XI. ON THE INOSITOL OF BRAIN AND ITS PREPARATION.

By GORO MOMOSE.

From the Physiological Laboratory, King's College, London.

(*Received January 19th, 1916.*)

Shortly after Scherer's [1850] discovery of inositol in muscle, von Bibra [1854] carried out a careful but unsuccessful search for this substance in brain. The presence of inositol in brain was first demonstrated by W. Müller [1857], and L. W. Thudichum [1884] is the only author who has since given attention to its occurrence.

The preparation of inositol from brain (and other organs) presents certain difficulties owing partly to the small amounts present and partly to the lack of trustworthy methods. The usual procedure, which has not varied much since Scherer [1850] first used it in the case of muscle, consists in extracting the organs with water and in precipitating the inositol from the suitably purified extract as an insoluble metallic compound by means of either basic lead acetate, copper acetate or baryta. From its combination with lead, copper or barium the inositol is set free by means of sulphuretted hydrogen or by sulphuric acid and finally crystallises out from the concentrated solution on the addition of alcohol.

W. Müller [1857] ground the brain with neutral lead acetate and water, allowed the mixture to stand and thus obtained a blood-red extract, which could be easily filtered and from which he was able to isolate inositol by means of basic lead acetate. The objection to this method is that it offers no guarantee of the complete extraction of the inositol, which moreover is usually obtained in a very impure condition in the first instance.

The difficulty encountered by von Bibra [1854] of filtering a watery extract of brain is easily overcome by gradually introducing the finely minced brain into boiling water and by acidifying with acetic acid before filtration. It

was to be expected that by a repeated extraction under these conditions practically the whole of the inositol would be removed. To my surprise however I found that when working up 1 kilo. of fresh ox-brain in this way, I was unable to obtain even a trace of inositol.

This result seems at first sight to be in favour of the view of F. Rosenberger [1908], according to which inositol does not occur as such in fresh organs, but is formed post-mortem by an autolytic or enzymic process from a precursor called "inositogen." This hypothetical substance was, however, not isolated as such and its existence was only inferred from the failure to isolate inositol itself. Rosenberger elaborated a method for the isolation of inositol from organs which is open to serious objections. He made use of the resistance of inositol to strong alkali in order to set it free from the tissues in a way similar to that used for the estimation of glycogen. The tissues are boiled with potash until completely dissolved and the solution is concentrated to one-third of its original volume after the addition of an excess of strong nitric acid. Hot baryta is next added and the heating continued for fifteen minutes, after which time the solution is again acidified and concentrated to a syrup. This is heated with concentrated nitric acid until the violent reaction is over, and again neutralised with baryta. If necessary the treatment with nitric acid and baryta is repeated several times. The final solution is cleared by neutral lead acetate and from the filtrate any inositol, which has survived this prolonged treatment, is precipitated by basic lead acetate in the usual way.

The negative results so often yielded by this method may well be due to the destruction and oxidation of inositol by the repeated treatment with strong nitric acid, as has been pointed out by E. Starkenstein [1908]. Moreover, the presence of the large quantities of salts introduced during the process may easily lead to the loss in the first lead precipitate of the small quantities of inositol normally present. It had already been noted by Thudichum [1884] that under certain conditions neutral lead acetate may carry down inositol and Meillère [1906] has traced this to the presence of salts.

It is not unlikely that von Bibra's and my own failure to isolate inositol from a brain extract made with boiling water is due to the same reason. I have not examined this subject any further, but as I was able to isolate inositol, by a method to be described, from the same lot of brain which failed to yield a trace by the usual extraction method, I incline to the view that as far as the brain is concerned the existence of "inositogen" is very unlikely.

I finally succeeded in finding a method which enables the preparation of inositol from brain to be made with certainty by making use of an observation kindly communicated to me by Dr O. Rosenheim. In the course of the method introduced by him [1906, 1914] for the preparation of lipoids from brain, acetone was used in the first instance for the dehydration of brain and the preparation of cholesterol. Rosenheim observed that the first watery acetone extract prepared at room temperature was free from cholesterol and that from it inositol could be easily prepared.

