LIV. CUORIN.

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THE name cuorin was given by Erlandsen [1907] to a new lipin substance which he isolated from the ether extract of heart muscle. In many of its properties cuorin was very similar to kephalin, but differed from this substance in the relative amounts of nitrogen and phosphorus present in the molecule. It formed one of a constantly increasing group of alleged new lipins isolated chiefly by the German school about a decade ago. At that time it seemed as if the number of different lipins present in the tissues was very great, while their complexity of composition and the difficulties experienced in isolating them made this interesting field of biochemistry appear to be almost hopelessly difficult. Gradually, however, as the result of further research, it became obvious that many of these alleged new bodies had no claim to be regarded as unit substances and were largely mixtures or decomposition products of well-known lipins. Thus MacLean [1918] was able to show that the number of phosphatide lipins present in animal tissues could with certainty be reduced to four—lecithin, kephalin, sphingomyelin and cuorin—but that even this short list was probably too long, since the evidence for the existence of cuorin as a chemical entity was meagre in the extreme. In this connection he remarked that there was no definite proof that the substance was not a mixture, and that though undoubtedly a substance resembling Erlandsen's cuorin could be obtained from tissues, yet the conditions under which this substance was isolated did not exclude the possibility of the body being an artificial product. This opinion was recently substantiated by Levene and Komatsu [1919], who, in a very fundamental investigation into the structure of kephalin, came to the conclusion that cuorin was a mixture composed chiefly of kephalin with certain degradation products.

A review of the literature disclosed the fact that many of the cuorin-like substances isolated differed materially in their nitrogen to phosphorus ratio, and that while some of them had a N : P ratio of 1 : 2, in others this ratio was as high as 1 : 1.5 or even more. All this lent force to the view that cuorin was probably a substance which did not exist in fresh tissue as such, but was formed as the result of the various manipulations utilised in the extraction of the lipins. On this hypothesis an attempt was made to isolate the lipins *from*

very fresh tissues as rapidly as possible, using the purest of solvents, in the hope that decomposition would be largely prevented. If cuorin were a decomposition product it should be possible to prevent its formation, and no substance of this nature should then be found in the tissue. By very careful and rapid manipulation of heart muscle, we found that the substance which corresponded to Erlandsen's cuorin had a N : P ratio of nearly 1 : 1 instead of 1 : 2 and was really slightly impure kephalin. It is, therefore, certain that cuorin is an artificial product and has no further claim to be regarded as a definite lipin. This observation simplifies to a considerable extent the classification of the lipins, for it is now reasonably certain that the tissues contain only three phosphatides—lecithin, kephalin and sphingomyelin—and that the addition to these phosphatides of the two cerebrosides—phrenosin and kerasin—completes the list of lipin substances as we know them to-day. Although the constitution of all these bodies has not yet been definitely ascertained, much light has recently been thrown on the question, and soon we may hope to know as much of the structure of the lipins as we do of such a well-defined group as the carbohydrates.

EXPERIMENTAL.

Ox hearts, obtained immediately after death, were freed from macroscopic fat and passed through a mincing machine. The resulting material was dried by stirring it up for a short time in a fairly large volume of pure acetone; the mixture was then strained through a piece of muslin, bound up in sailcloth, and freed as much as possible from acetone by the use of a powerful press. The tissue, which was now almost dry, was broken up and spread on a glass plate under a fan for about an hour during which it was constantly broken up with a spatula. It was then passed through a coffee mill and a fine powder obtained. The whole process was carried through as expeditiously as possible, so that the tissue was obtained in the form of a fine dry powder ready for extraction, a few hours after the death of the animals. In our opinion, this is most important, as there is little doubt that decomposition often takes place while the tissue is being dried, especially when this is done more or less slowly at a moderately high temperature, as is often the case.

The next step was to extract the powder with absolute alcohol in the usual way until the extract contained little or no residue on evaporation of the alcohol. After thorough treatment with alcohol the tissue was finally extracted with ether.

The alcohol extracts were mixed, the alcohol removed at ordinary temperature by means of a vacuum pump, and the thick syrupy mass remaining taken up with pure ether. Insoluble substances were removed by the centrifuge and the ether extract treated with excess of acetone. This process was repeated several times. The final precipitate was purified by water and acetone [MacLean, 1912], and the waxy substance obtained dried *in vacuo*. By further treatment with ether followed by centrifuging, some remaining

sphingomyelin was separated, and ultimately a substance was obtained which gave a clear solution in ether. This ethereal solution was again treated with acetone and the precipitate dried *in vacuo* over sulphuric acid. The dry material was then thoroughly shaken up with excess of alcohol, when part of it remained insoluble. The alcohol-soluble fraction consisted of more or less pure "lecithin," and on analysis gave N 1.97 % and P 4.05 %; N : P = 1 : 1.07.

The alcohol-insoluble portion should of course contain kephalin and Erlandsen's cuorin. After dissolving in ether and reprecipitating with acetone it gave on analysis 1.6 % of N and 4.1 % of P. This substance was then treated exactly according to Erlandsen's directions for separating cuorin from kephalin, when a small amount of a body corresponding in solubility and physical properties to Erlandsen's cuorin was obtained. This product which, if it were cuorin, should have a N : P ratio of 1 : 2 gave on analysis 4.06 % P and 1.66 % N, *i.e.* a N : P ratio of 1 : 1.1. The substance was therefore more or less impure kephalin, and no evidence of the presence of a substance of the composition of cuorin could be obtained. It would thus seem that cuorin is only found under conditions where decomposition is liable to take place and is really a mixture containing some decomposition products.

Subsequent treatment of the alcohol-extracted tissue with ether showed that practically all the lipin material present was removed by the alcohol. The ether extract contained only mere traces of phosphatides, and no body of the nature of cuorin was present.

CONCLUSIONS.

The cuorin described by Erlandsen is not a chemical unit, but results from decomposition taking place in the tissues during the processes utilised for the extraction of the lipins. This decomposition is probably most active during the drying of the tissue. When precautions are taken to dry the perfectly fresh tissue very quickly by means of acetone, no cuorin is obtained.

The only phosphatides present in animal tissues are lecithin, kephalin and sphingomyelin.

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