Physiology. — *"The Action of Atropin on the Intestine depending on its amount of Cholin"*. By Dr. J. W. LE HEUX. (Communicated by Prof. R. MAGNUS).

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The action of atropin on the intact, isolated small intestine of mammals has been the subject of a considerable amount of experiments, but no explanation, could be found for the various and widely different results achieved by the several authors. [1])

While MAGNUS [2]) established that the isolated intestine of the cat, in RINGER's solution, is paralysed in large doses of atropin (0,3 %), but mostly reacts on moderate quanta of atropin (0,025 –0,075 %) with symptoms of stimulation, the pendulum movements, executed by the intestine, getting more regular, especially when the movements of the intestine were previously insignificant, also an inhibiting effect of very small atropin-doses (0,005—0,05 %) was demonstrated by UNGER [3]). Other researchers also sometimes found this (paralysing) effect, sometimes they did not. In the isolated small intestine of rabbits and dogs KRESS [4]) noted stimulation consequent on moderate amounts of atropin, paralysis through large quanta, whereas others again obtained widely varying results. In most cases small quantities of atropin caused inhibition in the small intestine of rabbits, whereas moderate atropin-doses alternately stimulated and paralysed the gut without any regularity.

According to P. TRENDELENBURG [5]) the intact intestine of the rabbit reacts regularly on small quanta of atropin with paralysis, but on moderate quantities now with symptoms of stimulation, now with paralysis.

According to the same writer [5]) an exception to irregular behaviour

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[1]) An extensive survey of the literature is given by G. LILJESTRAND, Pflüger's Archiv, Bd. 175, p. 111, 1919.

[2]) R. MAGNUS, Versuche am überlebenden Dünndarm von Säugetieren. I. Mitt. Pflüger's Archiv. Bd. 108, pag. 1, 1905.

[3]) M. UNGER, Beiträge zur Kenntnis der Wirkungsweise des Atropins und Physostigmins auf den Dünndarm von Katzen Pflüger's Archiv. Bd. 119, pag. 373, 1907.

[4]) K. KRESS, Wirkungsweise einiger Gifte auf den isolierten Dünndarm von Kaninchen und Hunden. Pflüger's Archiv. Bd. 109, pag. 608, 1905.

[5]) P. TRENDELENBURG, Physiol. u. Pharmacol. Untersuchungen über Dünndarmperistatiek. SCHMIEDEBERG's Archiv. Bd. 81, pag. 55, 1907.

of the gut of various species of animals towards atropin is afforded by the small intestine of the guinea-pig, which is regularly inhibited by atropin, a question to which we will revert in this paper.

It is evident from this short survey that the behaviour of the intestine of different mammals towards small and moderate quanta of atropin is varying and inconstant. No doubt the researchers who obtained these various results, have been working under incongruous circumstances. As yet no one has succeeded in accounting for their conflicting results.

From experiments by v. LIDTH DE JEUDE [1]) it appeared distinctly that the explanation is not to be looked for in the different composition of the salt-solution, in which the isolated intestine was examined.

LILJESTRAND [2]) showed that the various results could neither be ascribed to the different composition of the atropin-preparations; he is rather inclined to believe that the explanation can be found in the gut itself. The object of the present paper is to verify this conception.

WEILAND [3]) has demonstrated that from the stomach, the small intestine and the large intestine a coctastable substance can be abstracted through extraction with water, which has the property of urging on the movement of the gut, and that this stimulating action can be arrested antagonistically by small quantities of atropin. It afterwards turned out [4]) that this substance consists for the greater part of cholin and that it can be obtained from the small intestine of the rabbit to such a quantity that it must act a prominent part in evolving the automatic intestinal movements.

Now cholin belongs pharmacologically to the group of pilocarpin; the stimulating effect exercised by cholin, just as by pilocarpin, on the gut is antagonised by slight quantities of atropin.

VAN LIDTH DE JEUDE [5]) showed by his experiments that the quanta of atropin required to check the pilocarpin-action upon the gut, are very slight; already a concentration of atropin of 1 to 10—50 million will do. Now I found that the atropin-concentrations, necessary

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[1]) A. P. v. LIDTH DE JEUDE, Quantitatieve onderzoekingen over het antagonisme van sulfas atropini enz. Thesis. Utrecht 1916.

[2]) G. LILJESTRAND, l c.

[3]) WEILAND, Zur Kenntnis der Entstehung der Darmbewegung. Pflüger's Archiv. Bd. 147, pag. 171, 1912.

[4]) J. W. LE HEUX, Choline als Hormon der Darmbewegung. Pflüger's Archiv. Bd. 173, pag. 8, 1918.

