THE CHEMISTRY OF THE LIPINS*

By I. Smedley-MacLean
Lister Institute, London

The group of lipins[1] contains two main subdivisions:

(*a*) The phospholipins (phosphatides) comprising an unknown number of substances, grouped in three classes, the lecithins, the kephalins, and the sphingomyelins. It is now recognised that the lecithin, kephalin, and sphingomyelin prepared from plant and animal sources are not single substances but mixtures of individuals, conforming to the same type of structure but differing in the nature of the two fatty acid groups in the molecule. Another class of phospholipins of complicated structure have been isolated from the tubercle bacillus, but at present the constitution of these is unknown.

(*b*) The galactolipins (cerebrosides) comprising phrenosin (cerebrone), kerasin, nervone, and hydroxynervone. These are regarded as well-characterised substances of the same type of structure, differing only in the nature of the single fatty acid radical present. It is possible that small amounts of other yet unidentified galactolipins may be present in these as impurities.

The Phospholipins

1. *The Lecithins*

Structure.—$CH_2OR \cdot CHOR' \cdot CH_2O \cdot PO(O) \cdot OC_2H_4 \cdot N(CH_3)_3$.

In the formula of lecithin there is place for only two fatty acid radicals, but the list of fatty acids previously obtained from lecithins includes palmitic, stearic, oleic, linolenic, and arachidonic acids. Sueyoshi (1) claims that the palmitic acid present in egg-yolk lecithin melts at 57° and differs from normal palmitic acid. Klenk and Schoenebeck (2) have now isolated from brain lecithin an unsaturated

* Received January 18, 1932.

[1] The term lipoid is retained in this *Review* to denote the ether-soluble constituents of a tissue, without regard to their nature.

acid containing 22 carbon atoms with 4 ethylenic linkages and have definitely established its composition by its complete reduction to behenic acid and by the preparation of an octobromide. The same acid was found in liver phospholipins and in egg lecithin. Klenk (3) investigated the acids of the mixed ether-soluble phospholipins of the brain and showed that an unsaturated acid containing 20 carbon atoms was present, confirming the previous finding of Levene and Rolf (4); this must have come from the lecithin, since it was not detected when the kephalin fraction alone was examined. The percentages of the total acids isolated from this mixture were as follows: stearic, 21.4; palmitic, 8; unsaturated acids containing (*a*) 18 C atoms, 39.6; (*b*) 20 C atoms, 8.7; (*c*) 22 C atoms, 19.8. This analysis showed that a considerable proportion of brain phospholipins must exist in which both the acid radicals are unsaturated.

The occurrence of natural lecithins containing only unsaturated acid radicals has been confirmed by Hateyama (5) who found the proportion of arachidonic acid in egg yolk so large that the existence of a lecithin containing two arachidonyl radicals was indicated.

No natural lecithin containing two saturated fatty acid radicals had previously been described, although Ritter (6) had prepared a saturated lecithin from egg yolk which had been completely hydrogenated. Merz (7) has now prepared lecithin from the lipin residue from brain after removing all material soluble in ether and in acetone. This product resembled the synthetic distearyl lecithin of Grün and Limpächer (8) in that it dissolved only in ether or in light petroleum with great difficulty. Eighty per cent of the total acids present consisted of stearic and palmitic acids, the remaining twenty per cent being oleic acid. The proportions of acids showed that a dipalmityl lecithin must have been present. If a saturated lecithin is present, therefore, in a tissue, it will remain mainly in the alcohol-soluble fraction and not pass into the ether extract.

In addition to adding one more to the list of fatty acids already isolated from lecithin, the work of the past two years has therefore established the existence of natural lecithins containing, respectively, two unsaturated or two saturated fatty acid radicals.

Iwata (9) claims to have isolated from polished rice a monopalmityl lecithin similar to the lysolecithin prepared by Delezenne and Fourneau (10) from the action of cobra venom on egg yolk.

Merz's ether-insoluble lecithin contained both the α and β forms of glycerophosphoric acid, the β form accounting for 58 per cent.

Suzukui and Yokoyama (11) have described the separation of the isomeric lecithins derived from an alcoholic extract of soya bean containing, respectively, α and β glycerophosphoric acids by using the difference in solubility in acetone of their double compounds with cadmium chloride.

Synthesis of lecithin in the body.—Rosenfeld (12) found from feeding experiments on hens that lecithin could be formed in the body if phosphorus and fatty acids were supplied. When glycine was added to a basal diet containing ether-extracted casein, starch, sugar, stearic acid, and salts, the quantity of lecithin formed in the body was much increased. Rosenfeld therefore regards glycine as a forerunner of lecithin.

