the specific activity of liver inorganic phosphate or some other precursor in work on phospholipin turnover rates in which different lipid accumulations are to be expected.

The differences in specific activity of liver inorganic phosphate may be due to several factors. The accumulation of excess liver lipid may alter the permeability of liver cells to plasma inorganic phosphate. In this connexion it is of interest that Flock, Bollman & Mann (1936) have found a marked reduction in liver inorganic phosphate in dogs with fatty livers. Derangements of liver phosphate metabolism due to the presence of excess lipid are also indicated by the work of Ennor & Stocken (1948) who found increases in amounts of the labile phosphate fractions in fatty livers produced by carbon tetrachloride poisoning.

Our observations indicate a remarkable constancy of percentage molecular turnover rates even when adequate choline is available, despite the considerable differences in the nature of the dietary fat. No differences in the quotient of phospholipin radioactivities and the radioactivities of the total acid-soluble phosphorus of the small intestine were found by Zilversmit, Chaikoff & Entenman (1948), despite the feeding of single doses of different fats. In the present experiment, however, the greatest proportion of the weight loss occurred during the first half of the experimental period, and it is therefore possible that changes in the phospholipin turnover rates, due to mobilization and accumulation of fat, might have been detected if measured at some time before the twelfth day.

An increase in phospholipin turnover rates, caused by single doses of choline and high-fat diets, has been found by other workers. Perlman & Chaikoff (1939) state that the effect of a single dose of choline is of short duration. In the present experiment when choline was administered it was fed in the diet, and therefore a sharp rise in turnover rate, due to synthesis of new phospholipin molecules, was not to be expected. However, the continuous feeding of choline might have produced a steady state at a higher turnover rate in view of the overall increase in fat metabolism in the palmitic acid-fed animals.

The interpretation of the relationship between percentage and amounts of choline-containing phospholipins and fat accumulation is difficult. Artom & Fishman (1943*b*) using 2–3-month-old rats, prevented fatty infiltration of the liver by choline supplementation, but did not raise the low values of

choline-containing phospholipins observed in the animals on the unsupplemented low-protein diet. However, Fishman & Artom (1944), using weanling rats, not only prevented the infiltration of fat by choline supplementation, but raised the percentage and absolute amounts of the choline-containing phospholipins, with a corresponding decrease in the non-choline-containing phospholipins. Similar results were obtained by Fishman & Artom (1946) with 100 g. male rats. From the present work there is an indication that the amounts of non-choline-containing phospholipins vary directly with the amount of acetone-soluble lipids, while the amount of choline-containing phospholipins may be constant, as in the palmitic acid-fed animals. Channon & Wilkinson (1936) selected natural fats to give a series of iodine numbers and found no correlation between amounts of lecithin and fat accumulation, but unfortunately kephalin was not estimated. Our evidence for the different effects of dietary oleic and palmitic acids on the amounts of liver choline-containing phospholipins indicate the importance of considering the nature of the fatty acids in work on phospholipin metabolism.

If the rate of renewal of the fatty acid of the phospholipin differs widely from that of the phosphate, it is possible that tracer studies with marked fats would show that changes in amounts of phospholipins are accompanied by changes in molecular turnover rates. From the preliminary experiment on the molecular rate of turnover of phospholipin phosphorus it was estimated that a change in metabolism of approximately 30 mg. of fatty acid by the phospholipins could be detected, on the assumption that fatty acids were renewed at the same rate as the phosphorus. It might have been expected that there would be changes in phospholipin turnover rates related to either the changes in overall fat metabolism or to differences in liver lipid accumulations. The present work indicates no changes in turnover rates of liver phospholipins despite the mobilization of large amounts of depot fat and the greatly increased fat metabolism in the palmitic acid-fed animals. The possibility of removal of accumulated liver lipids by a mechanism involving an increase in phospholipin turnover rates was not apparent in the present investigations, since in the only experiment in which enough choline was supplied to palmitic acid-fed animals to allow increases in turnover rates there were only slight accumulations of fat in the livers of their litter mates not receiving choline.