[5]) A. P. v. LIDTH DE JEUDE, l.c

to induce a temporary inhibition of the intestinal movements, fall within the same limits.

When combining these facts, the question arises whether perhaps the inhibition of small atropin-doses is to be considered as an antagonism for the cholin present in the intestinal wall.

If this is the case, the inhibition of small quantities of atropin, will not appear when the cholin has been previously removed from the intestinal wall, but it will come forth again after the addition of cholin and subsequent administration of atropin.

Aside from this inhibition of small quantities of atropin, its actual influence is, according to MAGNUS's [1]) experiments, one that stimulates AUERBACHS's plexus. It is only large quantities that paralyse the nerve centra and muscles of the intestinal wall.

The action of these latter quantities we leave out of consideration.

Experimental evidence in support of the above hypothesis may be obtained in the following way:

1st A gut, inhibited originally by small quantities of atropin, is to be brought into a condition in which a small quantity of atropin is without effect, through repeated washings, so that cholin is removed from the intestinal wall.

2nd The atropin-effect is to reappear in this gut after giving cholin.

3rd A gut, which is originally inhibited by moderate quantities of atropin, is to be brought, through repeated washing, into a condition, in which the same quantity of atropin has only a stimu- appeared lating effect.

The experiments made to prove this, were performed with the isolated small intestine of rabbits and guinea-pigs, which, as had before, are provided with rich quantities of cholin and — as may be expected, readily give them off to the environing fluid.

Experiments with the small intestine of the rabbit.

In the experiments with the small intestine of the rabbit a difficulty arose in that after some days the spontaneous movements diminished with the washing out of the cholin, which made the results less clear.

Turning to account LAQUEUR's [2]) experience that loops of intestine

[1]) R. MAGNUS, Versuche am überlebenden Dünndarm von Säugetieren. V. Mitt. Pflüger's Archiv. Bd. 108, pag. 1, 1905.

[2]) E. LAQUEUR, Over den levensduur van geisoleerde zoogdier-organen met automatische functie. Verslagen Kon. Akademie v. Wetenschappen te Amsterdam. 24 April 1914, XXII, p. 1318.

kept in horseserum at a low temperature, retain mobility for days, we now proceeded as follows:

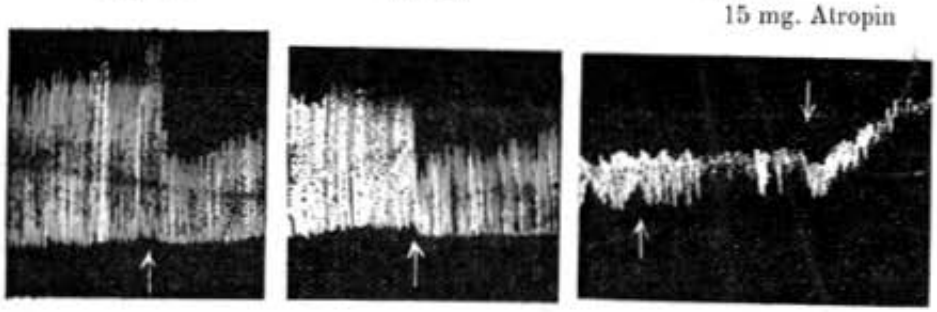
A certain number of loops were severed from the fresh small intestine, which had been cautiously cleaned with Tyrode solution; their movements were registered by MAGNUS's method. The vessels containing the loops were filled with 75 c.c. Tyrode solution of 38° C.

When small quantities of atropin (0,002—0,01) were added, the pendulum movements of the loops got invariably smaller.

This is illustrated in Fig. 1.

Fig. 1. Fig. 2. Fig. 3.

15 mg. Atropin



0.01 Atropin 15 mg. Atropin 0.01 Atropin

Fig. 1.

Pendulum movements of the rabbit's small intestine suspended directly after killing the animal. By administering 0,01 mgr. of atropin the magnitude of the movement is reduced by half.

Fig. 2.

The loop whose movements are registered here is the same as in fig. 1. The previous action of the atropin (0.01 mgr) is entirely eliminated through washing three times with Tyrode solution. 15 mgr. of atropin now yields a strong inhibition on the movements while the tonus is only slightly lessened.

Fig. 3.

A loop of the same gut, standing for 3×24 hrs. in the refrigerator in horseserum that was repeatedly refreshed. Subsequently the loop is washed out eight times with warm Tyrode solution and no longer reacts on 0.01 mgr. of atropin. After 15 mgr. of atropin a marked stimulation appears together with a considerable increase of tonus.