2. *The Kephalins*

Structure.—$CH_2OR \cdot CHOR' \cdot CH_2O \cdot PO(OH) \cdot OC_2H_4NH_2$

In investigating the fatty acids of pure brain kephalin, great difficulty has been experienced in completely freeing the kephalin from small amounts of galactolipins and sphingomyelin, both of which yield fatty acids on hydrolysis. Klenk (13) has overcome this by saponifying the crude kephalin under such mild conditions that the accompanying substances were left unattacked. This was effected by adding to the ether solution one-quarter of the amount of potash necessary for complete saponification, dissolved in hot methyl alcohol. The precipitate which separated overnight contained the saturated acids, the unsaturated ones remaining in the ether solution and being subsequently extracted by acetone. Stearic was the only saturated acid present. On complete hydrogenation, the unsaturated acids yielded only stearic and behenic acids. Arachidonic acid had previously been found in brain kephalin by Levene and Rolf (14), but if present the amount can only be exceedingly small. Corresponding to the behenic acid, dicosan tetrenoic acid ($C_{22}H_{36}O_2$) was isolated from the mixture of unsaturated acids. The unsaturated acid, previously isolated from brain by Brown and Ault (15) and provisionally identified as tetracosan pentenoic acid, is probably identical with Klenk's dicosan tetrenoic acid. Since stearic, oleic, and dicosan tetrenoic acids certainly occur in brain kephalin, at least two kephalins must be present.

Separation of lecithin and kephalin and the preparation of pure kephalin.—All the earlier preparations of kephalin from brain in

which the whole of the nitrogen was present in the amino form contained less than the 66 to 68 per cent of the carbon demanded by theory. Two explanations of this discrepancy have been put forward: (*a*) that some oxidation of the highly unsaturated acids always occurs; and (*b*) that the kephalin had not been completely freed from the degradation products, which are more easily split off than in the case of lecithin. The synthesis of distearyl kephalin by Grün and Limpächer (16) suggested useful improvements in the isolation of the natural product which have proved fruitful in the hands of Rudy and Page (17). The marked differences in the properties of synthetic lecithin and kephalin are probably related to the presence in lecithin of the strong base choline and its substitution in kephalin by the weak base amino ethyl alcohol. Kephalin is much more sensitive to the action of acids and alkalies than is lecithin and is more readily hydrolysed by alcoholic potash in the cold. Lecithin has an anhydride structure and in benzene or ether-alcohol solution is neutral to phenol phthalein, whereas kephalin acts as a monobasic acid with a definite neutralisation value. This neutral character of lecithin probably accounted for the failure by Fabisch (18) to find any buffering action. Rudy and Page found the determination of the neutralisation value in benzene-alcohol solution of great help in examining any mixture of phospholipins; the indication of the relative quantities of kephalin and lecithin thus obtained agreed very well with those obtained by estimating the proportion of the nitrogen present in the amino form. Since kephalin is soluble in the same solvents as lecithin but with more difficulty, the precipitation of kephalin by alcohol from an ethereal solution of mixed phospholipins must be incomplete; Rudy and Page investigated the petrol-alcohol mother liquors of brain phospholipins after removal of the alcohol-insoluble fractions. A concentrated solution in ether (or ethyl acetate) of the residual substance was fractionally precipitated with alcohol and a kephalin obtained containing 66.17 per cent of carbon and agreeing closely in composition with that of a pure linolenyl kephalin. Since the oxidation and decomposition products of kephalin are less soluble in alcohol than kephalin, the more soluble fractions furnish the best starting material for the preparation of kephalin. MacLean (19) had earlier pointed out that when a tissue was obtained in as fresh a condition as possible and quickly dried it might yield the whole of the phospholipin fraction in an alcohol-soluble condition but that after standing for some hours a considerable fraction which was regarded as the kephalin

fraction became insoluble. The insolubility of kephalin in alcohol seems to be largely due to its association with degradation or oxidation products.

Other methods found effective for the separation of the lecithin-kephalin mixture were: (*a*) the addition of a methyl-alcoholic solution of baryta to a benzene-alcohol solution of the mixture. The kephalin, unlike the lecithin, was converted to a barium salt, which separated on the addition of alcohol and methyl alcohol [Rudy and Page (17)]. (*b*) The fraction precipitated by alcohol from concentrated ether solution was dissolved in ether, poured into hot methyl-alcohol solution, and filtered hot. On cooling, kephalin was deposited and further purified [Wadsworth, Maltaner, and Maltaner (20)].

