XXVII. THE PREPARATION FROM ANIMAL TISSUES OF A SUBSTANCE WHICH CURES POLYNEURITIS IN BIRDS INDUCED BY DIETS OF POLISHED RICE.

PART I.

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(Received April 8th, 1913.)

Although several workers have isolated from various food-stuffs substances capable in small amount of curing polyneuritis in pigeons and have ascribed chemical formulae to them, attempts to prepare the active constituent in an amount sufficient for investigating its chemical constitution and properties have up to the present time been unsuccessful.

Funk [1911, 1913] has isolated from rice polishings, yeast, milk, and ox-brain substances curative in oral doses of 0.02 g. and possessing similar melting-points.

Edie, Evans, Moore, Simpson, and Webster [1912] have also prepared a substance from yeast curative in small amount, to which they have ascribed the formula $C_7H_{17}O_5N_3$, and Suzuki, Shimamura, and Odaki [1912] isolated an active substance containing nitrogen from rice-polishings in the form of a picrate, but they have not published any results of its analysis.

1. ISOLATION OF AN ANTI-NEURITIC SUBSTANCE FROM HORSE-FLESH.

In the present communication a method is described for preparing from horse-flesh a substance capable in minute amount of curing polyneuritis in pigeons.

The method employed was a modification of that described by Maclean [1912, 1] for the isolation and purification of the phosphatides of the kidney. The horse-flesh was minced, dried at 30° by means of an electric fan and ground, and the dry powder (weight 4000 g.) extracted at 37° with absolute alcohol on a shaking machine. The filtered extract was evaporated at 40° in

vacuo to remove the alcohol. The extract (weight 500 g.) was found to possess marked curative properties towards pigeons affected with polyneuritis, 4 g. being sufficient to bring about complete recovery within 24 hours. The extract was then treated with an excess of ether and the mixture allowed to stand in the cold room for 12 hours. The ether dissolved the fats and lipoids, but left undissolved a considerable amount of a white substance. This was washed thoroughly with ether and then tested on neuritic pigeons. Doses of 0.3 g. were sufficient to bring about complete recovery within 12 hours. This procedure therefore afforded a simple technique for separating a highly curative fraction from fats and lipoids and it was much more convenient than extracting the active substance by means of water.

The ether-soluble fraction was freed from ether by evaporating *in vacuo*. As much as 12 g. of the residue possessed only slight curative properties, while 2 g. of the lipoids separated from this fraction by means of acetone were inactive, but 7 g. were curative within 24 hours. It was thus possible to separate the bulk of the anti-neuritic substance from the alcoholic extract of horse-flesh by means of ether. These results support the explanation advanced by Maclean [1912, 2] for the curative properties of lecithin observed by several workers, namely, that the anti-neuritic substance is not a lipoid, but is present in ordinary lecithin as an impurity readily extractable therefrom by simple methods.

The ether-insoluble fraction was next treated with absolute alcohol, by which a large portion was dissolved. The insoluble residue contained both inorganic and organic material, but possessed no curative properties. The alcohol-soluble fraction however was strongly curative, and it was treated with excess of ether. This caused the separation of a yellow syrup (yield 50 g.) which was completely soluble in water and 0.2 g. of which was sufficient to cure pigeons affected with polyneuritis. The aqueous solution of the syrup was allowed to stand in a vacuum desiccator and a white crystalline substance gradually separated. This proved to be carnine and possessed no curative properties. The filtrate, which was highly curative, was next treated with finely powdered lead acetate, until there was no more separation of a flocculent precipitate, and the mixture was allowed to stand for 12 hours. The precipitate was filtered off, washed with water at 35°, decomposed with sulphuric acid, and the excess of acid removed by the careful addition of baryta. The filtered solution possessed no curative properties. The filtrate from the lead acetate precipitation was freed from lead by careful treatment with dilute sulphuric acid and was left very slightly acid. It possessed curative properties and was next treated with silver nitrate, which produced

