CVIII. ON THE CONCENTRATION-ACTION CURVE OF INSULIN PRÉPARATIONS, AND ON ANTI-INSULIN.

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In our laboratory we had to determine the strength at which the commercial preparations of insulin made in Holland had to be adjusted before they were brought into commerce. We then met with a peculiar fact, which has become the starting point of the following considerations. Of a new batch 1 cc. of a certain dilution was injected into two rabbits. Both animals showed convulsions and their blood sugar was reduced below 0.045 %, which is to be considered, when testing on rabbits, as the effect of at least three old clinical units of insulin. Two other animals, which were injected with 0.6 cc., did not show any convulsions but their blood sugar was reduced to 0.053 % and 0.046 % respectively. The minimal convulsant dose might then be supposed to lie between these two doses, and indeed 0.8 cc. did not cause any convulsions in two animals, but it did reduce the blood sugar below 0.045 %. So far the testing proceeded quite normally, and in the absence of more exact determinations 0.8 cc. of this dilution was preliminarily considered to be the minimal convulsant dose.

We had come so far, when, for reasons which it is not necessary to mention here, we had to inject a quantity of 1 unit into some rabbits. For this purpose we took a solution of this same preparation which was three times weaker than that referred to above and again used 0.8 cc. *Both animals soon showed convulsions* and a corresponding reduction of blood sugar. This fact, which seemed incredible at first, was repeated with eight rabbits in all, and these all behaved as is usual after injection of a convulsant dose of insulin. Details of the further exact standardisation of this preparation will not interest the reader. Briefly stated, the preparation appeared to possess two convulsant doses, between which there was a less effective zone, and of which the upper one came to 300 % of the lower one. This phenomenon has since then been observed with indubitable certainty in various batches. The schematic curve of the reduction of blood sugar by successive doses of insulin for one such preparation is shown in Fig. 1.

Each of these preparations corresponds more or less exactly to this scheme, because at that time we were unable to use enough rabbits to exclude individual influences entirely.

Therefore I have plotted in a curve the results obtained with 17 preparations of different degrees of purity, which all showed the double convulsant dose, on

Fig. 1.

the 217 rabbits, which were used for their standardisation (Fig. 4, p. 838). This was done by marking off on the ordinate the lowest blood sugar concentration in parts per thousand, while on the abscissa the quantity of injected insulin is expressed in old clinical units. Here again we considered as three units (= a convulsant dose) the smallest dose with which the convulsion limit might be attained. Now I have taken the average of the lowest blood sugar concentrations of all animals that have been injected with less than $\frac{1}{2}$ unit, and marked it off on the vertical zero line. The average of the animals which we injected with $\frac{1}{2}$ to $1\frac{1}{2}$ units is marked off on the vertical line, corresponding with 1 unit and so on. With each point thus obtained the number of animals is given from which the average blood sugar has been taken. Wherever that number was very small (3) I have connected the adjacent points by a dotted line. There is a striking resemblance between this curve and that of Fig. 1. Only the second elevations (*CD*) of the various batches accumulate more horizontally than vertically. This is quite intelligible as with one preparation the second convulsion limit is only 200, with the other sometimes even 400 % of the lower one. I particularly wish to draw the attention to the rectilinear course of the curve of 0–3 units as I shall discuss its importance later.

For the sake of completeness I must point out that, to save space, the curve has not been continued further to the right than to the upper convulsant dose. Hence the total number of the rabbits whose reactions are recorded in the curve does not amount to 217.

Connected with these phenomena it was observed that the clinicians, who are kind enough to test the preparations on patients before the factory hands them over to the trade, always complained of insufficient activity especially with these batches¹. Adhering to our definition that the clinical unit is one-third part of the *smallest* dose which is able to cause convulsions and reduction of blood sugar to 0.045 % in a rabbit of about 2 kg. the specimens for the clinics were diluted according to the “lower” convulsion dose. The information from the clinicians was always such, that we definitely had to assume as three units that quantity which we generally call the upper convulsant dose. In

¹ The insulin placed on the market, was then made 2–4 times stronger, according to need, so that these phenomena cannot be quoted as an explanation for eventual disappointment experienced with commercial insulin.

any case the deviation was no more than we also observed in the clinical data before we had observed the two convulsant doses. As these two convulsant doses deviated from 100 to 300 %, the continual correspondence of the upper dose with the effect on patients cannot be said to happen by chance.

