THE ACTION OF INSULIN ON THE ASEPTICALLY PERFUSED HEART.

By R. BODO and H. P. MARKS.

(From the National Institute for Medical Research, London.)

Cousy(1), of Noyon's laboratory, has recently repeated the perfusion experiments of Hepburn and Latchford(2) on the isolated rabbit's heart, in which they observed an increase in apparent sugar disappearance following the administration of insulin. But, whereas under ordinary conditions, the former observes a similar increase, when aseptic precautions are taken, he finds the sugar consumption to be practically abolished, instead of increased. Thus, in a number of experiments performed in the ordinary way, the apparent sugar disappearance was increased on the average from 1·08 to 1·85 mgr. per gr. of heart per hour under the action of insulin, while in similar aseptic experiments it was reduced from 0·85 to 0·15 mgr. per gr. per hour.

From these results Cousy concludes that insulin inhibits the glycolytic action of the heart, and attributes the increase in sugar disappearance normally observed after insulin to the action of bacterial contamination. Since this explanation appeared highly improbable in the light of previous work on perfused heart and skeletal muscle, carried out in this Institute by Burn and Dale(3), it seemed desirable to repeat Cousy's aseptic experiments.

Our first difficulty arose in connection with the preparation of sterile Ringer-Locke solution. Unfortunately, Cousy does not give any details concerning his perfusion solution, except that he boiled his solution and brought it back to the original volume. He does not mention the composition of the solution which he boiled, although it is well-known that this procedure leads to loss of carbon dioxide and precipitation of the calcium carbonate. We were only able to avoid this by sterilising the sodium bicarbonate solution separately from the other salts.

The procedure was as follows: The bicarbonate solution, to which was added a drop of phenolphthalein, was first boiled and cooled, during which process it became carbonate through loss of carbon dioxide. This latter was replaced by leading in a stream of the gas until the deep red

colour was discharged, and then the excess of carbon dioxide was removed by oxygenating the solution until the original faint pink colour appeared. The bicarbonate solution was then mixed into the solution of the remaining Ringer salts, previously boiled, cooled and thoroughly oxygenated, and, after addition of the glucose solution (sterilised three times in steam) was finally made up to volume in a sterilised one litre flask.

Aseptic precautions were observed throughout. The necks of all flasks were protected from contamination by inverted beakers, and were flamed before the transfer of solution. The tube for leading in carbon dioxide and oxygen was provided with a bulb containing a plug of sterile wool, and with a glass hood to protect the neck of the flask, and was previously sterilised in the autoclave. (The effectiveness of these precautions was always controlled by preparing agar plates from the final solution.) The solution so obtained was identical in its composition with that described by Locke and Rosenheim(5), and had the same *p*H, but was also sterile.

All flasks were of either Jena or Pyrex glass, and the distilled water used was previously redistilled in Pyrex glass apparatus in order to eliminate traces of heavy metals. The salts used were Kahlbaum's puriss. and the composition of the solution was as follows:

NaCl 0·9 p.c., KCl 0·042 p.c., $CaCl_2$ anhydr. 0·024 p.c., $NaHCO_3$ 0·02 p.c., Dextrose 0·1 p.c.

In one or two experiments a borate Ringer of the following composition was employed:

NaCl 0·9 p.c., KCl 0·042 p.c., $CaCl_2$ anhydr. 0·024 p.c., Dextrose 0·1 p.c., 20 c.c. Palitzsch's(4) borax-boric acid solution of *p*H 7·6 (3 c.c. M/20 borax + 17 c.c. M/5 boric acid, M/20 NaCl).

The preparation of this solution was quite simple, since it could be sterilised by boiling without fear of decomposition, but we preferred to use in most cases the more physiological Ringer-Locke solution.

The sterilisation of the apparatus did not present much difficulty. A suitable form of apparatus has been described by Noyons(6), and this was employed by Cousy. We used a closed circuit perfusion system similar in principle but modified so as to permit easier sterilisation and aseptic manipulation. By keeping as much of the apparatus as possible immersed in warm water jackets, it was found possible to dispense with the spiral heating tube employed by Noyons, and consequently to reduce the volume of the apparatus so that a perfusion could be performed with as little as 25 c.c. of fluid. Means were provided for the addition of fluid

to, or removal from the apparatus under aseptic conditions, through the graduated tap funnel (*a*) and the shielded jet (*b*) respectively. The apparatus was maintained at a temperature of 39° C. by means of a current of water heated by the electric immersion heater (*h*) and flowing first through the upper, and then through the lower water jacket, as indicated by the arrows in the diagram. A prolonged contact of the water with the heater was ensured by surrounding the latter with a copper wire spiral. The temperature of the water was regulated by adjusting the rate of flow past the heating element, this being done by raising or lowering the overflow tube (*e*).

The apparatus was sterilised (in steam) in three portions, viz. the part contained in the upper jacket, the heart chamber, and the heart cannula attached to a temporary reservoir by a length of rubber tubing.

