XVI. SPHINGOMYELIN, PHRENOSIN AND KERASIN IN FILMS ONE MOLECULE THICK ON WATER.

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THE cross section of the paraffin chain has been measured in crystals by the X-ray method and found by Müller [1927] to have the value 18.3 sq. Å. The area occupied in films one molecule thick formed upon water by compounds containing long chains of carbon atoms has been measured by Adam [1922, 1928] and has been proved to have as its minimum value about 20.5 sq. Å. He has described and defined conditions in which larger values than this are found for substances that, under other conditions, give this value, and has especially studied the influence exerted on the dimensions of the films by the character of the polar group at the end of the chain, by reason of which the formation of the film is made possible and the molecules are anchored to the water surface. Compounds with a large variety of such polar groups have been investigated and the foundations have been laid on which much that is of interest to biochemists must rest. Most of the substances of biochemical importance in which long fatty chains occur are not of a simple character; they contain commonly more than one such chain and their polar groups present great variety and complexity. Some of these Adam has dealt with, *e.g.* glycerides and lecithin. But many such substances present difficulties which must be approached with circumspection, and only after the ground has been thoroughly surveyed. Moreover they are in many cases difficult to obtain with sufficient guarantee of purity.

On two grounds however we felt justified in experimenting with some of the less familiar substances of biochemical interest even at this stage. Specimens were available that were probably as pure as any hitherto obtained; they were prepared by Dr Rosenheim and given by him to this department for work of this nature: and secondly, though their constitution has not in all particulars been finally settled, enough has been clearly established for them to present points of interest in a study of their behaviour in films on water.

The substances dealt with in this paper resemble one another in two respects; they contain two long aliphatic chains similar if not identical in all three; these are a primary amine sphingosine and a peculiar long-chain fatty acid, and these two components are united as an acid amide $R_1 \cdot CO \cdot NHR_2$.

The constitution of the base sphingosine, $C_{17}H_{35}O_2N$, is almost completely

known. Levene and Jacobs [1912] showed that the whole of the nitrogen was removed by nitrous acid; that since a triacetyl derivative could be obtained the two oxygen atoms were present as alcoholic hydroxyl groups; and that a doubly linked pair of carbon atoms was present in the chain, since by means of palladium a dihydro-derivative could be formed. Lapworth [1913] on oxidation of sphingosine with chromic acid obtained normal tridecylic acid. Levene and West [1914] by oxidation of dihydrosphingosine obtained normal pentadecylic acid. This leaves no doubt that the double bond is between the 13th and 14th carbon atoms and that one hydroxyl group is attached to the 15th; there is little doubt that the other is attached to the 16th and the amino-group to the terminal or 17th carbon atom, though this has not been proved.

The fatty acid, lignoceric acid, $C_{24}H_{48}O_2$, in sphingomyelin is, according to evidence furnished by Levene [1916], united to sphingosine as in an acid amide, a derivative having been obtained containing base and acid, but not the other components of the molecule, and this derivative gave up no nitrogen to nitrous acid. The same union exists apparently also in phrenosin and kersin [Levene and West, 1912], as neither of them contains a free amino-group. In kersin therefore the remaining component, galactose, must be condensed with one of the hydroxyl groups of the sphingosine, as a galactoside, since the compound has no reducing properties; and in phrenosin too, though there is here another possibility, seeing that phrenosinic acid is a hydroxy-acid, the probability is that the same arrangement holds. Similarly in sphingomyelin one of the hydroxyl-groups of the sphingosine must be condensed with the cholyphosphoric acid.

In all three compounds, therefore, the two long chains are connected to polar groups which are large enough to separate them much more than is the case with any of the compounds containing more than one long chain which have up to now been studied in this way, glycol esters, glycerides, lecithin or cetyl palmitate. Having regard to their power to form films one molecule thick on water they may be represented thus:

In phrenosin the right-hand chain of this latter formula becomes $C_{24}H_{48}O$ according to Levene and Taylor [1928], $C_{23}H_{47}O$ according to Klenck [1928;

see also Taylor and Levene, 1929]. In all these compounds the two paraffin chains are at the two ends of the same chain with polar properties consisting of four carbon atoms and one nitrogen atom. Attached to this polar chain, in the case of sphingomyelin, is the additional polar group cholyl-phosphoric acid, in the case of kerafin and phrenosin the additional polar galactoside group. Whether these additional polar groups attached to the chain common to all three are attached as represented above or by the other alcohol group further to the right is not known; but for present purposes this is immaterial.

EXPERIMENTAL.

The apparatus used was that of Adam and Jessop [1926] and throughout this work the authors have had the benefit of Dr Adam's guidance in the use of it. All the solvents used were specially distilled, so that there was no contamination of the surface of the trough due to the presence in them of foreign matter. The solvent used was a mixture of pyridine and benzene. The sphingomyelin, weighed out accurately, was first dissolved in a little pyridine, which was warmed gently until solution was complete; then after cooling, benzene was added, the volume used being roughly 2-3 times the volume of the pyridine. It was found that the addition of a small amount of pure ethyl alcohol gave a clearer and more stable solution. The solutions were then weighed and were used on the trough immediately after they had been prepared, because it was found that after standing for any considerable period of time, especially in cold weather, part of the sphingomyelin often came out of solution. This behaviour was carefully watched for throughout the experiments, and as soon as it was observed the solution was rejected.

The films were studied at a number of temperatures between 0° and 35°. In general three or more readings were taken for each solution at each temperature. Although certain small deviations were observed between different solutions, these were never large and most of the curves obtained were practically coincident.