I was able fully to confirm this observation and found that this method of extraction of inositol leads more quickly to a pure product than W. Müller's original method and that the yield also is greater. I have not yet applied the method to any other animal organs or plants, but it is to be expected that it might be found useful in this case also, especially as the amount of inorganic salts, which interfere with the preparation of inositol, is considerably decreased when acetone is used as a solvent.

In his work on inositol, Thudichum [1884] suggests that the inositol from human brain "is either altogether different from that contained in the brain of the ox, or is accompanied by another similar carbohydrate of less stable quality." This conclusion was arrived at by the analysis of the copper compounds of a well-crystallised and apparently perfectly pure specimen of inositol from human brain, which contained up to 6 % more copper than the corresponding compounds prepared from bovine inositol.

This statement of Thudichum's, so many of whose originally discredited observations have since been confirmed, warranted further investigation. It is now known that inositol may exist in several optically isomeric modifications, although its accepted formula does not contain an asymmetric carbon atom. It was pointed out by O. Aschan [1902] that van't Hoff and Le Bel's theory allows the existence of seven inactive forms besides the two active ones. Of these *d*- and *l*-inositol are known to occur as methyl esters in plants, whilst the ordinary inositol of animals and plants represents one of the seven inactive forms. It has, however, recently been shown by Johannes Müller [1907] that a second inactive modification of inositol is represented by scyllitol, a substance discovered by Staedeler and Frerichs [1858] in the kidneys, liver and other organs of certain plagiostomous fishes (i.e. elasmobranchs such as the shark, dogfish, etc.). The same substance occurs also in acorns, originally called "quercine" by Vincent and Delachanal [1887] and in the leaves of coconut palms (*Cocos nucifera* and *Cocos plumosa*) from which it was isolated as "cocositol" by Hugo Müller [1907, 1912]. The two inactive

inositols resemble each other in their solubilities and chemical properties, but possess melting-points differing by as much as 125°.

I prepared inositol from brain by the method described above and from a comparison of their physical constants I came to the conclusion that the inositol occurring in human brain is identical with that of ox brain and the ordinary *i*-inositol. Thudichum's analytical figures therefore only prove that the copper compounds of inositol are of varying composition and not suitable for the identification of inositol.

Experimental.

(1) *The preparation of inositol from brain.*

Several attempts to isolate inositol by the usual methods from a brain extract made with boiling water were unsuccessful, not even enough material for a colour reaction being obtainable. Through Dr de Souza's kindness an acetone extract of 10 kilo. of ox-brain, which he did not require for the purpose of his work, was put at my disposal. This was freed from acetone by distillation and the remaining watery extract filtered. To the filtrate neutral lead acetate was added in excess. The resulting precipitate was filtered and washed. To the filtrate basic lead acetate and ammonia were added, the precipitate again filtered, suspended in hot water and decomposed with sulphuretted hydrogen. The lead-free filtrate was concentrated to a small bulk and alcohol added to the hot solution until crystallisation of inositol began. The crude inositol, which weighed about 4 g., was recrystallised from water and a well-crystallised product, showing a melting-point of 225°, was easily obtained. Having made myself familiar with the properties of inositol, I proceeded to compare the new acetone method with that of W. Müller's [1857] by which he originally obtained inositol from brain and with the method of Scherer [1850] which is successful in the case of muscle.

For this purpose a large quantity of fresh ox-brain was passed through a mincing machine. Three portions of 1 kilo. each were weighed out and worked up by different methods.

(*a*) *Müller's method.* One kilo. of minced brain was ground with solid lead acetate and one litre of water into a thin emulsion. After allowing the mixture to stand for about 18 hours a clear blood-red solution had separated from the brain mass, and this was filtered through muslin. The solution was boiled and filtered again. Basic lead acetate and ammonia were now

added, the bulky precipitate allowed to settle for some hours and filtered. According to my experience it is not advisable to wash this precipitate with distilled water, as the inositol-lead compound seems to dissociate on prolonged washing. It may, however, be washed with a solution of ammonium carbonate. The precipitate is next suspended in hot water and decomposed with sulphuretted hydrogen. The lead-free solution is concentrated to a small bulk (about 20–30 cc.) and absolute alcohol added until the crystallisation of inositol begins. After standing for 24 hours in the ice chest, the inositol is filtered and washed with alcohol and ether. The yield of crude, rather yellowish inositol was 0·22 g.