The upper part of the apparatus was first inserted into its water jacket and the current of heating water adjusted to give a temperature of 39° C. in the jacket. The upper reservoir (*f*) and the temporary reservoir, with attached heart cannula, were then filled with the warm sterile Ringer's solution.

The actual experiment was performed under strict aseptic precautions, the rabbit's breast and neck having been depilated and painted with iodine. Under ether

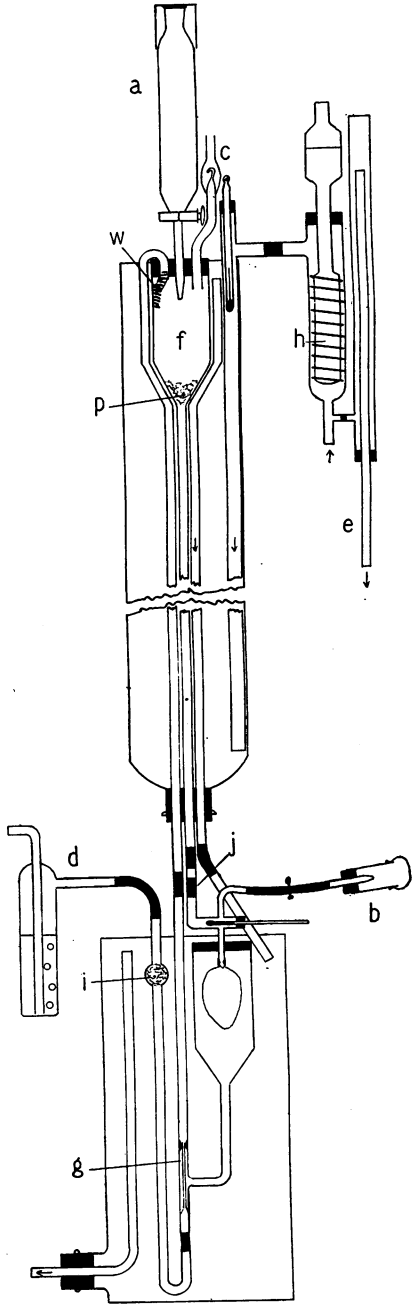

Fig. 1.

anæsthesia we bled the animal from the carotid artery, and, after opening the chest, the sterile cannula, connected with the temporary reservoir, was inserted and tied into the aorta. The heart was isolated and perfused in a sterile dish until the outcoming fluid contained no more blood. The temporary reservoir was disconnected and the cannula attached to the apparatus by the junction (*j*), the perfusion being continued from the upper reservoir, until the level of the Ringer had fallen to a mark which indicated that the amount of fluid left in the apparatus was exactly 10 c.c. The perfusion was then momentarily stopped while the heart chamber was attached, and 52·5 c.c. of fresh Ringer run in from the tap funnel (*a*). After perfusing the heart, the Ringer's solution drains from the heart chamber and is carried by a stream of oxygen issuing from the jet (*g*) back to the upper reservoir, from which it again passes to the heart through a vertical drop of about 60 cm. The mixed fluid and oxygen enter the upper reservoir through the greased spiral of silver wire (*w*) which effectively breaks up the froth, while the glass-wool plug (*p*) prevents any solid particles from reaching the heart and causing an embolism.

The oxygen enters the apparatus through the gas-washer (*d*) and the sterile wool plug (*i*), and leaves by the sterile trap (*c*). That the loss of fluid by evaporation is insignificant is shown by the fact that the theoretical amount of fluid can be recovered from the apparatus after a perfusion lasting for four or five hours.

About ten minutes after the commencement of the perfusion proper, samples of fluid for bacteriological control and determination of sugar were removed by means of a sterile syringe and needle inserted through the rubber junction (*j*).

Initially 12½ c.c. of fluid were removed, two portions of 5 c.c. being mixed with glucose-agar and poured into Petri dishes for incubation at 37°C., and 2 c.c. being deproteinised with zinc hydroxide for sugar determination, by the Hagedorn-Jennsen method.

Further samples were removed for sugar determination at half-hourly intervals, and samples for bacteriological control in addition, at the end of each perfusion period. After a control period of one hour, as much as possible of the perfusion fluid was removed by the jet (*b*) and replaced by an appropriate amount of fresh Ringer containing the required amount of insulin. By this means metabolic products were removed as far as possible, and the glucose concentration brought back nearly to the original value, so as to have the same conditions in the second period as in the first. After five minutes' perfusion to allow thorough mixing,

initial samples for the period were withdrawn. After one hour insulin period, the process was repeated for a second insulin period where possible.

The following is a typical experiment in full:

TABLE I.

