XVI. THE GALACTOSIDES OF THE BRAIN. III. LIQUID CRYSTALS AND THE MELTING POINT OF PHRENOSIN.

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The remarkable ease with which the brain galactosides are transformed into the liquid-crystalline state, was first observed by Rosenheim and Tebb [1908] in connection with some other work on the brain lipoids and has since been confirmed by Prof. O. Lehmann, who kindly examined phrenosin and kersin (as well as sphingomyelin) prepared by the author. This phenomenon offers an explanation for the discrepant statements with regard to the melting points of these substances recorded in the literature. It further led to the discovery of the conditions under which phrenosin can be obtained in true crystalline form, which in its turn satisfactorily explains a hitherto unexplained property of phrenosin, for which the provisional term "Umlagerung" was used by Thierfelder and Wörner [1900]. A short description of my observations in this direction seems therefore justified.

(1) LIQUID CRYSTALS, TRUE CRYSTALS AND MYELIN FORMS OF PHRENOSIN.

Phrenosin as usually obtained on cooling its solutions, consists of a white "crystalline" powder, which, however, does not possess any definite crystalline form in the usual sense. Under the microscope it is seen to consist of separate ball-shaped masses of approximately uniform size, which are only slightly anisotropic.

The formation of liquid crystals is best observed when a small quantity of the dry powder is carefully heated on a slide on Lehmann's polarisation microscope¹ [1910] until it is completely fused. In this condition it will be found to be completely isotropic, i.e. invisible in the dark field between crossed nicols. On allowing the slide to cool slightly a shower of separate anisotropic

¹ A very convenient electrical hot stage has been devised by Lorrain Smith and W. Mair [1910].

needle-shaped liquid crystals shoots out on the dark background (see Fig. 1). They may be best observed in the thinnest layers of the preparation and when pressure is exerted on the cover glass.

I made the further observation that phrenosin not only exists in the intermediary liquid-crystalline state when heated below its "clearing point," but also when it is allowed to settle out from its hot solutions in various solvents. This fact may also easily be demonstrated by means of Lehmann's microscope possessing a heating arrangement, using an alcohol solution of phrenosin. The liquid-crystalline globules which appear at first solidify on complete cooling to nodular masses, radially striated, thus forming the "crystalline" white powder as described above. The liquid-crystalline modification may even be observed macroscopically, if the dry substance is warmed with insufficient solvent. It then forms transparent globules which collect at the bottom of the flask and which were thought to be due to "decomposition" by the early investigators of brain lipoids.

It occurred to me that it might be possible to prevent the primary formation of the liquid crystals by some means and thus favour the formation of true crystals. After many unsuccessful attempts with various solvents under many conditions, it was found that true crystals were formed when a hot 2% solution of phrenosin in 85% alcohol was allowed to cool very gradually and without shaking, in an unsilvered Dewar flask. The flask was firmly plugged with cotton wool and kept in a water-bath at 75°. The temperature of the water-bath was allowed to fall and when the solution had reached a temperature of 65°, the first crystals appeared. Their quantity increased slowly and at 61° the whole solution was filled with a mass of glittering crystals, which were seen under the microscope to consist of thin, transparent, well defined plates, closely resembling cholesterol crystals (see Fig. 2). Highly purified phrenosin may thus be obtained completely crystallised. After filtration and drying it appears as silky flakes, its macroscopical appearance also resembling that of cholesterol. The substance may be recrystallised in the same way, but on allowing its solution to cool gradually in the ordinary way, the usual micro-crystalline powder (solidified liquid crystals) is obtained.

The air-dry crystals, when obtained from dilute alcohol, contain one molecule of water¹. The water of crystallisation is not given up on drying *in vacuo* over concentrated H₂SO₄, but escapes on drying the substance at 105°.

¹ It is possible that the crystals obtained from absolute alcoholic or methyl alcoholic solution contain alcohol of crystallisation, but this question has not yet been experimentally investigated.

Fig. 1. Liquid Crystals of Phrenosin. $\times 90$.

Fig. 2. Crystallised Phrenosin. $\times 50$.

Fig. 3. *Left*: Phrenosin swelling up with water. *Right*: Early stage of Myelin forms. $\times 250$.

0.4253 g. crystallised phrenosin, on drying in a toluene bath, lost 0.0100 g. = 2.35 % H_2O . Calc. for $\text{C}_{48}\text{H}_{93}\text{O}_9\text{N} + \text{H}_2\text{O} : 2.13 \% \text{H}_2\text{O}$.

On combustion of the substance, dried at 105° , the following results were obtained:

0.1300 g.; 0.3305 g. CO_2 ; 0.1291 g. H_2O . 0.0960 g.; 1.7 cc. N at 17.5° and 722.5 mm.