a copious yellowish-white precipitate. This was filtered off, decomposed by hydrochloric acid and the resulting solution was nearly neutralised with soda and was found to be curative. The filtrate from the silver nitrate precipitation was also curative, but its content of active substance was completely precipitated by silver nitrate when baryta was added. By carrying out several animal experiments it was found that at least $\frac{3}{5}$ of the total amount of anti-neuritic substance present in the filtrate from the lead acetate precipitation was precipitated by the addition of silver nitrate only, and about $\frac{1}{4}$ was carried out of solution by the subsequent addition of baryta. The remaining $\frac{3}{20}$ was probably destroyed by the alkali. The residue obtained by the evaporation of the curative solution resulting from the decomposition of the first silver nitrate precipitate with hydrochloric acid was next extracted with chloroform. Only a small amount of substance was extracted and this possessed no curative properties. The anti-neuritic substance was therefore insoluble in chloroform. It was also found to be insoluble in benzene and ethyl acetate. The residue insoluble in chloroform was next extracted with absolute alcohol; a large amount was insoluble, but the curative substance was found to have been entirely dissolved, and 0.10 g. of the soluble fraction was sufficient to cure a pigeon affected with polyneuritis. This fraction was then extracted with acetone and a considerable amount was dissolved. The insoluble residue, which contained all the active substance, was dissolved in 50 % alcohol, and the solution allowed to stand in a desiccator for some days. A white substance gradually crystallised out, 0.06 g. of which administered to each of two pigeons ameliorated the symptoms of polyneuritis in a few hours and effected a complete recovery within 24 hours. The substance dissolved with moderate readiness in water, but was insoluble in absolute alcohol.

It was not possible to obtain a sufficient amount of the active substance to determine its chemical composition, and the work is therefore being repeated on a much larger scale. As, however, it was found [Cooper, 1913] that cardiac muscle contained considerably more anti-neuritic substance than voluntary muscle, ox-heart has been substituted for the horse-flesh and the results so far obtained justify this change.

Some experiments have also been carried out to ascertain if the anti-neuritic substance could be precipitated by means of ether from the fats and lipoids derived from other animal-tissues. It was found that considerable amounts of curative fractions could be obtained by this procedure not only from horse-flesh and ox-heart, but also from horse-kidney, beef, ox-brain and liver.

Only a small amount of material, however, was separated by ether from the alcoholic extract of egg-yolk, and its curative power was very feeble. The results indicate that this fractionation affords quite a general method for separating the anti-neuritic substance from the fats and lipoids of animal tissues.

2. THE ABSORPTIVE CAPACITY OF ANIMAL CHARCOAL FOR THE ANTI-NEURITIC SUBSTANCE.

Chamberlain and Vedder [1911, 1912] found that an extract of rice-polishings containing the anti-neuritic substance after being filtered through bone-black could no longer prevent polyneuritis in fowls and that the active substance could only be extracted to a small extent from the charcoal by water or alcohol. As these results are of great importance, the experiments have been repeated. Powdered animal charcoal was extracted three times with boiling water and then dried. A curative solution of known activity (prepared by dissolving the ether-insoluble fraction derived from an alcoholic extract of horse-flesh in water) was filtered slowly through a bed of the charcoal six times. The filtrate was still curative, but its activity was reduced to the extent of 30 %. Separate portions of the charcoal were then extracted with water and alcohol, and the extracts were found to be highly curative. The anti-neuritic substance was thus partially absorbed by charcoal and could be recovered from the latter by extraction with water or alcohol. It is seen that the results do not agree entirely with those obtained by Chamberlain and Vedder, but it is unlikely that the use of bone-black as an absorptive agent will be of much value for the isolation of the anti-neuritic substance.

3. THE EFFECT OF SULPHURETTED HYDROGEN UPON THE ACTIVE SUBSTANCE.

In a preliminary fractionation of the horse-flesh extract sulphuretted hydrogen was employed to decompose the lead and silver precipitates and to remove the excess of the metals from solution, and during these processes a rapid disappearance of the active substance occurred. By substituting acids for the sulphuretted hydrogen (sulphuric acid for lead, hydrochloric acid for silver) it was possible to conduct the operations with a much smaller loss of active material.

These facts suggested that sulphuretted hydrogen destroyed the anti-neuritic substance. Accordingly, the effect upon activity of passing this gas

through a curative solution for four hours was investigated, but no evidence was obtained of any destruction of the anti-neuritic substance. The diminished activity during the above manipulations would therefore appear to have been due to the absorption of the curative substance by the colloidal metallic sulphides.

4. THE EFFECT OF ALKALI UPON THE ACTIVE SUBSTANCE.

Several investigators have observed that a considerable loss of anti-neuritic substance occurs when chemical operations involving the use of alkali are employed in the fractionations. It was thought desirable to throw some light on this matter by quantitatively studying the effect of treatment with alkali upon the curative power of the substance.

To a solution of known activity ammonia was added until its concentration throughout the total volume of liquid reached 10 %. The mixture was kept in a closed flask at ordinary temperatures for 24 hours, the ammonia then removed by a current of air, and the curative power of the solution redetermined. It was found that 50 % of the anti-neuritic substance had become destroyed by contact with the alkali.