Time		Sugar consumption in mgr. per. gr. per hour	Temperature °C	Heart-rate per min.	Outflow in drops per min.	Bacterial contamination per c.c.
Control period						
1.5	1st sample	0·6	39·0	165	165	0
1.35	2nd sample	1·0	39·0	174	171	—
2.5	3rd sample		39·2	171	168	·6
1st Insulin period (1 unit)						
2.25	4th sample	2·9	39·2	174	165	—
2.55	5th sample	2·0	39·0	174	156	—
3.25	6th sample		39·0	174	138	0
2nd Insulin period (1 unit)						
3.37	7th sample	1·8	39·0	153	126	—
4.7	8th sample	2·0	39·0	153	100	—
4.37	9th sample		—	—	—	·8

One unit of insulin was used in all such experiments. This amount is sufficient to produce a well-marked effect in the whole animal and should therefore suffice for the isolated heart. We did not use the large doses employed by Cousy, (20–40 units) as one of us (R.B.) has found that, in the heart-lung preparation, even of the dog, such doses produce a heart dilatation and constriction of the coronary vessels. On the other hand, one unit has no constrictor effect on the coronary vessels. The experiments were considered satisfactory only so long as the temperature, heart-rate, and coronary flow (measured by the drops falling from the heart apex) remained practically constant. Sometimes in the later periods the coronary flow would change, through capillary embolus or capillary œdema, and such periods are not included in the table summarising our experiments.

In order to observe the effect of the procedure of changing the Ringer on the apparent sugar disappearance, a few control experiments were carried out in the same way, but without the addition of insulin to the fresh Ringer used in the later periods. The following control experiment (Table II) is typical.

From Table III, which summarises our results, it is seen that insulin increases the apparent sugar disappearance in our aseptic experiments at least as much as in the non-sterile experiments of previous workers.

TABLE II.

Time		Sugar consumption in mgr. per gr. per hour	Temperature °C	Heart-rate per min.	Outflow in drops per min.	Bacterial contamination per c.c.
First period						
1.50	1st sample	0·5	39·2	180	180	·3
2.20	2nd sample	1·7	39·2	180	180	—
2.50	3rd sample		39·0	180	180	·3
Second period						
3.8	4th sample	1·4	39·0	180	180	—
3.38	5th sample	1·1	39·2	180	180	—
4.8	6th sample		—	—	—	·1
Third period						
4.20	7th sample	1·0	39·2	180	162	—
4.50	8th sample	0·8	39·0	165	102	—
5.20	9th sample		39·0	180	90	·5

TABLE III.

Expt. No.	Sugar consumption for each half-hour in mgr. gr. hr. 1st hour control		2nd hour insulin		3rd hour insulin		Bact. contamination per c.c. Initial	Final
1	1·0	1·0	2·0	1·2	1·4	—	0	5
2	1·0	1·0	3·2	2·0	1·8	1·9	1·4	0·9
3	0·6	1·0	2·9	2·0	1·8	—	0	0·8
	Control		Control		Control			
4	0·5	1·7	1·4	1·1	1·0	—	0·3	0·5
5	1·6	1·2	1·5	1·1	—	—	0·6	1·2
6*	1·4	0·8	1·2	1·3	2·2	1·8	—	—
7*	1·1	1·3	1·1	1·1	1·4	—	40	120

* Without aseptic precautions.

It will be seen from Table III that we did not achieve absolute sterility of working in these experiments. We doubt, indeed, the possibility of carrying out the relatively complicated technique of this perfusion in such a way as to exclude a very small number of bacteria with complete regularity. We took the precaution, however, of making regular bacterial counts from samples of the fluid taken at intervals; the results of these, as shown in the tables, make it clear that the contamination was minimal, and that no serious multiplication took place during the course of the experiments. Certainly there was no such growth in the experiments aiming at asepsis, as could account for any significant part of the large acceleration of sugar disappearance which followed the addition of insulin; nor was there any evidence that the addition of insulin influenced the rate of bacterial multiplication. In Cousy's paper, where the effect of insulin is apparently attributed to bacterial growth, there is no record of bacterial counts, or any other indication that direct evidence was

obtained of a connection between insulin and bacterial action. In two control experiments, without insulin or asepsis, we observed an apparent increase of sugar consumption in the final period, which could reasonably be attributed to the bacterial growth, then probably becoming active; but this could have no connection with the effect seen in the insulin experiments, where a much larger acceleration of sugar disappearance followed the introduction of insulin without any effect on the practically aseptic conditions.

Conclusion.

From the above results we draw only one conclusion, namely that the accelerated disappearance of sugar, from the fluid perfusing an isolated mammalian heart, under the influence of insulin, is a real phenomenon, and is not connected with bacterial contamination of the perfusion fluid. Our experiments throw no new light on the question of the fate of the sugar so disappearing. They show, as did those of Burn and Dale, that a large acceleration of sugar disappearance may occur without any significant acceleration of the heart beat. From analogy with the effect of insulin on skeletal muscle it would be expected that part of the glucose so disappearing is stored as glycogen; but it is not possible to carry out on the heart such adequate control analyses as would enable such a storage to be demonstrated.

Summary.

1. Apparatus and technique for aseptic perfusion of the isolated heart are described.

2. The increase in sugar disappearance after insulin, observed by previous workers, also occurs under sterile conditions.

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