Physiology. — "*On the Action of Insulin*". By Dr. J. G. DUSSER DE BARENNE and Dr. G. C. E. BURGER. (Communicated by Prof. H. ZWAARDEMAKER).

(Communicated at the meeting of March 29, 1924).

With regard to various fundamental questions about the action of insulin, opinions still differ widely, e.g. on the question whether the respiratory gaseous exchange, notably the respiratory quotient (R. Q.), is influenced by the substance. BANTING, BEST, MACLEOD and their co-workers, and also KROGH assert that insulin induces a rise of the R. Q. Others on the contrary, e.g. DUDLEY, LAIDLAW, TREVAN and BOOCK maintain that insulin evolves a lowering of the R. Q.

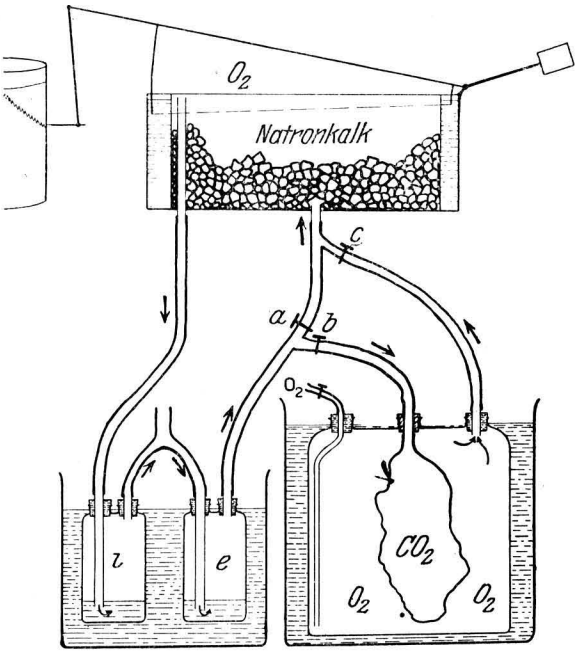
The latest number of the Klinische Wochenschrift (N°. 15, March 25, 1924, p. 555) contains a report of a communication by A. BORNSTEIN in the "Arztlicher Verein" Hamburg on 15 Jan. last, in which he gives, according to the reporter WOHLWILL the following summary of the researches hitherto made into this subject and of the results of his own experiments: "Die Untersuchungen welche eine Steigerung des R. Q. by Insulinwirkung ergeben haben, woraus dann auf vermehrten Zuckerverbrauch geschlossen wurde, sind deshalb nicht zu verwerten, weil dabei nicht der Einfluss erhöhter Lungenventilation auf den R. Q. berücksichtigt ist. Zieht man nur solche Tierexperimente in Betracht, bei denen die Lungenventilation nachweislich auf gleicher Höhe gehalten wurde, so zeigt sich, dass Insulin keine oder nur eine ganz unbedeutende, innerhalb der Bestimmungsmethode gelegene Erhöhung des R. Q. bedingt".

We should not like to endorse the first statement expressed in this summary; in our opinion it cannot relate to the inquiries prosecuted in the laboratory of TORONTO nor to KROGH's researches upon the gaseous exchange under insulin. When we consider the fact that KROGH's experiments have been conducted on curarised rabbits under artificial respiration, the argument contained in the first sentence falls to the ground. Neither would we be responsible for the statement contained in the second sentence, which sums up BORNSTEIN's own results in this matter.

By means of our method of the "CO_2-trap" which enables us to record graphically the oxygen-consumption, the carbon-dioxid-output,

therewith the R. Q., and also the pulmonary ventilation, we have of late also studied the respiratory exchange under the influence of insulin and have attained results entirely opposite to those achieved by BORNSTEIN. We might, therefore, briefly record them here; surely the question of the respiratory exchange under insulin must be looked upon as one of the fundamental questions of the insulin-problem.

First of all we might set forth the principle of our new method, which may be done best in referring to Fig. 1. For a fuller discussion of this method we refer to our paper in the Klinische Wochenschrift, 1924, p. 395, and especially to that in the Journal of Physiology, Vol. 59, 1924.



Natronkalk = Soda lime. Fig. 1

The oxygen-consumption is determined graphically with the aid of a small spirometer after KROGH [1]) (of ca 1,5 liters capacity for experiments on cats), to which is attached the "trap" for determining the CO_2-output.