After this the loops of intestine were washed out with fresh Tyrode solution three times every 10 minutes, by which, as we know from our own experience, the effect brought about by the preceding small atropin-dosis was again completely eliminated.

When thereupon a moderate dosis (15 mgr.) of atropin was added, the pendulum movements became considerably smaller in far and

away most cases, while at the same time the tonus was more or less lowered.

This is exemplified in Fig. 2. In a few cases the initial inhibition, caused by this moderate quantity of atropin, was followed by a rather strong stimulation.

This occurred, when the loops had already been cleaned *several* times, so that the gut had reached a stage, in which the inhibition of moderate atropin doses passes into a stimulating action. The next day some loops were again severed from the gut which had been kept standing during the night in horse-serum at a low temperature; after having been carefully washed free from the adherent serum they were suspended as before. Every ten minutes these loops were cleaned with fresh Tyrode solution. One of them (we always took the same) was experimented on to ascertain whether 0.01 mgr. of atropin still evolved inhibition on the movements. If it did not, the other loops of intestine were cleaned again some times and examined with regard to their behaviour towards atropin.

In the great majority of cases it appeared again that a small amount of atropin (0,01 mgr.) did not cause the slightest change in movements or tonus, but that after administering 15 mgr. of atropin a stimulation of the intestinal movements together with a large increase of tonus was noticeable. See Fig. 3. Over and beyond all this the primitive inhibition of the atropin could be elicited again in this stage of the experiment, if a small amount of cholin (1—2 mgr.) had previously been added to the loops.

We did not always succeed in reaching this stage already on the second day, so that it proved necessary to keep the gut in the repeatedly refreshed horse-serum some days longer, in order to arrive at a condition in which small doses of atropin do not affect the gut, which again had been cleaned repeatedly with Tyrode solution.

We also succeeded in obtaining this condition by merely cleaning the gut with Tyrode solution, i.e. without the appliance of horse-serum. It is true, though, that, as mentioned before, the movements will become smaller then, and the results less clear. This proves, however, that the results are not influenced by horse-serum.

In the foregoing we have thus shown for the rabbit's small intestine:

1st. that repeated washing, which, as demonstrated before, deprives the intestinal wall of cholin, evolves a condition in which the initial inhibition of small amounts of atropin, is arrested.

2nd. that by administering cholin this inhibition of atropin may be elicited again.

3rd. that the effect of moderate quantities of atropin, which was variable at first and in my experiments was mostly inhibitory, may be altered in a constant, stimulating effect by repeated washing.

Experiments with the small intestine of the guinea-pig.

We now took the small intestine of the guinea-pig as the object of our investigation.

Recently TRENDELENBURG [1]) has suggested an effective method to register graphically the peristaltic movements of the surviving small intestine of the guinea-pig and to determine to a certain extent in numerical values the action exercised on these movements by various poisons.

TRENDELENBURG records that atropin (acting on the small intestine of the guinea-pig) is *invariably* inhibiting peristalsis.

The great thing in our experimentation was to ascertain whether here also, as with the rabbit's small intestine, its behaviour towards atropin is governed by its condition. We used TRENDELENBURG's method and proceeded as follows:

The guinea-pig was killed by a blow on the neck, the small intestine was cautiously severed from the mesentery, and cleaned several times, with a warm fluid [2]) after LOCKE. Subsequently the intestine was cut into 5 parts, one of which was suspended immediately, the other pieces were put in separate dishes with LOCKE's solution, which was refreshed every now and then.

The loop of intestine which was suspended in a vessel of 150 c.c. capacity, was first left to itself with an interior pressure of 0 mm. H_2O.; then the pressure was gradually heightened and we determined at what pressure peristalsis first appeared (critical pressure). Then the interior pressure was lowered to 0 and after 3 minutes the critical pressure was again determined.

This determination was repeated after 0,1, 1, and 5 mgr. of atropin had been added respectively. In accordance with TRENDELENBURG's report, arrest of peristalsis took place, so that no peristalsis occurred any more even when the pressure was made considerably higher.

After being carefully cleaned, a second loop was suspended, which was kept standing for some hours in LOCKE's solution; the same determinations were made prior to and posterior to the administration of atropin. In most cases it appeared already now that the

[1]) TRENDELENBURG, l.c.

[2]) It is essential that the fluid should be prepared from pure salts and with pure water distilled from glass apparatus.

small atropin-dosis yielded a much weaker inhibition on the peristalis than with the first loop.

Subsequently another loop was examined, which had been kept standing in LOCKES's solution some hours longer again, and had been washed a few times more etc.

Table I comprises the results of the complete experiment.