The properties of lecithin and kephalin.—Remesow (21) has described methods for the preparation of sols of pure lecithin and cholesterol, the coagulation limit of the highest concentration of sol which he was able to reach being pH = 5.6 for lecithin and 6.2 for hydrolecithin. The lecithin sol was negatively charged and completely undialysable to water [Remesow (22)].

Recent studies on the colloidal properties of lecithin have been especially concerned with two questions, (*a*) the mutually antagonistic effect of solutions of lecithin and cholesterol; and (*b*) the effect of the addition of lecithin on the precipitation of serum proteins. Theorell and Widström (23) have confirmed the antagonistic action of lecithin and cholesterol on the haemolysis of red blood corpuscles. Spränger (24) studied the resistance to water, acids, and bases, respectively, of red blood corpuscles which had been previously treated with isotonic glucose solutions containing highly dispersed suspensions of lecithin or cholesterol in the concentrations in which they are present in blood serum. Whereas the untreated blood corpuscles of the sheep were haemolysed in a 0.62 per cent solution of NaCl, after treatment with lecithin they were hydrolysed by a 0.72 per cent solution; after treatment with cholesterol, a 0.50 per cent solution of NaCl produced haemolysis. The lecithin-treated red blood corpuscles were haemolysed by one-hundredth of the amount of ammonia necessary to haemolyse the controls. Degkwitz (25) found that if an isotonic solution of glucose containing lecithin was injected intravenously into rabbits the concentration of chloride in both urine and serum was diminished and a diminished volume of urine excreted. If cholesterol was substituted for lecithin, the opposite effect was produced. This result was also obtained by Dahmlos and Solé (26).

Dahmlos (27) found that when dogs were injected with solutions of lecithin or cholesterol these substances were picked out by the liver since no lipaemia followed their injection into the portal vein, whereas injected into the general circulation lipaemia followed.

Went and Farago (28) found that addition of aqueous emulsions of lecithin to a serum altered the proportion of protein precipitated in the fraction containing euglobulin and pseudoglobulin I and in that containing pseudoglobulin II and albumin. Merklen, le Breton, and Adnot (29) also found that the serum lipins exercised an inhibiting influence on the precipitation of the globulins. Theorell and Widström (23) added lecithin to serum, removed the excess by centrifuging and found that the additional lecithin was precipitated with the various fractions of protein salted out. When the proteins were fractionally precipitated by electrodialysis or salted out, the quotient $\frac{\text{Cholesterol}}{\text{Lipoid P.}}$ sank successively from fibrinogen through globulin to albumin. According to Macheboeuf (30) this association of lipin with protein plays an important part in regulating the passage of water to and from the tissues.

Wadsworth, Maltaner, and Maltaner (31) compared the actions of lecithin and kephalin in producing coagulation of the blood. An alkaline solution of heart lipins added together with a drop of calcium chloride solution to oxalated blood produced coagulation. A solution of lecithin was active only after it had undergone preliminary hydrolysis by heating for some minutes in alkaline solution. Kephalin solution was active; on adding to it a drop of calcium chloride solution, free acid was liberated and a precipitate of calcium salt formed which was inactive as a coagulant. The active substance was again obtained by shaking the calcium salt with acid. The precipitation of proteins in the presence of lipins was also studied and the conclusion reached that lipin and protein form an insoluble complex in the presence of free acid. The inactivity of lecithin before hydrolysis is explained by its internal anhydride structure and furnishes another instance of the difference of properties of lecithin and kephalin due to the presence of a free acid group in kephalin. The oxidation which kephalin undergoes in air was studied by Page and Bülow (32), who found that as the percentages of carbon, hydrogen, and nitrogen and the iodine value fell, the neutralisation value rose. These changes were catalysed by the presence of iron and prevented by keeping the kephalin under alcohol.

3. *The Sphingomyelins*

Structure.—$CH_2OH \cdot CH \cdot CHNHR \cdot CH : CH(CH_2)_{12}CH_3$

$$\begin{array}{l} CH_2OH \cdot \underset{\displaystyle |}{CH} \cdot CHNHR \cdot CH : CH(CH_2)_{12}CH_3 \\ \qquad\qquad\; O \\ \qquad\qquad\; | \\ O = P(OH) \cdot OC_2H_4N(CH_3)_2OH \end{array}$$

In 1929 Klenk (33) established that the base sphingosine contained 18 and not 17 carbon atoms as had been hitherto accepted. Lapworth (34) had shown that the amino- and the two hydroxyl groups were attached to the three terminal carbon atoms; Klenk and Diebold (35) have now shown that the hydroxyl groups occupy the two terminal positions, the amino group being in the α-position to the ethylene linkage. Tri-acetyl sphingosine was ozonised, the product hydrolysed, and the resulting aldehydes oxidised. Myristic acid and dihydroxy aminobutyric acid were obtained, the latter giving on reduction with hydriodic acid α-aminobutyric acid.

