Physiology. — *"A Quantitative Inquiry into the Antogonism Pilocarpin-Atropin on the Surviving Cat-gut"*. By Prof. W. STORM VAN LEEUWEN and Miss C. VAN DEN BROEKE. (Communicated by Prof. R. MAGNUS).

(Communicated at the meeting of April 23, 1920).

At our Institute we often felt the want of a correct physiological determination of the strength of atropin-containing solutions. With one of the usual methods which is based on the property of atropin to restore pulsation after the muscarin-standstill of the frog's heart our results proved unsatisfactory. We, therefore, endeavoured to find a method that should yield more reliable results, viz. by taking the antagonism of atropin on the action of pilocarpin on the surviving gut, as an index of atropin-action.

Laborious investigations of this antagonism have been carried out by VAN LIDTH DE JEUDE [1]). His publication also contains complete references on this subject.

He conducted his experiments as follows: The contractions of pieces of a rabbit's small intestine were recorded on a kymograph. The pieces of the intestine were suspensed in vessels of 15, 75 and 150 cc. The experimenter disposed of an apparatus that enabled him to work with twelve pieces at a time. The vessels were filled with Tyrode solution to which varying quantities of pilocarpin were added. As v. LIDTH DE JEUDE used vessels of varying sizes he was able to vary in his experiments the dosis of pilocarpin and atropin, with or without varying at the same time the concentrations of these drugs. As soon as, in his experiments, the pilocarpin had produced a contraction of the isolated gut, every 20 seconds $^1/_4$ c.c of a definite atropin-solution was added. This was repeated until an atropin-action was clearly noticeable.

VAN LIDTH DE JEUDE points to several errors to be guarded against in a similar investigation. The rate at which the oxygen bubbles through the vessels during the experiment, should not vary too much, since a strong current of oxygen causes the atropin to mix sooner, and consequently an antagonistic action to manifest itself sooner than a weak current will do. The concentration of the atropin-solution, of which always $^1/_4$ c.c. is added, should be the same in all experiments, otherwise erroneous results will be obtained, etc.

With due precaution v. LIDTH DE JEUDE undertook a series of careful experiments of which we here record the results that bear upon the question under consideration.

The pilocarpin-action depends on the concentration of the poison in the Tyrode solution, and is not dependent on the absolute amount of pilocarpin present in the solution.

The atropin-action *per se* (inhibitory effect of small doses) depends rather on the absolute quantum than on the concentration of the poison in the solution. The concentration is decisive with large atropin-doses (12,5—150 mgr. to 75 cc. of liquid).

According to v. LIDTH DE JEUDE also the antogonism of atropin hinges upon the absolute quantity, and not upon the concentration of the poison in the solution.

Furthermore, v. LIDTH DE JEUDE found, that generally the atropin doses to be added, differed little with highly varying pilocarpin-doses and pilocarpin-concentrations.

The only relation, found by him between the values of the two poisons, was that with a considerable rise of the pilocarpin-dosis (100 times the initial dosis), the atropin-dosis increased but little (3—5 times). Hereby the results published by MAGNUS in 1908 [1]) were confirmed, as MAGNUS also found that with a rise of the pilocarpin dosis (up to 50 times), the atropin doses required for the antagonism did not augment — anyhow less than ten times.

Although v. LIDTH DE JEUDE's method suited his purpose very well, it could not, as such, be utilized in cases concerning the physiological determination of atropin-containing solutions, because large individual differences occur in the reactions of the guts of various animals, nay, even in the reaction of different pieces of the gut of the same animal. For this reason we have modified the method by utilizing the familiar fact that the action of various poisons can be abolished by removing the drug-containing solution and substituting it by a fresh solution, so that the organ resumes its former condition and will react again in the same way on a similar quantum of poison. This enabled us to observe repeatedly the action of a poison on the same strip of intestine. This was also BARGER and DALE's [2]) method when they examined the action of various poisons upon the uterus. NEUKIRCH [3]) has demonstrated that the effect of pilo-

[1]) A. P. v. LIDTH DE JEUDE. Quantitatieve onderzoekingen over het antagonisme van sulfas atropini tegenover hydrochloras pilocarpini, salicylas physostigmini en hydrochloras muscarini (Grübler) op overlevende darmen van zoogdieren. Acad. Proefschrift. Utrecht, 1916.

[1]) R. MAGNUS. Kann man den Angriffspunkt eines Giftes durch antagonistische Giftversuche bestimmen? Pflügers Arch. B. 123. S. 99. 1908.