From it we see that the gut, which is arrested by quantities of 0,1, 1, and 5 mgr. of atropin, is brought after a 2½ hours' washing with LOCKE's solution into a condition in which 0,1 and 1 mgr of atropin produces a *much weaker* inhibition.

TABLE I.

Number of the loop of intestine.	Time of washing.	Critical interior pressure in mm H_2O.			
		normal.	after 0.01 mgr. of atropin.	after 1 mgr. of atropin.	after 5 mgr. of atropin.
1	0	7	Inhibition	Inhibition	Inhibition
2	2.5	20	25	30	Inhibition
3	6	20	16	20	Quick 20 pendulum movements.
4	8	12—16	13	—	—
5	11	10	7	—	—

After the process of washing had been prolonged for 6 hours the inhibition of these atropin quantities had entirely stopped, but now peristalsis appears after 0,1 mgr. of atropin already at a lower interior pressure as in the normal period. Also with loops of intestine that have been washed 8 and 11 hours atropin causes distinct stimulation, so that peristalsis comes forth already at a lower interior pressure.

With loop N°. 3 a very considerable increase of the pendulum movements was also perceptible after 5 mgr. of atropin.

It has thus been proved also for the small intestine of the guinea-pig that the inhibition of atropin, already established by TRENDELENBURG, can be arrested by washing and that the atropin-action proper, stimulation of AUERBACH's plexus, can be elicited by small quantities of atropin.

Now it is obvious also why TRENDELENBURG noted only the inhibition of atropin. It was because he did not allow the loops to stand in a solution, but always experimented with fresh ones taken from the guinea-pig under urethannarcosis.

The results obtained by our experiments put us in a position to view the variable action of atropin on the gut in a new light and to interpret the conflicting results of the various researchers.

In the living animal cholin is present in the intestinal wall in such quantities that they stimulate AUERBACH's plexus. On removal, to a certain extent, of the cholin from the surviving gut by a prolonged washing, the real action of atropin manifests itself distinctly. According to the earlier experiments of MAGNUS [1]) it consists in a stimulation of AUERBACH's plexus by moderate quanta, whereas only very large doses paralyse the centra, the nerve, and the muscle. Originally the intestinal movements are not affected by small quantities of atropin.

It is, therefore, upon the presence of more or less cholin in the intestinal wall that the atropin-action depends.

Cholin has a stimulating effect upon AUERBACH's plexus, which is antagonised by atropin. So long as an adequate quantity of cholin is present in the intestinal wall, a small dosis of atropin will inhibit the cholin action and consequently inhibit the intestinal movements.

The result of the action of moderate quantities of atropin will depend on the circumstance whether the immediate stimulating action on the plexus, or the antagonism for cholin preponderates.

In case the gut contains little cholin the stimulating action comes to the front, in case it contains much cholin the antagonism (inhibition) predominates. With a moderate cholin-content a stimulation will succeed an initial inhibition.

Likewise we are now enabled to account for the results of earlier researches.

The fact that with the cat's small intestine the stimulating effect of moderate quanta of atropin occurs more often than with the rabbit's or the guinea-pig's, tallies with our experience that the former contains less cholin than the latter two.

On the other hand it stands to reason that with the guinea-pig gut, which was always found to be rich in cholin, the inhibition of atropin appears regularly.

It is obvious now why the isolated rabbit's gut, according to the previous treatment, is now inhibited by atropin, now again is stimulated, while on the other hand the intact gut, which could not be liberated from cholin by washing, is according to TRENDELENBURG [2]) inhibited regularly.

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[1]) R. MAGNUS, l.c.

[2]) P. TRENDELENBURG, l.c.

Summary.

We have demonstrated in an earlier paper that the isolated small intestine yields to salt-solutions quantities of cholin, which are capable of stimulating AUERBACH's plexus.

This loss of cholin results in a changed behaviour of the gut towards atropin.

The rabbit's small intestine that is inhibited previous to washing by small doses of atropin, no longer reacts on them; on the other hand it is now stimulated by moderate doses.

The normal guinea-pig gut is invariably inhibited by atropin. This effect also here disappears after washing and is substituted by a stimulation through moderate quanta of atropin.

This is to be interpreted as follows: the real action of moderate quanta of atropin on the gut is stimulation of AUERBACH's plexus; if the gut contains much cholin, so that the plexus is readily stimulated, this stimulation is arrested through the antagonism of a small amount of atropin, occasionally weakened, and the result is inhibition. Moderate quanta of atropin are also inhibitory when this antagonism is strong enough, but in the presence of small quanta of cholin in the gut the latter will be stimulated.

It is clear, therefore, that here we have to do with a case in which the presence of a well known chemical substance (cholin) in the tissue determines the manner in which this substance reacts on a poison (antropin).

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