Merz (36) showed that starting with a pure sphingomyelin fraction three fatty acids, stearic, lignoceric, and nervonic, could be isolated from it. Three sphingomyelins must therefore be present, each containing a different acid radical.

Phospholipins of Unknown Constitution Isolated from Tubercle Bacilli

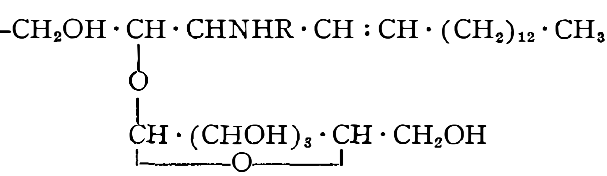
Anderson and Roberts (37, 38, 39) have made a further study of the complicated phosphorus compounds isolated from tubercle bacilli. The amounts of these substances isolated from human, bovine, and avian strains formed, respectively, 27.5, 11.5, and 15.0 per cent of the total lipoids extracted from the dry bacilli. The cleavage products are similar in all three cases, though the proportions of water-soluble and ether-soluble constituents differ somewhat. The percentages of nitrogen (0.4) and of phosphorus (2.2) were the same in specimens derived from avian and human strains. The lipin from the bovine strain was hydrolysed with more difficulty and contained 1 per cent of nitrogen. From all three samples oleic, palmitic, and stearic acids were isolated. In the phospholipin from the human and avian strains, the saturated liquid and optically inactive phthioic acid $C_{26}H_{52}O_2$ isomeric with cerotic acid was present; from the bovine strain the liquid tuberculostearic acid $C_{18}H_{36}O_2$ isomeric with

stearic acid was isolated. On hydrolysis, mannose [Anderson and Renfrew (40)] and inosite [Anderson (41); Anderson and Roberts (42, 43)] were found. The preparation from the human strain also yielded invert sugar. Phosphoric and glycerophosphoric acids were identified, but nothing is yet known as to the nature of the nitrogenous constituent. Pangborn and Anderson (44) showed that similar substances were present in the Timothy bacillus.

Estimation of phospholipins.—Bang first used an oxidative process for estimating the amount of lipin, the carbon and hydrogen being completely oxidised to carbonic acid and water; this process was subsequently improved by Bloor (45). Boyd (46) found that Bloor's method was entirely satisfactory for the estimation of amounts of the order of 2 milligrams of phospholipins but could not be used with safety for less quantities. Osato and Heki (47) have recommended the preliminary freezing, moistening, and grinding of the tissue before extracting it with a mixture of alcohol and ether; Bloor's nephelometric method was then used to determine the phosphorus in the extract. Lintzel and Fomin (48) have described a method for the estimation of lecithin which relies on the separation of the choline by oxidation with permanganate. Any ammonia and methyl- or dimethyl-amines were converted into non-volatile compounds by treatment with formaldehyde and the trimethylamine then estimated. The same principle was used by Lintzel and Monasterio (49) as the basis of a micro-method for the determination of lecithin in blood plasma. This method has the advantage of estimating only the lecithin in any mixture of lecithin and kephalin. A comparison of the various micro methods for determining the iodine values of the lipins was made by Yasuda (50), who found the Rosenmund-Kuhnheim method most satisfactory.

The Galactolipins (Cerebrosides)

Structure.—



Klenk and Härle (51) have studied the fatty acids present in the ether-insoluble portion of the brain lipides, 90 per cent of which are

present in amide combination, hydrolysable only with difficulty. Of the acids in this fraction 90 per cent contain 24 carbon atoms, the remaining 10 per cent containing 18 carbon atoms. Cerebronic, lignoceric, nervonic, and hydroxynervonic acids were all identified. All these acids contain chains of 24 carbon atoms such as would be formed by the condensation of four hexose molecules.

Hydrolysis with baryta of a mixture of cerebrosides consisting essentially of nervone and hydroxynervone yielded a galactosidosphingosine identical with that obtained from kerasin. Klenk has made clear the close relationship existing between the four acids isolated from the cerebrosides, phrenosin, kerasin, nervone, and hydroxynervone, respectively.