The inspiratory-, and expiratory valves (watervalves after MÜLLER *i* and *e* of 2 mm. resistance only) are connected with the animal through the trachea-cannula and through rubber tubes with the spirometer, filled with oxygen. On the bottom there is sodalime for the absorption of the expired CO_2.

[1]) A. KROGH. Sur un appareil respiratoire enrégistreur servant à déterminer l'absorption d'oxygène et les échanges caloriques chez l'Homme. C. R. de la Soc. de Biol. de Paris, 1922, Tome II, blz. 458.

When writing an O_2-curve the tube is open at *a*, but the tubes, leading to and from the CO_2-trap, are closed at *b* and *c*. The CO_2 given off by the animal during this O_2-period, is absorbed by the soda-lime; only the unconsumed O_2 comes back again in the spirometer. The respiratory curve of the animal on the kymograph is a rhythmically moving, gradually falling curve; its fall at the end of an experimental period of 10—20 minutes, after calibration of the spirometer, gives the amount of O_2, taken in by the animal.

For registration of a CO_2-curve the tube is closed at *a* and the other tubes are opened at *b* and *c*. The animal now expires into a rubber balloon enclosed airlight in a large reservoir, filled with O_2. This rubber balloon of 14 liters capacity in our experiments on cats, serves as the "*CO_2-trap*", in which the expired air is caught as in a trap, so that it cannot mix with the O_2 in the spirometer, but can act volumetrically as if entering the spirometer. On reaching the trap the expired air extends this balloon, and expels exactly its volume of O_2 from the reservoir, in which the rubber balloon is suspended, back into the spirometer. The curve that is now traced by the respiration of the animal, is less abrupt in its descent than the O_2-curve, because the expired CO_2 is not absorbed by the soda lime, but only retained in the trap and acts volumetrically as if it entered the spirometer. *The difference in fall between the CO_2- and the O_2-curve corresponds to the amount of expired CO_2.* Of course due regard should be given to an even temperature of the whole apparatus; therefore the O_2-reservoir and the trap it contains should be put in a water-bath or another heat-isolating device. (See Fig. 1).

The advantages of this method are manifest. The apparatus enables us to determine graphically the CO_2-output, just as easily as KROGH's, or BENEDICT's method does for the O_2-intake. As the bloodpressure may be studied from a bloodpressure-curve, so the bearing of all sorts of operations and substances upon the gaseous exchange may be represented graphically, while it can also be readily ascertained whether with contingent changes, as with an increase of the R. Q., hyperventilation comes into play. From these CO_2-curves, in combination with the O_2-curves, the R. Q. can be computed. But even at first glance some information about the R. Q. may be gathered from the CO_2-curve. If this curve descends it implies a R. Q. smaller than 1, if it runs horizontally we have in this experimental period a R. $Q = 1$, and if the curve rises above the horizontal, it means that the R. Q. during that rise is higher than 1.

Numerous problems may, therefore, be studied in this way; some of them we have already taken in hand, e. g. the problem of the gaseous exchange under the influence of insulin. Thus far our experiments were made on cats which had fasted for at least 24 hours, mostly 36—48 hours. The experiment proper was made on the decerebrate, normally breathing animal, when it had been left alone after the decerebration for one hour, at the very least, often much longer (3—4 hours), in order to allow the anesthetic (ether)

to disappear from the animal. In order to maintain a constant body-temperature, the animal was placed after the decerebration in a waterbath at 37°; only the paws and a small part of the chest and the abdomen being submerged, so that the breathing of the animal was not hindered by the pressure of the water.

After the anesthetic could be expected to have left the animal, first a "normal curve" of the O_2-intake and of the CO_2-output was registered, then insulin was administered during the tracing of a CO_2-curve, and while more CO_2-curves were being written, the course of the CO_2-output was followed; finally the experiment was ended by an O_2-curve. In the intervals between the several CO_2-curves the balloon was emptied everytime and some c.c. of arterial blood were drawn for bloodsugar estimation (method of SHAFFER-HARTMANN, c.q. of COHEN TERVAERT [1]).

The result of the six experiments made up to now, is that in five of them a distinct rise of the R. Q. was observed after the injection of insulin, without the occurrence of hyperventilation; in two of them the R. Q. rose even above 1. In one animal the R. Q. was already 1 before the injection of insulin and maintained itself after the injection.