[2]) G. BARGER and H. H. DALE. Chemical structure and sympathomimetic action of amines. Journal of physiology. Vol. XVI. 1910, page 19.

[3]) P. NEUKIRCH. Pflügers Arch. 147. 171. 1912. Physiologische Wertbestimmung am Dünndarm. Pflügers Arch. 147. 151. 1912.

carpin upon the surviving small intestine could also be washed out. It was, therefore, incumbent on us to find out whether this was the case also for atropin. Originally we supposed this was not so, because v. LIDTH DE JEUDE had stated that the atropin-action depended on the absolute quantity of atropin, and not, as is the case with most other poisons, on the concentration in which the poison is presented. We believed that the atropin-action could depend on the absolute quantity *only then*, if all or nearly all the atropin had been adsorbed from the liquid by the gut. Now we deemed it improbable that in that case the whole quantity of atropin could be washed out again. On further examination, however we found that the atropin action, like the pilocarpin-action, could, indeed, be abolished by washing out. This induced us to ascertain whether the atropin-action indeed depended only on the absolute quantity and not on the concentration.

We will not delay the statement that also for atropin only the concentration of the poison was found to be conclusive. In this inquiry we made use of an apparatus differing from that of v. LIDTH DE JEUDE. Also our technique differs considerably from his.

The fact was namely that — unlike v. LIDTH DE JEUDE — we did not add to the gut pilocarpin only once and subsequently some drops of an atropin solution till the pilocarpin-action began to be neutralized; but, in order to ascertain in the same gut the action of several doses and concentrations of atropin, we wanted to be able to transfer the gut to different vessels every time without breaking the contact between gut and lever. To this end we used an apparatus, that was already described on a previous occasion [1]). With this apparatus (fig. 1) the gut is not fastened to the bottom of the vessel, but to the bent arm of a glass rod, which reaches into the glass vessel. The glass rod is attached to a metal bar, which also supports a lever for the registration of the contractions of the organ. The metal bar is movable in a vertical direction, in a metal mantle, so that it can be moved upwards by a single motion of the hand which lifts the gut out of the solution without interfering with the contact between lever and gut. The glass vessel, in which the gut is contained, and which has a capacity of 75 c.c. stands in a copper vessel, in which there is, moreover, a second glass vessel of 150 c.c. capacity. In this metal vessel there is also a thermoregulator, connected with a small burner under the vessel. The metal vessel and the burner under it are attached to a revolving disc. All is arranged in such a way that as soon as the bar, which supports the gut and the lever, is moved upwards, the metal vessel can be turned by a single motion of the hand, so that the gut, on being lowered, reaches the vessel of 150 c.c. where the poison is washed out. The removal from the one vessel into the other can be accomplished so quickly that the curve on the kymograph is hardly interrupted. So, if necessary, the whole washing process may be registered accurately.

[1]) W. STORM v. LEEUWEN. Physiologische Waardebepaling van geneesmiddelen. Acad. Proefschr. Utrecht. 1919.

The contrivance just described is a part of the large apparatus represented in fig. 1, which consists of three metal vessels, mounted, together with their revolving disc, on a plank that can be moved to and fro. The gut can now be transferred at will to each of the 6 vessels of the apparatus (this is also of importance when examining the action of poisons at a different temperature).

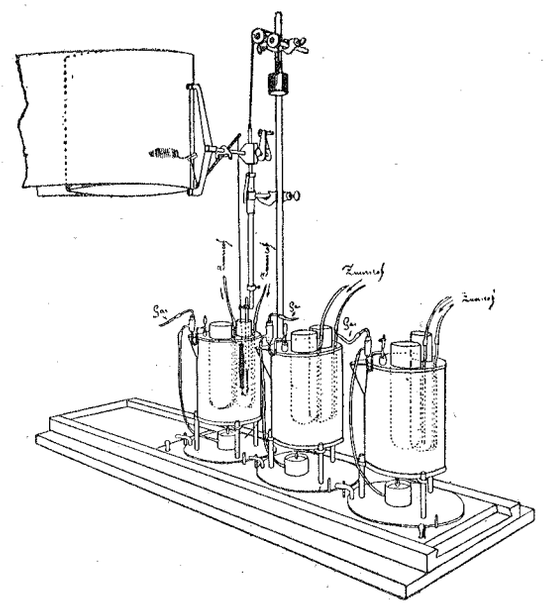

Fig. 1.
Apparatus for the registration of the movements of a surviving organ, provided with a simple arrangement for washing out the added poisons and for operating at various temperatures.

