Physiology — *"On the Nature of the Constituent of Intestine-Extracts, which exerts a Stimulating Influence upon the Gastro-Intestinal Movements."* By Dr. J. W. LE HEUX. (Communicated by Prof. C. A. PEKELHARING.)

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In 1912 an article was issued from the Pharmacological Institute of the Utrecht University entitled: "Zur Kenntniss der Entstehung der Darmbewegung" of Dr. WALTHER WEILAND [1]).

In this article WEILAND reports his striking experience that when different parts of the gastro-intestinal tract (stomach, small intestine, large intestine) of various animals (rabbit, cat, and dog), after being duly cleaned, are put in water of 38°, the fluid possesses after some time the quality to largely modify the movement of the surviving small intestine of these animals.

The effect of these aqueous extracts on the surviving small intestine, which is not specific for any type, evinces itself in a broadening of the contractions or in an increment of tonus, or in both, an effect, therefore, resembling that of pilocarpin also in that it can be counteracted by a small dose of atropin. On further inquiry WEILAND found that the active constituent of these extracts is not of a fermenting nature, but that after boiling and filtering the solution and evaporating it down to small bulk, the remaining dark-coloured residue was as active as the primary extract. Another purification was effected by treating with absolute alcohol the extract that had been evaporated to dryness on the waterbath and filtering it, by again evaporating the filtrate and subsequently extracting the residue with ether; the ether was then removed from the limpid filtered ether solution and the rest was dissolved in water. According to WEILAND a solution is obtained in this way that contains nearly the original quantity of the active constituents. To litmus the reaction of this solution is distinctly alkaline; it contains, however, only traces of nitrogen. The reaction after MILLON is negative, biuret-reaction faintly positive. Phosphotungstic-acid and phospomolybdic acid yield a large white precipitate, platinic-chloride a slight one. No precipitate resulted from potassium-mercury-iodide, potassium-

[1]) Arch. f. d. ges. Physiologie Bd. 147 S. 171 1912.

periodide, sublimate, and picric acid. In acetone the substance appeared to be insoluble. It now turned out that the extracts thus purified, did not only act upon the surviving small intestine, but also upon the intact animal. WEILAND'S RÖNTGEN-tests showed that a potent positive influence was exerted on the movements of the stomach and of the small intestine in intact cats and rabbits. The essential influence this substance (or these substances) seemed to have in originating the movement of the small intestine rendered further investigation necessary. Prof. MAGNUS now suggested to me to endeavour to abstract the active constituent of these extracts in a pure condition and to determine the chemical structure. The present paper is a provisional report of this inquiry.

Initially I prepared the extracts from the small intestines of a cat and proceeded as follows:

The cat was profoundly anaesthetized under the bell and killed by a blow on the neck, the small intestine was removed from the animal and put in a dish with warmed Tyrode solution, washed out twice with this liquid, each time transferred to a fresh solution, and at last it was irrigated by a powerful jet of water. Subsequently the gut was tied up on both ends, and put in about 100 c.c. of distilled water of 38°; only the ends of the gut did not touch the water. After an hour the gut was taken out, the slightly opalescent but slightly coloured fluid was boiled up on a copper gauze, evaporated down to a volume of 30 c.c., filtered, and the filtrate was then evaporated to dryness in a porcelain dish on the waterbath. The brown residue was subsequently extracted with absolute alcohol, the alcoholic solution was filtered and evaporated to dryness, the remainder was extracted with ether. After filtration the ether was evaporated and the residue was taken up in water.

The extracts prepared and purified in this way proved (though not always) to include the looked for active constituent. During our inquiry, however, it appeared that in using more material the alcohol abstracts the active substance only slowly and partially from the tough residue of the aqueous extract. We, therefore, evaporated the residue to dryness on purified quartz-sand and extracted it with alcohol for some time in extracting apparatus.

From the then obtained dark coloured alcoholic solution the solvent was removed by distilling, the remainder was dried in vacuo over sulphuric acid and after this extracted with a large amount of ether. The difficulty I encountered here was that only a portion of the active substance was transmitted to the ether, especially after a preliminary purification with acetone. Also without this a consider-

able portion remained undissolved even when the extraction was performed with a large amount of ether. In a small amount hardly anything was dissolved.