The reduction in amounts of total phospholipin on a body weight basis in the palmitic acid-fed as compared with the oleic acid-fed animals was the only clearly significant change produced in the phospholipins by the nature of the dietary fat.

Low-protein, high-fat diets did not produce any changes in the molecular or total turnover rates of the liver phospholipins on an absolute or body weight basis, from the values observed for animals on stock diet. This is in agreement with Bollman & Flock (1946).

The present work indicates that increases in liver fat accumulations caused by feeding saturated as compared with unsaturated fatty acids may be due to increased fat mobilization and metabolism superimposed on a deficiency of lipotropic factors.

SUMMARY

1. An investigation has been made of the amounts of liver lipid accumulating during the feeding of rats with large amounts of pure saturated and unsaturated fatty acids.

2. Low-protein high-fat diets containing pure oleic and palmitic acids were fed to rats. Analyses were made of carcass and liver lipids, and liver phospholipins. ^{32}P was employed in the measurement of phospholipin turnover rates.

3. The metabolism of large amounts of dietary palmitic acid is accompanied by simultaneous release of large amounts of depot fat. Metabolism of oleic acid does not impose a similar demand on the fat depots.

4. There is a pronounced difference in the percentage of liver fat of different litters of rats when oleic and palmitic acids are fed; in the animals from the litters in which liver fat accumulates, significantly higher percentages of liver fat are obtained for palmitic acid-fed than for oleic acid-fed animals.

5. Feeding palmitic acid reduces the amount of total liver phospholipin per unit of body weight as compared with animals fed oleic acid or stock diet.

6. There is some evidence that different relationships exist between the choline-containing and non-choline-containing liver phospholipins when oleic and palmitic acids are fed.

7. A correlation has been found between the amount of liver lipid expressed on a dry-weight basis and the amount of radioactivity per unit of liver inorganic phosphate 4 hr. after injection of radiophosphate.

8. Phospholipin turnover rates, measured by employing ^{32}P to mark the phosphate component of the molecule, show no correlation with amounts of liver lipids or with gross changes in fat metabolism caused by palmitic acid feeding. This indicates that either phospholipin turnover rates may not be involved, or that the rate of turnover of radioactive phosphate is not a measure of that of the fatty acid on the phospholipin molecule.

9. The factors causing liver lipid accumulations in animals fed diets containing large amounts of saturated fatty acids are discussed.