Kerasin yields lignoceric acid,

$$CH_3 \cdot (CH_2)_7 \cdot CH_2 \cdot CH_2 \cdot (CH_2)_{12}CH_2COOH$$

Phrenosin yields α-hydroxylignoceric acid,

$$CH_3 \cdot (CH_2)_7 \cdot CH_2 \cdot CH_2 \cdot (CH_2)_{12} \cdot CHOH \cdot COOH$$

Nervone yields nervonic acid,

$$CH_3 \cdot (CH_2)_7 \cdot CH : CH \cdot (CH_2)_{12}CH_2COOH$$

Hydroxynervone yields α-hydroxynervonic acid,

$$CH_3 \cdot (CH_2)_7 \cdot CH : CH \cdot (CH_2)_{12}CHOH \cdot COOH$$

Confirmation of the constitution of kerasin was obtained by combining the galactosidosphingosine split off from a mixture of brain galactolipins with lignoceric acid, when a synthetic kerasin was obtained. This agreed closely in its properties with the kerasin obtained by Rosenheim (52) from brain. The kerasin derived from spleen has a slightly higher rotation and may contain a different acid radical.

Preparation of the galactolipin fraction from brain.—Rosenheim (53) after removing the cholesterol and phospholipin fractions by extracting with acetone and light petroleum, respectively, dissolved the galactolipins by means of pyridine. Page (54) has modified this method by replacing the solvent pyridine by the cheaper tetralin,

which is also pleasanter to use in large quantity. From the tetralin solution the galactolipins are precipitated in the usual manner by means of acetone.

Properties of sphingomyelin, phrenosin, and kerasin.—The similarity in the properties of these three lipins all of which contain a single saturated fatty acid radical bound in amide-like combination with the base sphingosine, was brought out by Turner and Watson (55) in the measurements they have made of the areas occupied by films of these substances, one molecule thick, on water. In these three substances, two saturated paraffin chains containing, respectively, 15 and 23 carbon atoms are united by a chain with polar properties containing one nitrogen and four carbon atoms. At room temperature, the area per molecule is about 60 sq. Å; as the pressure is increased, the molecules pack closer until the area occupied is 42 Å, agreeing with that which should be given by a film in which two parallel paraffin chains are packed closely together.

The Function of the Phospholipins

In 1920, Meigs, Blatherwick, and Cary (56) described experiments on the mammary gland which seemed to offer conclusive evidence that milk fat was derived from phospholipins, for the blood left the gland richer in inorganic phosphate and poorer in phospholipin in comparable amount. Jost (57) has now brought forward further evidence in support of the view previously advocated by Leathes (58) that the phospholipins act as intermediate substances in fat metabolism. In one set of experiments the organs perfused were "normal livers" from dogs well fed on a normal diet and subsequently starved for 24 hours; these livers contained from 0.5 to 1.5 per cent of glycogen and 3 per cent of fat. In the other set, "fatty livers" from dogs, previously treated with phlorhizin for four days, were used; these contained about 10 per cent of fat and were glycogen-free. When blood was perfused through them, its content of inorganic phosphate increased, the increase being 2 to 3 times as great in the "fatty" as in the "normal" livers; the lipin phosphate in the blood showed no significant change. The deduction was made that the breaking down of phospholipin was proceeding more actively in the fatty livers, phosphate being thrown into the blood stream. When a finely divided emulsion of heart kephalin was added to the perfusion fluid, it disappeared at first rapidly and then more slowly,

the lipin phosphate of the blood corpuscles remaining practically unchanged. The fatty livers contained slightly less lipin phosphate after the perfusion than at the beginning of the experiment. The fatty livers always contained more inorganic phosphate than the normal ones and were richer in glycerophosphoric acid, a substance which is very easily broken down by the phosphatase known to be present in liver. In a study of the hydrolytic splitting of lecithin by tissue enzymes, King, King, and Page (59) found that liver extract and bone phosphatase were without action on lecithin, whereas extracts of the intestinal wall and of the kidney had a very marked action [King (60)]. Jost found that the breaking down of the lipin molecule appeared to be associated with the oxidation of its fatty acid, for when an emulsion of phospholipin was added to the circulating liquid a marked increase in the oxygen intake of the liver followed and the respiratory quotient fell from 0.95 to 0.62. The acetone and acetoacetic acid, however, increased only from 30 to 40–60 mg. per litre, an increase very much less than that corresponding to the increase in fat combustion. When glycogen-free livers from dogs starved for four days previously were perfused with blood the amount of acetone formed was 50 to 100 per cent more than in the glycogen-containing livers, while perfusion of the phlorhizinised "fatty livers" produced still larger amounts. The gas metabolism appeared, however, to furnish a better index of the increased fat oxidation of the isolated liver than the slight rise of acetone in the blood. Smedley-MacLean and Pearce (61) have pointed out that all available evidence points to the production of acetone from the four terminal atoms only of the carbon chain.