Calc. for $\text{C}_{48}\text{H}_{93}\text{O}_9\text{N}$

C	69.34	69.65
H	11.11	11.24
N	1.91	1.70

The fact that phrenosin may, under certain conditions, be obtained in the true crystalline condition offers an explanation for an interesting observation made by Thierfelder and Wörner [1900] many years ago. They found that when "cerebron" (= phrenosin) was kept at 50° suspended in 85 % alcohol or at 60° in methyl alcohol-chloroform [Thierfelder and Loening 1910], the previously "crystalline" product was gradually transformed into true crystals. This process was assumed to be due to an intra-molecular change (Umlagerung) but Thierfelder was at a loss to explain this "Umlagerung," which he showed was not due to the entrance of alkyl-groups into the molecule. I have repeated this experiment successfully with all my samples of pure phrenosin in the solidified liquid-crystalline condition and would suggest, in the light of the above observations, as an explanation of Thierfelder's "Umlagerung," that it consists simply in the gradual change of the solidified liquid-crystalline modification into the true crystalline condition at the temperature which is favourable for its formation¹.

Another characteristic property of phrenosin, which is related to the liquid-crystalline condition, consists in its tendency to give rise to myelin forms in the presence of water. If a fine suspension of phrenosin in water is gently warmed on a slide under the microscope, the small ball-shaped nodules swell up and are seen to be suddenly covered with spicules, which gradually develop into myelin forms (see Fig. 3). I may add the further observation that under the polarising microscope and a selenite plate, these peculiar forms behave exactly like the myelin forms obtainable from ammonium oleate or lecithin. In the paragonal position they show the subtraction colour (yellow) and in the epigonal position the addition colour (purple to blue).

¹ Phrenosin can only be obtained in the true crystalline state if certain conditions are observed, a fact which brings out the analogy of the brain galactosides to the vegetable glucosides, especially to those containing galactose like the digitalis glucosides. The latter have also so far only been separated by physical means, and digitonin, for instance, can only be obtained crystallised under certain conditions. Even then it may still contain the amorphous galactoside, gintonin, as has recently been shown by Windaus and Schneckenburger [1913].

The myelin forms appear on slight warming far below the boiling point of water, and indications of them may be seen even at 37°¹. If a slide is kept at this temperature and then allowed to cool it will be seen that water has been taken up by the phrenosin powder, transforming it into transparent globules. Under the polarising microscope they are now strongly anisotropic, show the black cross of spherocrystals and behave with a selenite plate exactly like the spherocrystals of phrenosin as obtained from pyridine [see Rosenheim, 1914].

(2) THE MELTING POINT (CLEARING POINT) OF PHRENOSIN.

The melting points recorded in the literature for phrenosin (pseudo-cerebrin, cerebrin, cerebron) show remarkable variations. Thudichum gave it at 176° and the subsequent statements vary from 170° (Parkus) to 212° (Thierfelder and Wörner). The last investigators state that the substance becomes "moist" at 130° and gradually covered with fine droplets, at 200° it becomes slightly yellowish, and fuses at 209° on slow heating, at 212° on rapid heating, to a clear yellowish fluid.

In my first observations on phrenosin (prepared according to Thudichum as well as by the pyridine method), I noticed a similar behaviour. On slow heating in a Thiele apparatus the samples soften between 130°–140°, shrink together and partially fuse between 170°–190°, and melt to a clear fluid at 205°–206°. On rapid heating the same substances melt, after the preliminary softening at 130°–140°, fairly sharply at 212°–215°. According to the rate of heating and the width of the tube, it is possible to obtain what appears as a melting point at any temperature between 170°–215°. Crystallised phrenosin behaves exactly like ordinary "crystalline" phrenosin.

An explanation for this peculiar behaviour was found in the above described discovery that this substance exists in the liquid-crystalline condition between the temperatures of 95° and about 210°. This fact may also be very conveniently demonstrated, without a polarising microscope, by the help of the arrangement recommended by Stoltzenberg [1911]. A thin glass rod is introduced into the melting point tube and the liquid-crystalline condition is recognised by pressure exerted on the glass rod. Under these conditions it was found that phrenosin enters into the liquid-crystalline

¹ G. F. Göthlin [1913] was unable to observe this fact, because he evidently made his observations in the absence of any heating arrangement. The myelin forms of pure phrenosin were first observed on my preparations in Prof. Aschoff's laboratory [see R. Kawamura 1911]. L. Smith and W. Mair [1910] have also made similar observations on the "cerebrosides" prepared by their method.

condition below 100° (approximately at 95°) and forms on pressure a perfectly clear viscous fluid at 130° , showing a final clearing point at 212° – 215° . The liquid-crystalline phase evidently possesses a greater viscosity than the liquid-isotropic phase and the change from one to the other may be readily observed.

The discrepant statements about the melting point of phrenosin, which were quoted as evidence against the identity of the various preparations, are satisfactorily explained by the existence of the liquid-crystalline modification. Some observers evidently considered the substance as fused during the liquid-crystalline phase, whilst others took the temperature of formation of the isotropic-liquid phase as the melting point.

In view of the above observations there is no justification to speak of a "melting point" of phrenosin, the proper expression being the "clearing point," i.e. the temperature at which it changes from the liquid-crystalline anisotropic condition into the isotropic liquid-amorphous condition. [See also Lapworth, 1913.]