These results indicate that the isolation of the anti-neuritic substance in large amount is only likely to be effected by employing chemical methods that do not involve the use of alkali and sulphuretted hydrogen.

5. THE EFFECT OF ALKALOIDS UPON PIGEONS AFFECTED WITH POLYNEURITIS.

Strychnine was found [Cooper, 1913] to prolong the lives of birds affected with polyneuritis but to exert no curative action. It was accordingly of interest to investigate the action of other alkaloids. Experiments have been carried out with quinine, cinchonine, and cinchonidine. The substances were dissolved in a trace of hydrochloric acid and administered to the birds orally.

Doses of quinine ranging from 0.01 to 0.1 g. exerted a temporary curative action. In six cases the symptoms were ameliorated for about three days and then became once more acute, further administration of quinine having no effect. In two other cases however the neuritic symptoms completely disappeared within a few hours, and one of the birds remained free from polyneuritis for four days, whilst the other, receiving daily in addition to polished rice 0.025 g. of quinine, remained healthy for 10 days and then died exhibiting

symptoms merely of weakness and not of acute polyneuritis. When given in amounts exceeding 0.1 g. however quinine had no ameliorative effect, but actually appeared to hasten the fatal issue.

Daily doses of quinine ranging from 0.01 to 0.025 g. administered to several pigeons fed on polished rice did not prevent or delay the development of neuritic systems.

Cinchonine in doses varying from 0.05 to 0.1 g. like quinine effected a marked improvement in the condition of pigeons suffering from polyneuritis, but within 48 hours the birds again exhibited the acute neuritic symptoms and died notwithstanding further doses of the alkaloid. Cinchonidine on the other hand had no ameliorative effect at all.

As quinine and cinchonine are extracted from cinchona-bark by means of acid, it was thought that their curative action might possibly be due to contamination with traces of the anti-neuritic substance present in the plants. The active substance is readily destroyed by heat, so that some light could be thrown on this matter by observing whether the alkaloids retained their curative properties after strong heating. It was actually found that after being heated at 125° for six hours quinine no longer had any ameliorative effect upon neuritic pigeons. As the alkaloid is not chemically altered by such treatment, it would appear that its curative action is due to the presence as an impurity of a minute amount of the anti-neuritic substance.

6. THE EFFECT OF ALCOHOL UPON BIRDS FED ON POLISHED RICE.

The administration of small doses of alcohol (0.5 c.c. three times daily) to pigeons fed on polished rice had no measurable effect upon the period of time elapsing before the occurrence of symptoms of polyneuritis, and thus appeared not to influence the utilisation of the supply of anti-neuritic substance distributed in the tissues of the birds.

This is presumptive evidence that alcoholic neuritis is not caused by any diminished capacity on the part of the organism to utilise the anti-neuritic substance which might be expected to result from the disturbing effects of alcohol upon the metabolism of the nervous system.

SUMMARY.

1. A fraction rich in the anti-neuritic substance can be precipitated from the fats and lipoids (alcoholic extracts) of various animal tissues by means of ether.

2. A method based on this observation is described for isolating from horse-flesh a substance small amounts of which can cure polyneuritis in pigeons.

3. The substance is insoluble in absolute alcohol, benzene, chloroform, ether, and ethyl acetate, but is moderately soluble in water.

4. The substance is absorbed to some extent by animal charcoal and is readily destroyed by alkali. It is not inactivated by sulphuretted hydrogen, but disappears in large amounts during chemical operations in which colloidal metallic sulphides are formed.

5. Quinine and cinchonine exert a temporary curative action upon birds affected with polyneuritis. After being heated at 125° for 6 hours, however, quinine has no ameliorative effect, so that its curative properties would appear to be due to its contamination with traces of the anti-neuritic substance derived from the cinchona bark.

6. The administration of small doses of alcohol to birds fed on polished rice does not affect the period of time elapsing before the occurrence of polyneuritis, and thus appears not to influence the utilisation of the supply of anti-neuritic substance distributed in the tissues of the birds. This suggests that alcoholic neuritis does not result from a diminished capacity of the organism to utilise the anti-neuritic substance.

In conclusion I am glad to have this opportunity of expressing my indebtedness to Dr Hugh MacLean for drawing attention to the curative properties of the residues obtained by treating alcoholic extracts of voluntary muscle with ether and for much valuable help in the course of the investigation.

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