Here, then, we have an arrangement of three vessels of 75 c.c. and three vessels of 150 c.c. to which the surviving gut can be transposed. One of the three metal vessels was displaced in some of the experiments by a large glass vessel containing 1300 c.c. of Tyrode solution and in which also a thermoregulator was placed and a tube through which the fluid was oxygenated. When the gut was put into this large vessel, the action of a definite dosis of atropin could be watched with a dilution twenty times stronger than when an equal dosis of atropin was examined in one of the small vessels, which contained only 65 c.c.

In a series of experiments we tried to ascertain whether the pilocarpin-action indeed depended only on the concentration. This appeared to be the case, so that in this respect we quite agree with v. LIDTH DE JEUDE and consequently our experiments pertinent to the matter in question may readily be left out.

In another series we examined the question whether atropin can be washed out.

This was to the following effect:

Atropin-action is completely reversible, for when the gut is put

in a vessel containing pilocarpin, after this in a vessel with pilocarpin + atropin, then again in pure Tyrode, and subsequently again in pilocarpin, the second dosis of pilocarpin will act in the same way as before, while this action can, just as the first time, again be arrested by atropin in the same way. This experiment may be repeated as often as six times, without interfering with the action of pilocarpin or atropin. The experiment just described also proved that, while the antagonism is being accomplished, only very little atropin is adsorbed by the gut, because the experiment (pilocarpin-action subsequently arrested by a minimal dosis of atropin) can be repeated six times without the necessity of a fresh solution in the vessel with pilocarpin + atropin. The fact that during the action of atropin only very small quantities of it are absorbed by the gut, renders it highly improbable that the atropin action should not depend on the concentration, but on the absolute quantity, for this would be possible only if during the antagonistic action the greater part of the atropin were adsorbed from the solution by the gut, whereas our experiments showed that the gut can take up only very small quantities of atropin. To settle the question whether the atropin-action depends on the absolute quantity or on the concentration, a new series of experiments was undertaken, in which vessels of 65 and of 1300 cc. were used, so that action of a certain dose of atropin could be examined in various concentrations. The result of one of these experiments was, for instance, the following: 0.01 mgr. of atropin in 65 cc. Tyrode solution produced a stronger action than 0.15 mgr. of atropin in 1300 cc. of solution; 0.03 mgr. of atropin in 65 cc. of Tyrode had a greater effect than 0.45 mgr. in 1300 cc., but as great an effect as 0.6 mgr. of atropin in 1300 cc. of solution.

From these experiments, and others that had been conducted in precisely the same way, we are, therefore, justified in concluding that the atropin action, like the pilocarpin action is completely dependent on the concentration and not on the absolute quantity of the poison. This result differs from v. LIDTH DE JEUDE'S, which is owing to the circumstance that our technique differs largely from his. VAN LIDTH DE JEUDE took a different piece of gut for every experiment. Besides this, v. LIDTH DE JEUDE'S using very small vessels (15 cc.) led to many errors, as in this case it is not possible to fix a correct dosage — especially because the solution most often foams considerably. In the third place the way in which v. LIDTH DE JEUDE administers the atropin and his index of the antagonistic atropin-action differ from ours: VAN LIDTH DE JEUDE added to the solution that contained the gut, first a definite quantity of pilocarpin

and when the stimulating influence of pilocarpin was distinctly noticeable, every time $^1/_4$ cc. of a constant atropin-solution was instilled by drops, at intervals of 20 seconds, until a distinct atropin-action revealed itself.

We first put the gut in the vessel containing 10 mgr. of pilocarpin, left it there precisely $1^1/_2$ minutes, then transposed the gut to a vessel that contained, beyond the 10 mgr. of pilocarpin, also the quantity of atropin under examination, and watched for an arrest (after a definite time mostly 1—$1^1/_2$ minutes) of the increase of tonus caused by the pilocarpin. This we assumed to be the case if the bases of the curves were again returned to the original level, no matter whether the "oscillatory movements" of the gut were still greater than before or were not. The criterion used by v. LIDTH DE JEUDE, on the contrary was, whether or no, after the administration of the atropin, a distinct *beginning* of the fall of the curve could be observed, in other words v. LIDTH DE JEUDE watched for the *beginning* of the antagonistic action, whereas we looked for the condition reached *after a certain lapse of time.*

VAN LIDTH DE JEUDE had established in his publication, which we quoted several times in this paper, that with an increase of the pilocarpin-dosis (up to a 500-fold) the atropin-dosis required for the commencement of the antagonism augments but very little (3—5-fold). We knew from earlier investigations that the curve, indicating the ratio between the concentration and the action of pilocarpin, runs as is shown in fig. 2. In the beginning of the curve (*a* to *c*) small