So far as to facts. Let us now try to give a plausible explanation of these strange phenomena.

1. *How can the two convulsant doses be accounted for?*

We can say without exaggeration that the straight line has always been taken for granted as the concentration action curve of insulin, for the unit is a biological one and so it does not concern the quantity but the effect. When a preparation is labelled 1 cc. = 20 units, it is already implied that 2 cc. are supposed to have the double effect of 1 cc. As 2 cc. must necessarily contain twice as much insulin as 1 cc., concentration and effect are directly proportional and the curve is a straight line (Fig. 2 *AB*). This supposition seems to accord

Fig. 2.

with the facts as there have never been any complaints from the clinical side that a greater number of cubic centimetres of the same preparation did not answer to what was expected from the effects of 1 cc.

The above-mentioned facts which we observed on rabbits during the period of August–November, 1923, deviated considerably from this supposition.

This becomes intelligible if we assume an inhibiting substance to be present in the solution which only begins to act with larger doses. The latter is quite possible if this inhibiting substance has a higher limit below which no action is detectable than has insulin, or even when the limit is the same, if the concentration of the inhibiting substance in the solution is less than that of the insulin¹.

Now in order to dominate the insulin, the CA curve² of our hypothetical second substance must rise more quickly than that of the insulin. If the height KK_1 indicates the effect of three old clinical units we can easily infer from

¹ For the insulin too has such a limit. Strictly speaking, the curve would consequently be AA_1B_1 and not AB .

² Concentration-action curve.

the course of both curves that with L the effect LL_1 (the perpendicular distance between the two curves, which indicates the effect of the mixture) corresponds to less than one convulsant dose, this perpendicular being shorter than KK_1 . If, however, the effect of the insulin is to increase again with still greater doses, the curve of the inhibiting substance must bend towards the horizontal (Fig. 2 *D*). If the perpendicular MM_1 is as long as KK_1 the upper convulsant dose is near point N . Now a curve answering to these conditions has been realised in the adsorption curve, a line, which, as a concentration-action curve, is known in pharmacology. So the appearance of two convulsant doses can be accounted for by assuming that the preparations with a double convulsant dose contain two substances which oppose each other. Insulin itself in its CA curve follows the straight line of the partition coefficient; the inhibiting substance follows the adsorption curve (Fig. 3).

Fig. 3.

For the sake of brevity I shall henceforth speak of anti-insulin (AI) without intending to say anything about the character of the inhibiting substance. It certainly is not an anti-toxin.

2. *Why do we not find two convulsant doses in every batch of insulin?*

In other words: must we suppose the anti-substance to be absent when we do not observe two convulsant doses? The answer is quite simple. The two relative concentrations are not always equal in the solution. If it contains relatively more anti-insulin (AI), the lower convulsant dose disappears. For then the entire AI curve (Fig. 2) moves to the left, and as soon as point C comes to the left of K_1 the lower convulsant dose disappears, as in that case the perpendicular distance of the two curves is not as long as MM_1 in any point to the left of N . The presence of AI can then be inferred from the blood sugar curve which follows the dotted line (Fig. 1) repeating the form of our first curve on a higher level.

But also when the anti-insulin proportionately decreases, only one convulsant dose is observed, viz. exclusively the lower one. For then point C is so far to the right that the minimum perpendicular distance of the two curves PD is always greater than the convulsant dose. In observations on rabbits we can then at most get an indication about the presence of the AI, for example, by three convulsant doses only producing one single convulsion within the time limit, perhaps even no convulsions at all but only a reduction of blood sugar to below 0.045 %.