When the "fatty" livers were perfused with Ringer's solution, sugar appeared in the perfusion liquid, but the amount of sugar formed and of oxygen absorbed fell rapidly after the first half-hour, the respiratory quotient rising. If at the end of this time phospholipin was added to the perfusing fluid, the output of sugar and intake of oxygen rose sharply, while the respiratory quotient fell; the ketone bodies remained unchanged. Jost regarded this as definite evidence that sugar was formed from the fatty acids of the lipin molecule, the amount of glycerol being quite insufficient to account for the sugar formed. Heart kephalin was much more effective than lecithin, the addition of highly unsaturated acid being apparently necessary.

Sinclair (62) has furnished confirmatory evidence that the lipins are concerned in the absorption and metabolism of fat, for he found

that during the absorption there was a pronounced change in the composition but none in the amount of the phospholipins of the liver and of the intestinal mucosa; on the other hand, the composition of the lipin fatty acids of both smooth and striated muscle showed no change. If the lipin feeding were continued for some days, the lipin fatty acids of all the tissues examined, liver, heart, kidney, smooth and striated muscle, intestinal mucosa, and brain, increased in unsaturation in animals fed on a more unsaturated diet. Beef kidney and beef muscle, respectively, were used as diets containing acids of different degrees of unsaturation. This result is in opposition to the finding of Terroine, Hatterer, and Roehrig (63, 64) who concluded that the composition and amount of the fatty acids was a fixed characteristic for each tissue, unaffected by diet. In Sinclair's experiments the amount of lipin fatty acid increased with the more unsaturated diet in the liver alone. Sinclair concluded that the fatty acids absorbed were transformed into phospholipins in the intestinal mucosa as an essential step in the resynthesis of neutral fat, and that the lipin either was an intermediary in the metabolism of fat or else that the phospholipins of the tissues underwent continual wear and tear and were replaced at the expense of the food fat. Boyd (46) working with a diet low in fat and rich in carbohydrate and using the micro-oxidative method of Bloor found abnormally low phospholipin values, e.g., 49 to 76 mg. per cent compared with values of 240 to 478 mg. for dogs fed on a normal diet. No similar variation was found in the lipin phosphate of the red blood corpuscles.

Sinclair (65) studied in young rats the ratio of the phospholipins to the tissue solids and found that they decreased rapidly after birth, the period of most rapid decline coinciding with that of most active growth. Bloor, Okey, and Corner (66) found that the percentage of lipin in the corpus luteum of the sow varied with the activity of the gland and before oestrus and during pregnancy was doubled or trebled. Bloor and his colleagues (67, 68) also showed that the amount of phospholipin per kilogram of liver tissue was approximately double that in other organs.

Changes in the Lipin Content of the Organs in Disease

Certain pathological conditions are characterised by marked increases or decreases in the amounts of lipins present in the blood or in various organs. Epstein and Lorenz (69) showed that in the case

of Gauber's disease, the total lipin content of the spleen remained normal but a large proportion of kerasin was present. In the splenohepatomegaly of Neumann-Pick's disease there was a very marked increase in the phospholipin content of the spleen. In both these cases the cholesterol content of the spleen remained normal. Schmitz and Koch (70) found that in the lipaemia which follows venesection in dogs, the phospholipins were largely increased. In rabies, from the moment of infection of the animal until its death, there was a very characteristic steady increase in the lipin phosphorus of the blood [Zuwerkalow and Goldenberg (71)]. In lipoidic nephrosis, Macheboeuf, Wahl, and Sandon (72) found that the phospholipins of the serum might rise to six times their normal amount and a very much greater proportion of these was precipitated with the albumin fraction of the serum proteins. Eckstein and Wile (73) found the phospholipin content of a xanthematous tumour to be 200 times that present in the normal subcutaneous fat, and to be greatly increased in the tissue surrounding the tumour. A study of the livers of rats with tumours led Roffo and Correa (74) to the conclusion that as the tumour developed the phospholipin content of the liver fell much below the normal. In pneumonia, tuberculosis, and fatty degeneration, Theis (75) found that the proportion of liver phospholipin decreased. Milbradt (76), working on the avitaminosis of pigeons fed on polished rice, found that the phospholipin content fell in the brain and in all parenchymatous organs, as did the ratio $\frac{\text{phospholipin}}{\text{cholesterol}}$. The inorganic and nucleotidic phosphorus was increased. Milbradt ascribed this to a lessened synthesis of phospholipin, though it seems also possible that it may be due to an increased breaking down of the tissue lipins.