To instance this we subjoin the following protocol and fig. 2, representing the curves obtained.

Experiment of 23 Feb. 1924:

Castrated male cat, body-weight 3.10 kg. Ether anaesthesia, during which moderate excitation. Tracheal canula. Vagi left intact. Carotids ligated. (Barometer at 2 p.m. 767.5 mm.; at 8 p.m. 768,5 mm. Hg).

8^{30} a. m. Blood I drawn (from carotid with potassium-oxalate).

8^{45} a. m. Decerebration. Hypophysis not removed [2]). Animal in waterbath at 37° C.

11^{15} a m. Animal quite well. Maximal decerebrate rigidity in the four legs.

11^{25} a. m. Blood II drawn.

11^{40} a. m. Hypophysis removed as a whole, sella turcica not cleaned [2]).

Curve 1. O_2—curve.

12^{19}—12^{30} p. m. Maximal decerebrate rigidity in the four legs; temperature spirometer 11,9°—12°. rectal temperature of the animal 39,1° C.

12^{33} p. m. Blood III drawn.

Curve 2. CO_2 – curve.

1^{56}—2^{08} p. m. Maximal decerebrate rigidity in the four legs. Temperature spirometer 12°—12°. Rectal temperature 39°.

2^{18} p. m. Blood IV drawn.

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[1]) See D. G. COHEN TERVAERT. Nieuwe methoden voor bloedsuikerbepaling, Nederlandsch Tijdschrift voor Geneeskunde, **1921**, II, p. 857.

[2]) These data are not important in our case. They are so, however, for another question that will be left for further discussion in a future paper.

Curve 3. CO_2 – curve 3a.

3^{01}—3^{12} p m. Temperature spirometer 12,4°—12,8°. Rectal temperature 39°.

3^{07} p. m. 4 units of Iletin Lilly (American insulin U 20) injected intravenously at the point marked ↓; at *m* beginning of the manipulations.

Curve 3b.

3^{31}—3^{34} p. m. Temperature spirometer 12,9°—12,9°. Rectal temperature 38,9°.

Curve 3c.

3^{57}—4^{10} p. m. Maximal decerebrate rigidity in the four legs. On either side vivid reflexes of the pinnae, the corneae and the whiskers. Animal well. Temperature spirometer 12,9°—12,9°. Rectal temperature 38,8°.

4^{35} p. m. Blood V drawn.

Curve 3d.

4^{41}—4^{54} p. m. Temperature spirometer 12,9°—12,7°. Rectal temperature 38,8°.

Curve 4. O_2 – curve.

5^{12}—5^{24}. Decerebrate rigidity in right foreleg almost quite disappeared, still rather marked in left foreleg and in hindlegs. Cornea-reflex on either side +, reflexes of the pinnae and the whiskers —. Temperature spirometer 12,8°. Rectal temper. 39°.

7^{22} p. m. Blood VI drawn. Calibration of the spirometer: 200 cc. = 29 mm.

The rest of the protocol is not essential in this case, so it is omitted here.

Result of the bloodsugar determinations:

Blood I	before Insulin	1,9 ‰
Blood II		2,5 ‰
Blood III		2,77 ‰
Blood IV		4,00 ‰
Blood V	after Insulin	2,15 ‰
Blood VI		1,3 ‰

Result of the determinations of the gaseous exchange:

Curve 1 fall in 10 min. = 32,7 mm.; consequently O_2-consumption per kg. hour (reduced to 0° and 760 mm. Hg, dry air) $\frac{200 \times 6 \times 32,7}{29 \times 3,10} \times 0,953 = 415,9$ cc.

Curve 2 fall in 10 minutes = 7,7 mm, consequently CO_2-output = *318,0* cc.

Curve 3a fall in 10 minutes = 6,7 mm., consequently CO_2-output = *330,1* cc.; R.Q. = *0,79.*

Curve 3b fall in 9 min. = 3.7 mm., so in 10 min. = 4,1 mm.; consequently the CO_2-output = *363,1* cc. So R.Q. in 3^b = *0,87.*

Curve 3c fall in 10 min. = 0 mm., so CO_2-outpul = O_2 consumption. So R.Q. in 3^c = 1.