It stands to reason that in our further experimentation we did not attempt to prepare an ether-extract, but that the residue of the alcoholic solution was only washed with a little ether. What was then left, was taken up in water and treated as follows:

Sulphuric acid was added to the solution to 5 per cent, and a concentrated solution of phosphotungstic acid was added, which produced a large, white precipitate. This was filtered by suction, washed and decomposed in the usual way with baryta water. The filtrate was freed from phosphotungstic acid by baryta and the solutions thus formed, were examined for their effect upon the surviving small intestine of a rabbit.[1]) It appeared from this that, when we work with concentrated solutions, about 80%—90% of the active constituent had been precipitated with phosphotungstic acid. Admixture of silvernitrate and silvernitrate with baryta yielded in the solution, through decomposition of the phosphotungstic precipitate, only inconsiderable residues, which generally contained no active substance. But in the filtrate of these precipitates we have not been able to find an undiminished quantity of the active substance. If this filtrate was evaporated down, after removal of traces of silver-salt and baryta, and subsequently extracted repeatedly with slight quantities of absolute alcohol, an addition of alcoholic sublimate solution gave a white precipitate, which contained the active substance, though not in toto. By extracting this precipitate with boiling water and concentrating this solution, we could not manage to abstract a pure mercuric chloride. Just as with sublimate we also obtained with platinic-chloride in alcoholic solution a precipitate that principally contained the active substance. This precipitate with platinic-chloride was soluble in a very small quantity of water. By concentrating the solution or by addition of alcohol no pure compound was set free. After decomposing the platinum-compound with hydrogen sulphide and addition of gold-chloride solution only a few crystals segregated, which melted at 215°—222°. A repetition of this experiment with a large amount of material did not enable us to isolate the active substance or a compound of it in a pure condition. The quantity of the gold-salt was very small and contained only a portion of the active constituent of the primary intestine extract.

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[1]) The extracts were always examined for their effect after MAGNUS' method with the excised small intestine of the rabbit in 50 cc. of Tyrode-fluid at 38°.

An attempt to obtain a larger amount of material from the intestines of pigs and oxen did not yield the looked for result, though here also the activity of the extracts was satisfactory. Meanwhile, since by the method, thus far employed, the activity disappeared almost entirely, I tried to obtain a purified condition by a simpler method, viz. through extraction with various solvents that could readily be removed.

With a view to this we prepared an extract from five small intestines of cats, the alcoholic solution was evaporated to approximate dryness and the residue was extracted first with ether and then with chloroform. What remained out of solution was treated with a little glacial acetic acid. After evaporating this solution the remainder was dissolved in 5 cc. Tyrode-fluid and the activity of these solutions was determined.

It now appeared that the portion dissolved in acetic acid exhibited a much stronger activity than could be expected in an extract not subjected to a preliminary treatment. By heating with acetic acid, or stronger still with acetic acid anhydride, the activity seemed to be largely increased, in some cases five-hundred fold. After acetylating the extract of one small intestine $^1/_{5000}$ part could still be seen to exert distinct influence, whereas of the primary extract $^1/_{10}$—$^1/_{12}$ was needed to obtain this result. I always availed myself of this quality of the extracts in my further experiments to ascertain to what extent the various precipitates and filtrates still contained part of the active constituents. The large increase of activity after acetylating, in connection with the property of the looked for substance of yielding precipitates with phosphotungstic acid and in alcoholic solution with sublimate and with platinic-chloride, naturally led us to suppose that cholin, or an analogous compound, might be the active constituent of the extracts as it was well known that the physiological effects of cholin are as a rule largely increased by acetylating.[1]) We, therefore, ascertained whether the effect of cholin upon the gut is also increased by acetylating. This proved to be the case as, indeed, GUGGENHEIM and LÖFFLER[2]) have also reported.

We did, however, not succeed in isolating cholin in a pure condition from extracts of cats' small intestines, nor in making an approximately quantitative determination of the amount of cholin

[1]) HUNT and TAVEAU, Bull. No. 73 of the Hygienic Laboratory of the Public Health and Marine Hospital Service Washington (1911).
DALE, Proc. Physiol. Soc. Journ. of Physiol. Vol. 48. 111 (1914).

[2]) GUGGENHEIM und LÖFFLER, Bioch. Zeitschr. 72 319 (1916).
Id. id Bioch. Zeitschr. 74. 208 (1916).

present. We, therefore, carried out another series of tests with extracts from rabbits' small intestines, which were always much more active than extracts of cats' intestines. Our procedure was the following:

The rabbit was killed by a blow on the neck and dehematized from the carotids; the small intestine was cautiously detached from the mesenterium and subsequently put in an abundant quantity of warm Tyrode-solution. Hereafter the gut was perfused with this fluid three or four times and every time transferred to another dish with fresh Tyrode-fluid. After being tied up on both ends and rinsed again it was suspended in 75 c.c. of water at 38°. The gut was taken out after an hour. A slightly alkaline reaction of the colourless and perfectly limpid aqueous extract was now noticeable. By infusion of carbonic acid or an admixture of some drops of 1/10 nHCl the reaction to litmus is made neutral, and rapidly boiled up. A flocculent precipitate is thrown down and on filtration there ensues a clear solution which (foaming being precluded) is evaporated down to a very small volume under a diminished pressure at 50°. A subsequent admixture of 25 cc. of methylalcohol produces a precipitate which contains little or nothing of the active substance and can be readily removed by filtration. The alcoholic solution thus obtained, possesses in undiminished degree the activity of the primary aqueous extract.