The lipins in tuberculosis.—The phospholipin fractions isolated by Anderson (*37 et seq.*) from various strains of tubercle bacilli when injected intraperitoneally into rabbits produced tubercle-like bodies with extensive infiltration of lymphocytes and with some diffuse epithelioid and giant Langerhans cells. Sabin and Doan (77) showed that there was a quantitative difference in the specificity of the phospholipins from the human, bovine, and avian strains which might be used to assist in identifying the type of organism causing any particular case of tuberculosis. Doan (78) showed that the phospholipin from the bovine type acted as an antigen in rabbits and was precipitated in high dilutions against the sera of tuberculous

cattle. This particular property of the phospholipin seems to be derived from the liquid-saturated fatty acid which it contains and for which possibly the lipin acts as a carrier.

LITERATURE CITED

1. SUEYOSHI, Y., *J. Biochem. (Japan)*, **13**, 145 (1931)
2. KLENK, E., AND SCHOENEBECK, O. VON, *Z. physiol. Chem.*, **194**, 191 (1931)
3. KLENK, E., *Z. physiol. Chem.*, **200**, 51 (1931)
4. LEVENE, P. A., AND ROLF, I., *J. Biol. Chem.*, **54**, 99 (1922)
5. HATEYAMA, T., *Z. physiol. Chem.*, **187**, 120 (1930)
6. RITTER, F., *Ber.*, **47**, 531 (1914)
7. MERZ, W., *Z. physiol. Chem.*, **196**, 10 (1931)
8. GRÜN, A., AND LIMPÄCHER, R., *Ber.*, **59**, 1350 (1926)
9. IWATA, M., *Biochem. Z.*, **224**, 430 (1930)
10. DELEZENNE, C., AND FOURNEAU, E., *Bull. soc. chim.*, **15**, 421 (1914)
11. SUZUKUI, B., AND YOKOYAMA, Y., *Proc. Imp. Acad. (Tokyo)*, **6**, 341 (1930)
12. ROSENFELD, G., *Biochem. Z.*, **218**, 48 (1930)
13. KLENK, E., *Z. physiol. Chem.*, **200**, 51 (1931)
14. LEVENE, P. A., AND ROLF, I., *J. Biol. Chem.*, **54**, 51 (1922)
15. BROWN, J. B., AND AULT, W. C., *J. Biol. Chem.*, **89**, 167 (1930)
16. GRÜN, A., AND LIMPÄCHER, R., *Ber.*, **60**, 151 (1927)
17. RUDY, H., AND PAGE, I. H., *Z. physiol. Chem.*, **193**, 251 (1930)
18. FABISCH, W., *Biochem. Z.*, **242**, 121 (1931)
19. MACLEAN, H., *"Lecithin and Allied Substances,"* Longmans, Green and Co., London, p. 81 (1917)
20. WADSWORTH, A., MALTANER, F., AND MALTANER, E., *Am. J. Physiol.*, **97**, 74 (1931)
21. REMESOW, I., *Biochem. Z.*, **218**, 86 (1930)
22. REMESOW, I., *Biochem. Z.*, **218**, 134 (1930)
23. THEORELL, H., AND WIDSTRÖM, G., *Z. ges. exptl. Med.*, **75**, 699 (1931)
24. SPRÄNGER, W., *Biochem. Z.*, **218**, 341; **221**, 315 (1930)
25. DEGKWITZ, R., *Klin. Wochschr.*, **9**, 2336 (1930)
26. DAHMLOS, J., AND SOLÉ, A., *Biochem. Z.*, **227**, 401 (1930)
27. DAHMLOS, J., *Biochem. Z.*, **242**, 88 (1931)
28. WENT, S., AND FARAGO, F., *Biochem. Z.*, **230**, 239 (1931)
29. MERKLEN, P., BRETON, E. LE, AND ADNOT, M. A., *Compt. rend.*, **192**, 1053 (1931)
30. MACHEBOEUF, A., *Compt. rend.*, **192**, 1413 (1931)
31. WADSWORTH, A., MALTANER, F., AND MALTANER, E., *Am. J. Physiol.*, **91**, 423 (1930)
32. PAGE, I. H., AND BÜLOW, M., *Z. physiol. Chem.*, **194**, 166 (1931)