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REFERENCES

Artom, C. & Fishman, W. H. (1943*a*). *J. biol. Chem.* **148**, 405.
Artom, C. & Fishman, W. H. (1943*b*). *J. biol. Chem.* **148**, 423.
Best, C. H. & Campbell, J. (1936). *J. Physiol.* **86**, 190.
Best, C. H., Lucas, C. C., Patterson, J. M. & Ridout, J. H. (1946). *Biochem. J.* **40**, 368.
Bollman, J. L. & Flock, E. V. (1946). *Fed. Proc.* **5**, 218.
Bollman, J. L., Flock, E. V. & Berkson, J. (1948). *Proc. Soc. exp. Biol., N.Y.*, **67**, 308.
Chaikoff, I. L. (1942). *Physiol. Rev.* **22**, 291.
Channon, H. J. & Wilkinson, H. (1936). *Biochem. J.* **30**, 1033.
Ennor, A. H. & Stocken, L. A. (1948). *Biochem. J.* **42**, 549.
Fisher, R. A. (1944). *Statistical Methods for Research Workers*, 9th ed. Edinburgh: Oliver and Boyd.
Fishman, W. H. & Artom, C. (1944). *J. biol. Chem.* **154**, 109.
Fishman, W. H. & Artom, C. (1946) *J. biol. Chem.* **164**, 307.
Flock, E., Bollman, J. L. & Mann, F. C. (1936). *J. biol. Chem.* **115**, 179.
Hevesy, G. (1938). *Enzymologia*, **5**, 138.
Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Stoneburg, C. A., Oleson, M. C. & Whitehead, R. (1941). *J. biol. Chem.* **139**, 897.
Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Welch, E. A., Kornberg, S. L. & Falkenheim, M. (1947). *J. biol. Chem.* **169**, 707.
Kaplan, N. O. & Greenberg, D. M. (1944*a*). *J. biol. Chem.* **156**, 511.
Kaplan, N. O. & Greenberg, D. M. (1944*b*). *J. biol. Chem.* **156**, 525.
Kaplan, N. O. & Greenberg, D. M. (1944*c*). *J. biol. Chem.* **156**, 543.
Loureiro, de, J. A. (1931). *Arquito Patalog.* **3**, 72.
MacLachlan, P. L. (1944). *J. biol. Chem.* **152**, 391.
Ozaki, J. (1927). *Biochem. Z.* **189**, 233.
Perlman, I., Chaikoff, I. L. (1939). *J. biol. Chem.* **127**, 211.
Popják, G. (1943). *Biochem. J.* **37**, 468.
Taurog, A., Entenman, C., Fries, B. A. & Chaikoff, I. L. (1944). *J. biol. Chem.* **155**, 19.
Yasuda, M. (1931). *J. biol. Chem.* **94**, 401.
Zilversmit, D. B., Chaikoff, I. L. & Entenman, C. (1948). *J. biol. Chem.* **172**, 637.

Renal Function as Affected by Experimental Unilateral Kidney Lesions

2. THE EFFECT OF CYANIDE

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An earlier paper (Nicholson, Selby & Urquhart, 1938) described some of the functional changes occurring when a mild degeneration of the cells of the proximal convoluted tubules is produced by sodium tartrate. One effect is an interference with the selective permeability of the tubular cells such that there is back diffusion of substances to which the walls of the tubules are normally impermeable (e.g. inulin, creatinine, ferrocyanide). Bobey, Longley, Dickes, Price & Hayman (1942) reported a similar effect with uranium nitrate. It was thought possible that any substance which caused tubular degeneration or which interfered with tubular function might also produce changes in the permeability of the cells lining the tubules. To test the latter possibility the effect of cyanide has been studied. In the isolated kidney Starling & Verney (1925) found that cyanide completely inhibits tubular activity and results in the excretion of a urine which is essentially an ultrafiltrate of the plasma, i.e. unchanged glomerular filtrate.

The present experiments show, however, that when cyanide is added to the blood flowing through the kidney *in situ* its nephrotoxic action is more selective.

METHODS

Physiological techniques

Preparation of the animals. Female dogs of from 7 to 8 kg. were used. Under nembutal anaesthesia the femoral artery and vein on both sides and one jugular vein were exposed. The kidneys were exposed through a long mid-line incision, and the renal artery and vein on each side were gently freed from the surrounding perirenal fat. When the cut surfaces had ceased oozing the animal was heparinized. Thin-walled silver cannulae, of approximately the same internal diameters as the vessels in which they were to be used, were inserted into the renal and femoral vessels. The femoral and renal veins and the femoral and renal arteries were joined by moderately thick-walled rubber tubing running through the abdominal cavity. Each length of rubber tubing had a glass T tube inserted in its course so that one end of each T tube was in immediate juxtaposition with the free end of the respective femoral cannula. The side arms of the arterial T tubes were each attached to a mercury manometer. From each venous T tube a short piece of rubber tubing, closed by a pinchcock, led into a 500 ml. cylinder. A graduated reservoir contained 1500 ml. of heparinized blood, obtained immediately before the experiment from dogs whose blood gave no cross agglutination with that of the experimental animal, was attached by a rubber tube, closed by a pinchcock, to a cannula inserted into the jugular vein. The urinary