It may be mentioned here that kersin behaves in every respect similarly to phrenosin. It gives rise to liquid crystals and to myelin forms, although the latter are not so readily produced as with phrenosin. Its clearing point is, however, much lower, namely 180° . Further I have so far been unable to obtain it in the truly crystallised condition like phrenosin. This fact may be connected with the constitution of the fatty acid (lignoceric acid) contained in kersin, which, in distinction from phrenosinic acid, does not contain the hydroxyl group in its molecule. It may further be assumed that, in analogy to the derivatives of cholesterol, the ability of the galactosides to exist in the liquid-crystalline condition is mainly dependent on their basic constituent sphingosine, which resembles cholesterol in so far as it has the constitution of an unsaturated alcohol¹.

Prof. O. Lehmann, to whom I submitted samples of phrenosin and kersin for more detailed investigation of their liquid-crystalline modification, has kindly given me permission to communicate the following abstract of his observations which he intends to publish in full elsewhere.

"The first of the two preparations of phrenosin received (labelled "crystallised") consists of thin transparent plates, approximately rectangular, and

¹ The property to form liquid crystals is not limited to the brain lipoids. I have found that "carnaubon" which was first obtained by Dunham and Jacobson [1910] from ox kidneys also possesses a liquid-crystalline phase. According to some preliminary experiments of the author the main fatty acid of carnaubon seems to be identical with lignoceric acid and carnaubon itself appears to be a mixture of a galactoside of the kersin type with a phosphatide of the sphingomyelin type. The fact that these lipoids form mixed liquid crystals explains the difficulty of their separation.

slightly anisotropic; the second of small globules of fairly uniform size which show between crossed nicols the black cross of spherocrystals and which had probably been formed by solidification of globular myelin forms. The behaviour of both preparations is identical. On warming the dry substance it is suddenly transformed into the anisotropic liquid-crystalline modification and on further heating into the isotropic melt. The liquid crystals depositing from the latter do not show any tendency to run together, but they are deformed by pressure on the coverslip just like other liquid crystals. If the solid crystals are brought into water and gradually warmed, the change into the liquid-crystalline modification takes place below the boiling point of water. The liquid crystals assume the shape and structure of myelin forms. The ball-shaped solid structures are suddenly covered with radiary processes which, however, are not pointed but of uniform thickness and with rounded ends. On cooling, all the spicules suddenly contract to about one half of their size and their contents are transformed into an aggregate of fine plates of the solid modification. By repeated warming and cooling the extension and contraction of the rod-like myelin forms (liquid crystals) may be produced over and over again. We have to deal here with changes of form of a similar kind to those observed previously by Lehmann with protocatechuic acid [see Lehmann, 1911, p. 333]. Phrenosin forms with lecithin and ammonium oleate mixed liquid crystals in all proportions. Even with a high percentage of phrenosin the liquid crystals which separate out of dilute alcohol show the phenomenon of flowing together just as those of pure ammonium oleate (or lecithin).

By means of some aniline dyes the myelin forms of phrenosin may be artificially coloured, similarly to those of ammonium oleate. The phenomena are most striking, if the water in which the phrenosin granules are allowed to swell up contains just sufficient methylene blue to colour it faintly. The myelin forms, appearing on warming, will be seen to be stained intensely blue. On contraction by cooling, the blue colour changes into a reddish violet. If the processes are extended again to their former length by warming, the reddish violet colour just as suddenly changes into blue.

Kerasin behaves similarly to phrenosin, when heated in the dry state. At a certain temperature it suddenly passes into the liquid-crystalline modification, which changes on further heating into the isotropic melt. Warmed with water it swells up enormously, forming myelin forms which are only slightly more refractive than water. In consequence of their high water content they are of a slimy fluid consistency. With methylene blue they only stain very slightly, their size being reduced by the admixture of

the dye. On heating a few grains of the original substance with water to a higher temperature, it is transformed into the liquid-crystalline modification and then myelin forms appear which are distinctly anisotropic and easily stained. On cooling, the two kinds of myelin forms solidify, suddenly undergoing a contraction just like those of phrenosin. The solidified myelin forms are not brittle, but still soft and plastic. Kerasin forms with lecithin and ammonium oleate mixed liquid crystals in all proportions just like phrenosin."

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SUMMARY.

1. The galactosides phrenosin and kerasin exist in the liquid-crystalline condition at temperatures from below 100° up to 180° (kerasin) and 215° (phrenosin).

2. This fact explains the divergent statements with regard to the melting point of these substances. Instead of a melting point they possess a "clearing" point, i.e. a point at which the anisotropic liquid-crystalline phase changes into the isotropic liquid-amorphous phase.

3. They give rise to myelin forms on warming with water which possess the same optical properties as the myelin forms of lecithin, etc.

4. The conditions are described under which true crystals of phrenosin may be obtained.

5. An explanation is thereby afforded for Thierfelder's "Umlagerung" of phrenosin.

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