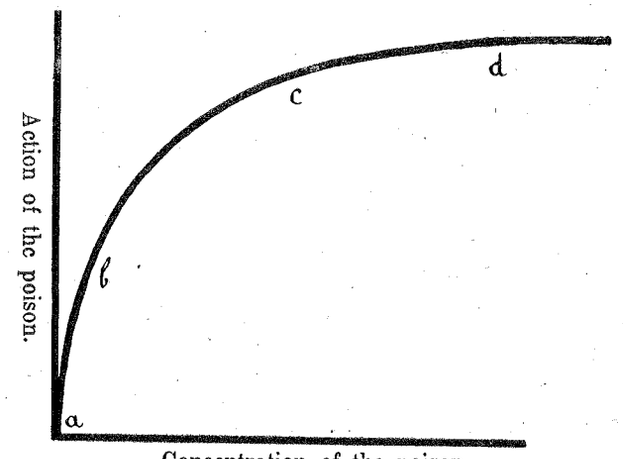

Fig. 2. Scheme of a Concentration-Action curve.

differences in concentration bring about a large difference in action; while with the higher concentrations the action increases only very

little when the concentration rises. We were naturally led to suspect that the small augmentation of the atropin-dosis, observed by VAN LIDTH DE JEUDE with a rise of the pilocarpin-dosis, would take place at the very beginning of the concentration-action-curve, i. e. we suspected that with very small doses of pilocarpin, the increase of the atropin doses would be relatively large when the pilocarpin-concentration increases, while in the higher pilocarpin-concentrations the quantum of atropin necessary for the antagonism would be the same.

In another series of experiments we have attempted to solve this problem.

We used pieces of a cat's gut contained in vessels with 75 c.c. of Tyrode solution. At the commencement of the experiment, several times pilocarpin was added to these guts (and after this the pilocarpin was washed out again) till the sensitiveness of the gut to this poison had become constant. This done, we ascertained how much atropin had to be added to arrest the pilocarpin action almost completely after 3 minutes.

In this procedure the intensity of the "oscillatory movements" was not regarded, but the pilocarpin-action was considered to be arrested, when the base of the curve had nearly resumed its normal niveau again. It became evident from these experiments that the quantum of atropin necessary for arrest of the pilocarpin-action does not depend on the *quantity of the pilocarpin doses*, but on the *intensity of the action* incited by the pilocarpin, that is to say, when at one moment in one and the same experiment a given dosis of pilocarpin exerts a weak action and has a stronger effect at another moment, then the quantum of atropin required in the first case will also be smaller than the one required in the second. The same holds both for the action of pilocarpin upon one and the same piece of gut, and upon different pieces. So, if at a given moment the sensitivity of the gut to pilocarpin, is such that 0.1 mgr. of pilocarpin produces a weak action, the quantity of atropin, required to arrest this action, will be equal to that, required to arrest the same weak pilocarpin-action if at another moment it is elicited by a dosis of pilocarpin as small as 0.01 mgr. In all we have performed 33 experiments in this manner. When arranging these experiments so as to place all the cases of a weak pilocarpin action in one group, in another all the cases of a moderately strong pilocarpin-action, and lastly all the cases of a submaximal pilocarpin-action (corresponding with the point *c* of the concentration-action curve of fig. 2) in a third group, it appeared that the average quantum of atropin required for the antagonism

was for the three groups respectively 0.0005 mgr., 0.001 mgr., and 0.0014 mgr. This implies that when the intensity of the pilocarpin-action rises from *a* to *c* of the concentration-action-curve, three times as much atropin is wanted as before. The quantum of pilocarpin required for a definite intensity of action, did not modify the quantum of atropin which would afterwards be necessary to arrest the pilocarpin action.

Now that it had been demonstrated that in the zône *a* to *c* of the concentration-action-curve the atropin-action depends on the intensity of the pilocarpin-action, we suspected that with still higher pilocarpin-concentration, the atropin-dosis required for the antagonism, would not increase any more.