Curve 3d Rise in 10 min. = 1,7 mm.; consequently CO_2-output= *379,9* cc.

Curve 4. Fall in 10 min. 28,2 mm. consequently O_2-consumption = *358,0* cc.

When assuming that during curve 3^d, traced about 10 minutes earlier, there was an O_2-consumption equal to the one during curve 4, then the R.Q. during the period of curve 3^d has been $\frac{379,9}{358,0} = 1,06$.

In this experiment we observe, therefore, that after the introduction of insulin a progressively increasing rise of the R. Q. reveals itself from 0.75 upwards to 0.87, and from 1 to 1.06. This increase

of the R. Q. appears distinctly from the curves of fig. 2, while we can also infer from them that hyperventilation is not answerable for this increase, neither in this experiment, nor, indeed, in any of the others. This will be readily understood, if we consider that from curve 3^a, just before the insulin-injection, we register a ventilation of 1095 cc. per minute and from 3^d, after insulin, a ventilation of 1140 cc. per minute, that is an increase of only 4 %. That this inappreciable hyperventilation should be answerable for the rather prolonged rise of the R. Q. is in itself not admissible; moreover this view is disproved directly by the fact illustrated by curve 3^a, that the much more pronounced increase of ventilation, occurring immediately after the insulin-injection, does not involve the least change in the course of CO_2-curve.

Besides, in this stage of the experiment there was also a decrease in the bloodsugar-content from 4 ‰ (hyperglycemia after decerebration) down to 1.3 ‰ [1]). The O_2-consumption displayed a fall from 415.9 cc. per kg-hour to 358.0 cc. per kg.-hour.

That the rise of the R. Q. under the influence of insulin cannot possibly be attributed to hyperventilation is proved additionally by our experiments, which yielded curves that showed an increase of the R. Q. even with smaller ventilation than existed before the insulin-injection.

The rise of the R. Q. generally begins 20 min. after the injection of the insulin, and is most often gradual, as in the experiment recorded here, sometimes, however, it is rather abrupt.

Of course, the principal interest centres in the question how is the increase of the R.Q. to be explained? So long as the R.Q. does not rise above 1, this increase may be assumed to have evolved from the increased combustion of carbohydrates under the influence of insulin. This view has been adopted by BANTING, BEST, COLLIP, HEPBURN and MACLEOD, when they found that insulin raises the R.Q. in the pancreas-diabetic dog and in the normal dog. Other researchers, e.g. KROGH and DALE have also accepted this view. However, this is presumably not the only factor in this process, as is borne out by those cases in which the R.Q. rises above 1 under the influence of insulin. For the present these cases are perhaps best interpreted when assuming, in accordance with MACLEOD's and GEELMUYDEN's opinion, that under the influence of the pancreas hormone also glycose is converted into substances that contain less of O_2 in their

[1]) In a later phase of the experiment the well-known hypoglycemic phenomena revealed themselves, from which we conclude that, in the stage of the experiment recorded here, the insulin-action had not yet culminated.