The curve for sphingomyelin, which is reproduced, represents the one which was most frequently obtained at room temperature and it forms a good average of the small experimental variations. At this temperature the area per molecule at the commencement of packing (*i.e.* the area of the head of the molecule) is about 60 sq. Å. As the pressure on the film is increased, the molecules pack closer until the area occupied by each molecule is about 42 sq. Å., the area, that is, that should be given by a film in which two parallel paraffin chains are packed closely together. Further compression decreases the area per molecule to 39 sq. Å. but, at the pressure necessary for this (42 dynes/cm., more than 150 atm.), the film shows signs of collapse. At room temperature the film is liquid at low and medium compressions but solidifies at high compressions. At 0° the film solidifies at lower pressures, but at 25° and above the film is liquid under all pressures.

At 0° the head of the molecule occupies a smaller area (50 sq. Å.) but as the pressure is increased the curve becomes coincident with that obtained at room temperatures. At 25° and 35° the films of sphingomyelin become increasingly difficult to compress, and under the highest pressures obtainable without collapse of the film (about 45 dynes/cm.), the limiting area of 42 sq. Å. is never reached.

Fig. 1.

The curves obtained with phrenosin and kerasin were practically identical and closely resemble that given by sphingomyelin. The size of the head is about 70 sq. Å. at room temperature, and the limiting area per molecule under the highest compressions is about 42 sq. Å. At 33° the head occupies a larger area (about 100 sq. Å. per molecule) but as the pressure is raised, the curve gradually approaches that obtained at 15° . At room temperature the films are solid under moderate pressures, and at about 30° they are liquid under all compressions.

DISCUSSION.

In the measurements here recorded it is to be noted that with sufficient compression the area occupied by molecules of these substances in films one molecule thick on water can be reduced nearly to that which their two paraffin chains alone would occupy when vertical to the surface and packed closely. The complexity of the polar groups connecting the two paraffin chains makes this remarkable. Compounds containing a single paraffin chain have been shown by Adam to resist compression to the area accounted for by that chain in varying degrees according to the nature of the polar group holding them

to the water. Thus the areas of films of α -bromo-fatty acids, acetamides, ureas and monoglycerides are larger than those of saturated fatty acids, alcohols or esters. Monopalmitin and monomyristin for instance [Adam, Berry and Turner, 1928] at 11° and a linear compression force of 5 dynes/cm. occupy about 25.5 sq. Å.; as the compressing force is increased up to 30 dynes/cm. the area contracts uniformly to 23 sq. Å., altogether about 10 %. With our compounds containing two paraffin chains the area at 5 dynes/cm. is for sphingomyelin 54 sq. Å., or 27 for each chain and for kersin 64, or 32 for each chain; at 30 dynes/cm. the area for sphingomyelin is reduced to 40, a contraction of 27 %, for kersin to 46, a contraction of 28 %; so that with this substance the area per chain at this pressure is the same as in the case of the monoglyceride. The compression curve is however not rectilinear with either sphingomyelin or kersin. With these substances therefore the area at low compression is definitely larger than for the monoglyceride, but they are more compressible than it is. It is remarkable that this should be so; not so much that at low pressure the completely immersible glyceryl group in $\text{R.COO.CH}_2\text{.CHOH.CH}_2\text{OH}$ should occupy less than half the area occupied by the complex polar group in these substances suspended between the two paraffin chains at its two ends, as that the latter should be more compressible. There seems to be more pliability in it than in the glyceryl ester group. The rigid bulkiness of this latter is not accounted for by the ester linkage; for with ethyl stearate the area of the molecule is reduced to that of the single paraffin chain at about 10 dynes/cm. [Adam, 1923, 2].

Measurements for compounds containing more paraffin chains than one exist only for the ester union of fatty acids to carbon atoms directly united to one another as in tripalmitin, glycol dipalmitate [Adam, 1922] and hydrolecithin [Leathes, 1923; Adam and Jessop, 1926, p. 368] of which the last is especially comparable with sphingomyelin. The unsaturated acid chains in lecithin make it non-comparable. At 5 dynes/cm. hydrolecithin occupies a smaller area, 47 sq. Å. or 23.5 for each fatty chain; at 20 dynes/cm. it contracts, 12 %, whereas sphingomyelin and kersin contract 22 and 23 % respectively; but the area it occupies at this pressure is 41.4 sq. Å., which is not more than is accounted for by the fatty chains.

Other compounds containing more than one paraffin chain for which data have been published by Adam [1923, 1] are cetyl palmitate, which is insufficiently anchored to the water to give stable films, and pentaerythritol tetrapalmitate [Adam and Dyer, 1924], which at 5 dynes/cm. occupied 22.5 sq. Å. per fatty chain and at 20 dynes/cm. 20 sq. Å.

Measurements such as those here recorded are clearly insufficient to enable a precise conception to be formed of the disposition assumed by the polar groups immersed in the water. But some idea is shown of the amount of distortion that they undergo under compression and of the amount of force necessary to overcome the strains set up by this compression. The thickness of a condensed film of palmitic acid is 24 Å., and in that case 1 dyne/cm.

means a pressure of about 4 atmospheres. The thickness of the layer formed by the polar chains in these molecules buckling under pressure must be very much less than this and the pressure when the force is 30 dynes/cm. accordingly very much more than 120 atmospheres.

The fact that the films formed by these substances on water should be definitely liquid at room temperature, at any rate under the action of a moderate compression force, is worthy of note.

SUMMARY.

The surface films of sphingomyelin, phrenosin and kerafin have been studied. It is found that for each substance the area occupied by the molecule in a closely packed surface film can be compressed to about 42 sq. Å. This is in good agreement with what would be expected from the configurations of the molecules as deduced from their chemical behaviour, on the supposition that the limiting value is the area occupied by the paraffin chains set side by side vertical to the water.

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