(*b*) *Scherer's method.* One kilo. of minced brain was gradually introduced into one litre of boiling water and acidified with acetic acid. After cooling a clear filtrate was easily obtained. The extraction with boiling water was repeated once more. The combined filtrates were precipitated with neutral lead acetate, the precipitate filtered and well washed. The filtrate from the neutral lead acetate was treated with basic lead acetate and ammonia as before. The final solution remained perfectly clear on the addition of absolute alcohol and I was unable to separate from it any substance giving the colour reactions typical for inositol.

(*c*) *Acetone method.* One kilo. of minced brain was well shaken up with one litre of acetone, left standing for about 18 hours and strained through several layers of muslin. The treatment with acetone was repeated, the combined extracts heated to boiling and filtered. From the clear yellowish filtrate the acetone was removed partly by distillation and finally, after the addition of water, on the water-bath. The watery solution was treated with lead acetate as before. The final lead precipitate was much less bulky than those obtained in the previous methods and the inositol, crystallising out on the addition of alcohol, was nearly white. The yield of crude inositol was 0·32 g.

(2) *The identity of the inositol of human and ox brain.*

A sample of inositol from human brain was prepared by the acetone method above described. If Thudichum's [1884] suggestion that the inositol of human brain is not identical with that of ox brain were correct, we should expect the inositol from human brain to represent either one of the optically active isomeric forms of inositol or another of the possible seven inactive forms. In both cases an examination of the behaviour towards polarised light and a determination of the melting-point

would form a sure criterion. The melting-point of scyllitol, the only other known form of *i*-inositol, is 350° [H. Müller, 1912], whilst ordinary inositol melts at 225°.

I examined 10 % solutions of inositol prepared from human and ox brain in a large Lippich-Polarimeter (with three field division) and failed to find in either case any optical activity.

The melting-point of two carefully purified specimens was 225°. A mixed sample showed no alteration of melting-point. Another sample mixed with *i*-inositol prepared from seeds (phytin) fused also at 225°.

Both preparations possessed the same solubilities and crystalline form and gave Salkowski's [1910] modification of Scherer's reaction, alike qualitatively as well as quantitatively.

The inositol of human brain agrees in its behaviour towards polarised light, in its melting-point and colour reaction with the inositol of ox brain, and both are identical with the ordinary *i*-inositol of plants.

The expenses of this investigation have been defrayed out of a grant made to Dr Rosenheim from the Government Grant Committee of the Royal Society.

Conclusions.

1. A trustworthy method for the preparation of inositol from brain, based on its solubility in dilute acetone, is described.

2. The inositol of human brain is identical with that of ox brain and with the ordinary *i*-inositol of plants.

REFERENCES.

Aschan, O. (1902), *Ber.* **35**, 3389.
Bibra, E. von (1854), *Vergl. Unters. u. d. Gehirn, etc.* (Mannheim), 63
Meillère, M. G. (1906), *J. Pharm. Chim.* (6), **24**, 241.
Müller, H. (1907), *J. Chem. Soc.* **91**, 1767.
—— (1912), *J. Chem. Soc.* **101**, 2383.
Müller, J. (1907), *Ber.* **40**, 1821.
Müller, W. (1857), *Annalen*, **103**, 131.
Rosenberger, F. (1908), *Zeitsch. physiol. Chem.* **56**, 373.
Rosenheim, O. (1906), *J. Physiol.* **34**, 104.
—— (1914), *Biochem. J.* **8**, 110.
Salkowski, E. (1910), *Zeitsch. physiol. Chem.* **69**, 478.
Scherer, S. (1850), *Annalen*, **73**, 322.
Staedeler, G. and Frerichs, F. Th. (1858), *J. pr. Chem.* **73**, 48.
Starkenstein, E. (1908), *Zeitsch. physiol. Chem.* **58**, 162.
Thudichum, L. W. (1884), *The Chemical Constitution of the Brain* (London), 205.
Vincent, C. and Delachanal, B. (1887), *Compt. rend.* **104**, 1855.