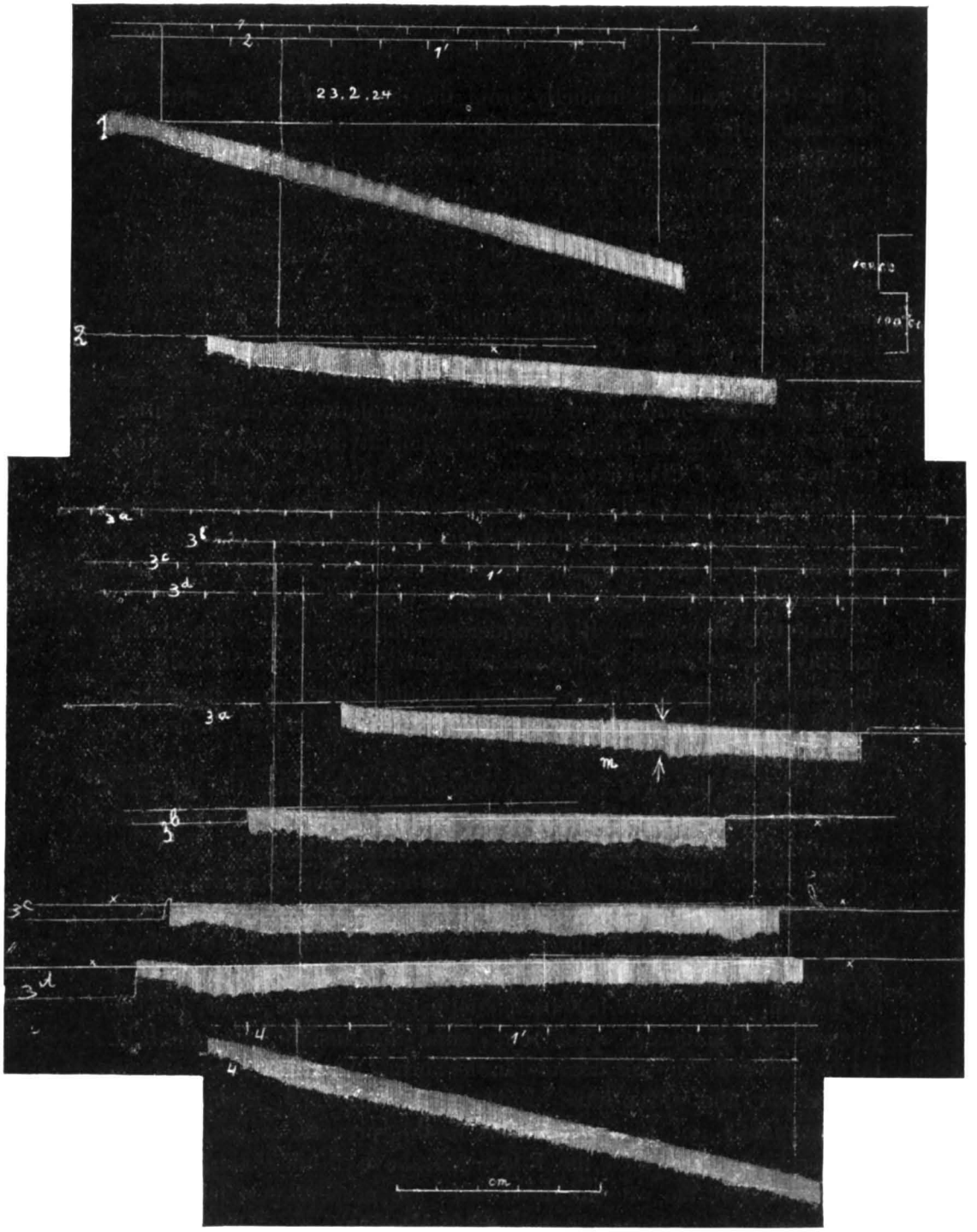

Fig. 2.

Curve 1 and 4: 2 O_2-curves, 1 *before*, curve 4 *after* insulin.
Curve 2: CO_2-curve *before*, curves 3^b, 3^c and 3^d *after* insulin.

In curve 3^a at ↓↑ intravenous injection of 4 units Iletin (U_{20}); at *m* beginning of the manipulations for the intravenous injection; the lines *x* in the curves 2, 3^a, 3^b etc. parallel to the control-lines of the "trap"-system, are used to measure jn the middle of the curves the extent of the fall, resp. the rise.

Under curve 4 5 cm. illustrate the magnitude of the diminution. The time-lines, given for each of the curves separately, because the kymograph was not absolutely regular, indicate minute-intervals. By the side of curves 1 and 2 calibration of the spirometer (200 cc. = 29 mm.).

molecule, e.g. into fat. This assumption tallies with the long known fact that animals, fattened on a carbohydrate-containing diet, have a R.Q. higher than 1. Of course it is highly presumable that this factor exists also in those cases, in which the R.Q. does not rise above 1, but that it is superseded there by other factors, or performs an inferior function. Doubtless, the action of insulin is a very complicated problem, and still far from being set at rest. However, these experiments have shown:

1°. *that under the influence of insulin (Iletin Lilly and Insulinum Neerlandicum) in the decerebrate fasting cat mostly (in* 5 *out of* 6 *cases) a distinct rise of the R.Q. appears, which cannot be ascribed to hyperventilation.*

2°. *that in some cases* (2 *out of* 5) *the R.Q. rises even above* 1.

This method of graphical registration of the CO_2-output with the aid of our "CO_2-trap" is peculiarly adapted for similar researches.

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From the Physiological Laboratory of the University of Utrecht.