If this were so our results would tally completely with those of VAN LIDTH DE JEUDE, notwithstanding the difference between his criteria and ours. Contrary to our expectation, however, it appeared that with a further rise of the pilocarpin-dosis, also the atropin-dosis had to be largely augmented, anyhow if we stuck to our criteria. So the latter result differs from that of VAN LIDTH DE JEUDE, which finds a satisfactory explanation in the different techniques. In addition it is just with the high pilocarpin-concentrations that the difference between the criteria applied by VAN LIDTH DE JEUDE and by us comes much more to the front than in the previous experiment. For after these very high pilocarpin-concentrations the interval of 3 minutes, after which the atropin-action was observed, is too short. In the experiments with small amounts of pilocarpin we observed that, if after 3 minutes the pilocarpin action was not yet arrested by the atropin, the atropin action increases but little with a longer interval, so that three minutes proved to be the proper time after which the action of the atropin should be registered. It is not so with the very high pilocarpin-concentrations, here it occurs repeatedly that after 3 minutes only a very insignificant effect has been produced by the atropin, whereas after 4 or 5 minutes it is sometimes complete. Now, since with high pilocarpin-concentrations the space of 3 minutes is doubtlessly too short, and with low pilocarpin doses it must not be made much longer (or the chances are that the pilocarpin-action decreases spontaneously, so that an atropin-action could be presumed where it did not really exist) our method is not trustworthy in comparing the antagonistic atropin-action of small and very large pilocarpin-quanta. This is why we have not continued our inquiry in that direction and are only able to record that with a strong increase of the pilocarpin dosis in

the zône c to d (and farther) of the CA-curve, the atropin-dosis is sure to increase still more, without our being able to procure accurate data on this head.

CONCLUSIONS.

I. In accordance with what has been found by v. LIDTH DE JEUDE and others, the pilocarpin-action upon the surviving gut is entirely dependent on the concentration of the pilocarpin in the solution in which the gut is suspended. The pilocarpin-action is completely reversible.

II. Contrary to VAN LIDTH DE JEUDE's assumption, also the antagonistic atropin-action depends entirely on the concentration and not on the absolute dosis of the poison present.

The atropin-action is also completely reversible anyhow when the atropin dosage is not too large. A surviving piece of gut does not adsorb so much of the smallest active dosis of atropin present in the 75 c.c. of Tyrode solution, as to alter the atropin-concentration *appreciably*.

III. With the relatively small quanta of pilocarpin (i. e. such as exert an action corresponding with the zône a—c of the C.-A-curve) the amount of atropin, required for the antagonism, does not depend on the quantum of pilocarpin administered, but chiefly on the *action* exerted by that quantum. The quantity of atropin, necessary to arrest a sub-maximal pilocarpin-action is about three times larger than the quantity of atropin, required to exert antagonism on a pilocarpin dosis with only a slight action. With pilocarpin-doses with a maximal action, a strong rise of the doses is still accompanied by a rise of the atropin-doses. The reason given above rendered it impossible for us to examine this phenomenon in detail.

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Physics. — "*Discontinuities in the Magnetisation*". By Dr. B. VAN DER POL JR. (Communicated by Prof. H. A. LORENTZ).

(Communicated at the meeting of June 26, 1920).

In a recent paper, "*Zwei mit Hilfe der neuen Verstärker entdeckte Erscheinungen*", in the Phys. Zeitschr., Sept. 1919, Prof. H. BARKHAUSEN describes some experiments by which discontinuities in the magnetisation were made detectable by a telephone. To this end an iron rod was placed vertically in a small solenoid which was connected to a triode-amplifier. When a small permanent magnet was brought by hand near the iron rod, so that the latter became magnetised, a rustling sound in a telephone connected to the amplifier could be heard, which sound was due to the induction pulses caused by the discontinuities.

Repeating and extending these experiments we observed some new phenomena, which may be described here briefly.

At the outset it may be remarked, that the mentioned rustling was known already in the technics of wireless telegraphy where it was regarded as troublesome in the use of the magnetic detector of MARCONI [1]).

In our experiments we used a so-called three-stage low-frequency amplifier, in which the energy-transport from triode to triode took place by means of small transformers. The terminals of the solenoid that contained the iron to be magnetised were connected with the filament and the grid of the first triode either directly or by means of a small transformer of suitable dimensions.

The rustling in the telephone, due to the induction-pulses in the solenoid must primarily be caused by a discontinuous change of the total flux through the solenoid which accompanies the sudden changes of magnetisation-direction of molecule-groups or of iron-crystals. When in this phenomenon the magnetisation of the separate iron-crystals is reversed suddenly by the external field, we should expect that the change of the number of lines of force that are already present in the case of spontaneous magnetisation and which must describe in the air small curves near the surface of the iron, will be best observed by means of a solenoid fitting narrowly round the iron.

[1]) ECCLES. Wireless Telegraphy and Telephony, 2nd Ed. p. 284, 285.