Now we can still ask the question: how is it possible that in the series of preparations following each other quickly, the double convulsant dose has appeared so suddenly and frequently? This will most probably be connected with a change of manufacture which was introduced just then, where the same process of purification was applied three times instead of twice, to decrease the solids. In doing this more anti-insulin than insulin seems to be lost according to comparative curves, and consequently the curve of the AI has moved to the right, thus making two convulsant doses possible, and consequently therefore a large discrepancy between clinical and biological standardisation.

So we must suppose that in previous batches there was relatively even more anti-insulin in the preparations, but that it escaped our observation by the two curves practically covering each other.

At the same time these considerations indicated how it might be possible to avoid the unfavourable action of AI. By purifying once more or even oftener in the same way, the AI always decreases relatively (the AI curve always goes more to the right). One has to bear in mind that the AI curve moreover must proceed relatively less steeply. If point *C* lies very far to the right, the upper convulsant dose will not only disappear, but practically we cannot speak of the presence of AI any longer. In fact, we never saw any indication of the presence of AI in later preparations that had been purified four or five times. But also by applying other methods of preparation known from the literature (short heating, making use of the selective action of substances which flocculate albuminoids, such as picric acid according to Dudley, $(\text{NH}_4)_2\text{SO}_4$ according to Shaffer, NaCl according to Murlin, and so on) we could avoid the appearance of AI with ever greater certainty. After November, 1923, all preparations we had controlled were free from it.

3. *How can it be explained that insulin always has an effect on man according to the upper convulsant dose?*

In order to make this intelligible one must bear in mind that, if there are two convulsant doses, point *C* cannot be far to the right of point *K*. If that distance is slight, the greatest insulin effect which can be obtained in this zone (± 4 units) is undetectable in most human patients.

If we try to give more the AI begins to act. In rabbits, however, these 4 units already produce the maximum effect.

4. *In support of the theory* developed above, we have the fact that under the lower convulsant dose, where theoretically we may not suppose an anti-insulin effect, the CA curve of the insulin really is a straight line. We have observed this several times and in the scheme (Fig. 1) as well as in the curve (Fig. 4) it has been shown by the steeply descending straight line which represents the blood sugars 2 hours after the injection of increasing insulin doses.

In an attempt to eliminate by conversion the deviations which occur on account of the unavoidable differences in the weights of the rabbits, we got still nearer to this straight line. So in this zone the concentration-action curve

Fig. 4.

of the insulin is certainly a straight line. Now if this holds good for the only part of the CA curve that happens to be controllable we have no reason to suppose that it should not also apply to the further part. We can, of course, hardly speak of the CA curve after the convulsion dose, but when we once more consider the dotted line of Fig. 1 from this point of view, the supposition that its elevation is caused by a second substance becomes very probable indeed. Why then should not the same reasoning apply to the uniform curve with the two convulsion doses?

Finally one might suppose that not a constituent part of the solution but a reaction product of the body, appearing when greater doses are injected, causes these phenomena. But then the connection between the upper convulsant dose and the clinical action, the existence of which cannot be denied, would be incomprehensible.

SUMMARY.

1. The CA curve of insulin, with regard to blood sugar, seems to be a straight line; that of anti-insulin an adsorption curve.

2. In the "Insulin Neerland.," as it was issued some months ago ("purified three times") there was present an inhibiting substance. This substance was called anti-insulin for the time being, which does not imply that it is identical with or akin to other constituents or fractions as having the same effect and the same name, which have often been described, but which mostly either appear at a very early stage of the preparation or whose antagonistic effect can be compared with that of trypsin. This anti-insulin caused (a) the phenomenon of the double convulsant doses, (b) the resultant enormous differences between experiments on rabbits and on diabetics. As preparations have only been brought into commerce with the upper convulsant limit, which has always been done in Holland, these phenomena (double convulsant limit) cannot have any influence on clinical activity.