33. KLENK, E., *Z. physiol. Chem.*, **185**, 169 (1929)
34. LAPWORTH, A., *J. Chem. Soc.*, **103**, 1029 (1913)
35. KLENK, E., AND DIEBOLD, W., *Z. physiol. Chem.*, **198**, 25 (1931)
36. MERZ, W., *Z. physiol. Chem.*, **193**, 59 (1930)
37. ANDERSON, R. J., AND ROBERTS, E. G., *J. Biol. Chem.*, **85**, 509 (1930)
38. ANDERSON, R. J., AND ROBERTS, E. G., *J. Biol. Chem.*, **85**, 519 (1930)
39. ANDERSON, R. J., AND ROBERTS, E. G., *J. Biol. Chem.*, **85**, 529 (1930)
40. ANDERSON, R. J., AND RENFREW, A. G., *J. Am. Chem. Soc.*, **52**, 1252 (1930)
41. ANDERSON, R. J., *J. Am. Chem. Soc.*, **52**, 1607 (1930)
42. ANDERSON, R. J., AND ROBERTS, E. G., *J. Biol. Chem.*, **89**, 599 (1930)
43. ANDERSON, R. J., AND ROBERTS, E. G., *J. Biol. Chem.*, **89**, 611 (1930)
44. PANGBORN, M. C., AND ANDERSON, R. J., *J. Biol. Chem.*, **94**, 465 (1931)
45. BLOOR, W. R., *J. Biol. Chem.*, **82**, 273 (1929)
46. BOYD, E. M., *J. Biol. Chem.*, **91**, 1 (1931)
47. OSATO, S., AND HEKI, M., *J. Biol. Chem.*, **87**, 541 (1930)
48. LINTZEL, W., AND FOMIN, S., *Biochem. Z.*, **238**, 452 (1931)
49. LINTZEL, W., AND MONASTERIO, G., *Biochem. Z.*, **241** (1931)
50. YASUDA, M., *J. Biol. Chem.*, **94**, 401 (1931)
51. KLENK, E., AND HÄRLE, R., *Z. physiol. Chem.*, **189**, 243 (1930)
52. ROSENHEIM, O., *Biochem. J.*, **10**, 142 (1916)
53. ROSENHEIM, O., *Biochem. J.*, **8**, 110 (1914)
54. PAGE, I. H., *Biochem. Z.*, **219**, 161 (1930)
55. TURNER, K., AND WATSON, M. M., *Biochem. J.*, **24**, 113 (1930)
56. MEIGS, E. B., BLATHERWICK, N. R., AND CARY, C. A., *J. Biol. Chem.*, **37**, 1 (1919)
57. JOST, H., *Z. physiol. Chem.*, **197**, 90 (1931)
58. LEATHES, J. B., *"The Fats,"* Longmans & Co., London (1910)
59. KING, H., KING, E. J., AND PAGE, I. H., *Z. physiol. Chem.*, **191**, 243 (1930)
60. KING, E. J., *Biochem. J.*, **25**, 799 (1931)
61. SMEDLEY-MACLEAN, I., AND PEARCE, M. S. B., *Biochem. J.*, **25**, 1252 (1931)
62. SINCLAIR, R. G., *J. Biol. Chem.*, **86**, 579 (1930)
63. TERROINE, E. F., HATTERER, CH., AND ROEHRIG, P., *Bull. soc. chim. biol.*, **12**, 657 (1930)
64. TERROINE, E. F., AND HATTERER, CH., *Bull. soc. chim. biol.*, **12**, 674 (1930)
65. SINCLAIR, R. G., *J. Biol. Chem.*, **87**, xxiii (1930)
66. BLOOR, W. R., OKEY, R., AND CORNER, G. W., *J. Biol. Chem.*, **86**, 291 (1930)
67. BLOOR, W. R., OKEY, R., AND CORNER, G. W., *J. Biol. Chem.*, **86**, 307 (1930)
68. BLOOR, W. R., AND SNIDER, R. H., *J. Biol. Chem.*, **87**, 399 (1930)
69. EPSTEIN, E., AND LORENZ, K., *Z. physiol. Chem.*, **192**, 145 (1930)
70. SCHMITZ, E., AND KOCH, F., *Biochem. Z.*, **223**, 257 (1930)
71. ZUWERKALOW, D., AND GOLDENBERG, I., *Biochem. Z.*, **226**, 278 (1930)

72. MACHEBOEUF, M. A., WAHL, R., AND SANDON, G., *Bull. soc. chim.*, **12**, 504 (1930)
73. ECKSTEIN, H. C., AND WILE, U. J., *J. Biol. Chem.*, **87**, 311 (1930)
74. ROFFO, A. H., AND CORREA, L. M., *Bull. soc. chim. biol.*, **12**, 1247 (1930)
75. THEIS, E. R., *J. Biol. Chem.*, **82**, 327 (1929)
76. MILBRADT, W., *Biochem. Z.*, **223**, 278 (1930)
77. SABIN, E. R., AND DOAN, C. A., *J. Biol. Chem.*, **85**, 521 (1930)
78. DOAN, C. A., *Proc. Soc. Exptl. Biol. Med.*, **26**, 672 (1929)

LISTER INSTITUTE OF PREVENTIVE MEDICINE
CHELSEA BRIDGE ROAD
LONDON, S.W. 1, ENGLAND