After removing the greater part of the methylalcohol by distillation, acetone is added to 4—5 times the quantity of the extract, which gives a large precipitate, containing only a small portion of the active constituent. After filtration the solution is evaporated down to a small volume under a lower pressure and subsequently placed in a vacuum-exsiccator. The pale yellow residue is then repeatedly extracted with small amounts of absolute alcohol until KI_3, added to a few drops of the filtrate, arrests the precipitation. As STANĚK has pointed out this is a delicate reaction on cholin. By saturating this solution with sublimate a white precipitate is produced that, after some hours, is filtered by suction and washed out first with an alcoholic sublimate solution and subsequently with absolute alcohol. The precipitate is then extracted with a little warm water, acidulated with a drop of hydrochloric acid. After cooling down, or if necessary concentrating, lightly tinged crystals segregate in the shape of little columns. After repeated crystallization the melting point was found to be 244°; when mixed with the mercurous double salt of pure cholin (melting point 246°) we found 244°—245°. To identify our experience still further platinum-salt was prepared, which melted at

215° and did not exhibit a lowering of the melting-point in the mixture-experiment. For the melting point of the gold double salt we found 238°—239°, corresponding with that of the gold-salt prepared from pure cholin.

Through some microchemical reactions we could also demonstrate that we had to do here with cholin. With sodium gold chloride the precipitate consisted of yellow obliquely truncated columns, completely resembling those prepared from pure cholin; with potassium mercury iodide (MAYER's reagent) a double salt was given, crystallizing into fine needles; likewise the precipitate with picrolonic-acid as well as with potassium periodide looked outwardly like that obtained from cholin.

Likewise the action upon the isolated rabbit's small intestine in Tyrode fluid appeared to resemble that of cholin.

A solution containing 0,3 mgrms of the compound isolated from the small intestine, possessed a distinct influence upon the excised rabbit's small intestine of about similar extent to the effect of 0,3 mgrms of cholin-hydrochloride; after heating with acetic acid anhydride an amount corresponding with 0,003 mgrms of the detected compound appeared to possess a considerable stimulating activity, approximately similar to an equal amount of acetyl-cholin.

This, then, proves that cholin is an active constituent of the small-intestine extract. The quantity of cholin, derived in this way as a mercurous double compound from the extract of a small-intestine, amounts to about 1 mgrm. This is certainly not all the cholin present in the primary aqueous extract, much less the total amount in the intestinal wall, as we know that the precipitate, obtained with acetone, contains also a small part of the active substance. The alcoholic filtrate of the sublimate precipitate is always more or less active and in this the presence of cholin can be readily demonstrated, by means of the periodide test. By acetylating a greater activity could be obtained, as with cholin, in the acetone precipitate as well as in the alcoholic filtrate. Assuming that the activity of these fractions, occurring with the isolation of cholin, may also be ascribed to cholin, it appears that at least 75 % of the looked for active constituent of the rabbit's small intestine may be attributed to cholin. However, I wish to accentuate that not in all experiments such a high percentage of the cholin, present in the extracts, could be isolated, as part of it gets lost more or less in the various experiments. This was evident from the control-tests in which cholin was added to the primary extracts; after the usual experimentation only part of the cholin could be found again.

MAGNUS [1]) has previously pointed out that the intestinal movements are effected under the influence of AUERBACH's plexus and points to metabolic processes in these nerve centres as the most probable cause of the stimuli.

From WEILAND's [2]) experience that a substance can by a simple method be derived from the intestine, which largely increases the movements of the surviving small intestine, he concludes that, just as with the automatic respiratory movements, the cause of the automatic intestinal movements must also be a chemical stimulus.

From the above we conclude that the intestinal extracts contain an amount of cholin that may in a high measure be made responsible for their stimulating influence upon the gut. Further inquiry showed that cholin is a substance, which occurs abundantly in various parts of the animal body. The rôle which this substance has to play in the body, has as yet not been discovered. The above inquiry has rendered it highly probable that cholin plays an important part in bringing about the automatic intestinal movements.

1) MAGNUS Ergebnisse der Physiologie. 7e Jahrgang 8. 47.

2) WEILAND Loc. cit.

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