THE EFFECT OF ADRENALINE ON THE UTILIZATION OF GLUCOSE

by

J. A. COHEN
Biochemical Department, Cambridge (England)

INTRODUCTION

During the last 15 years there has been some controversy as to the effect of adrenaline upon glucose utilization by the tissues. CORI (1928 a b c) is of the opinion that the tissues (of which muscle is quantitatively the most important) show lowered utilization after adrenaline injection. After glucose absorption rats can use 250 mg glucose/h for several hours; after adrenaline injection the glucose coming from the liver was calculated to be only about 50 mg/h; yet this was enough to cause prolonged hyperglycaemia. Other authors arrived at the same conclusion (LUNDSGAARD, 1938; CONN *et al.*, 1940; DILL *et al.*, 1939; COURTICE, 1939; WIECHMANN, 1927).

This view of the effect of adrenaline has been contested, *e.g.*, by SOSKIN (1927) and his colleagues, who used measurements of blood flow and converted arteriovenous bloodsugar differences into amounts of sugar retained by the muscles per unit of time. Their results were confirmed by the work of other authors (*e.g.*, HIMSWORTH AND SCOTT, 1927; JONKERS, 1945). They found no evidence for an effect of adrenaline on glucose utilization.

Since experimental work on this subject has all been done *in vivo* and the relevant data were usually obtained by calculation involving approximations and assumptions, it seemed of interest to try to obtain some more direct information from experiments *in vitro*. Rat diaphragm was used for these experiments on the assumption that results obtained on this object would be representative for muscle in general. The glucose utilization investigated and referred to in this paper was regarded as the uptake of sugar by the tissues, thus being governed by the intrinsic activities of the enzyme systems reponsible for this uptake at a constant supply of substrate. More explicitly this is taken to mean the rate of the reaction:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

assuming that this reaction determines the rate of glucose uptake. This reaction was followed by determining the rate of anaerobic glycolysis of diaphragm on the assumption that the rate of glycolysis is limited by the hexokinase reaction.

EXPERIMENTAL METHODS

Groups of 4 animals were used of which 2 were injected and 2 served as controls. They were all starved for a period of 19–24 hours before the experiment. They were killed by decapitation 40–70 minutes after the injection of adrenaline into the experimental animals of the group. Littermates

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(weighing 100–120 g) were used for the great majority of the experiments. It proved necessary to standardize the conditions rigorously from the moment the animals were killed. It was so arranged that the first manometer reading was performed exactly 35 min after the first animal of the group of 4 had been killed. Two of every group of 4 animals provided tissue for the 2 control manometers (with and without glucose); the remaining 2, which had been injected with adrenaline, furnished tissue for the 2 experimental manometers (with and without glucose). Thus every manometer received approximately equal portions of diaphragm from each of 2 identically treated animals. The 4 diaphragms were taken out and quickly washed in KREBS-HENSELEIT salt solution. The membranaceous part was dissected out and discarded. They were then divided, distributed and placed in 4 manometers. Two of these manometers contained KREBS-HENSELEIT-RINGER bicarbonate solution with 0.2% glucose. The 2 others contained the same solution without glucose. Thus the final distribution over the manometers was, that the diaphragms of the adrenaline injected and the control rats were tested both in the presence and in the absence of glucose. BARCROFT manometers were used. The gaseous medium was N_2 containing 5% CO_2 to maintain a pH with the bicarbonate in the fluid medium of 7.4. The bath temperature was 38° C.

For the purpose of determination of phosphate hydrolysis curves 2 rats weighing approx. 100–120 g were killed and the diaphragms taken out as usual. These were weighed on a torsion balance and transferred at once to a cooled mortar and immersed in 5 ml trichloroacetic acid (T.C.A.) (5%). After crushing in the mortar this extract was filtered through a GOOCH funnel into a cooled flask and the residue washed with 5 ml T.C.A. After neutralization to phenolphthalein with sat. soda the volume was made up to 15 ml (STOCK solution). 1.5 ml of the stock solution was used for the determination of inorganic plus creatine phosphate. This was estimated by diluting this volume of the stock solution and adding the calculated amount of ammonium molybdate-H_2SO_4-reagent (1.5 ml) of the FISKE AND SUBARROW reaction. After standing for 20 min 0.6 ml of the FISKE SUBARROW reducing reagent was added, the volume made up to 15 ml and the phosphate estimated in the colorimeter. To 3 other samples of 1.5 ml of the stock solution were added 0.75 ml of 3N HCl. These samples were hydrolysed in a boiling water bath for periods of 7, 15 and 180 minutes. Total phosphate was determined in a sample of 1.5 ml using H_2SO_4 for digestion followed by addition of 1 drop of nitric acid. All phosphates were determined using the FISKE AND SUBARROW method.

Adrenaline was injected subcutaneously 40–70 min before the experiment in a dose of 1 mg/kg rat and in a volume of approximately 0.5 ml. It was made up by dissolving adrenaline (base) in water acidified with HCl.

EXPERIMENTAL RESULTS

Glycolysis of diaphragm of rats after treatment with adrenaline

Anaerobic glycolysis was determined in diaphragms of rats injected with adrenaline and of untreated control rats. Fig. 1 gives a clear picture of the type of result that is usually obtained under the experimental conditions which have just been described in the technical part. Studying this figure, it will be seen at once that there is an inhibition of glycolysis in the diaphragm of the animals which had been treated with adrenaline. At first sight this inhibition seems to apply to the glycolysis in the absence as well as in the presence of glucose; closer examination of a great number of curves however reveals that most of the differences between treated and untreated diaphragms in these blanks (in the absence of glucose) disappear after an initial period of incubation of app. 10–20 min. In other words the small overall difference found between blanks from treated and untreated animals is usually generated in the first period only; after that period, when glycolysis becomes very low in either case, a significant difference no longer exists between the rates of glycolysis in blanks from diaphragms of treated and untreated animals. Faced with the existence of an initial glycolysis in the blanks which practically disappeared after 20 min and which differed slightly in treated animals and controls it was necessary to allow for the possibility that this difference would, to a certain extent, account for the differences found between treated animals and controls in the presence of glucose. It is the object of these experiments to interpret glycolysis of added glucose in terms of glucose uptake. If therefore the difference in glycolysis

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rate between controls and treated animals in the presence of glucose were no greater than the difference observed in the absence of glucose, this difference in the presence of glucose would have no significance. Since the difference in the rate of glycolysis in blanks from treated animals and controls disappears after 20 min when glycolysis becomes minimal in both cases, it was decided to estimate the glycolysis in all experiments (involving diaphragms of treated animals and controls with and without glucose) during the period of 30 min elapsing between 20 min and 50 min from the commencement of incubation. The rate of glycolysis during that period served as a basis for the calculation of the conventional $Q^{N}_{CO_2}$. The figures thus obtained ought to reflect the true utilization of added glucose. The blanks of diaphragm from treated and untreated animals were the same under these conditions.

Tables I and II show the results of a series of experiments in which $Q^{N}_{CO_2}$ has been thus computed. Table I shows that a significant difference between the blanks of both groups of animals no longer exists, whereas

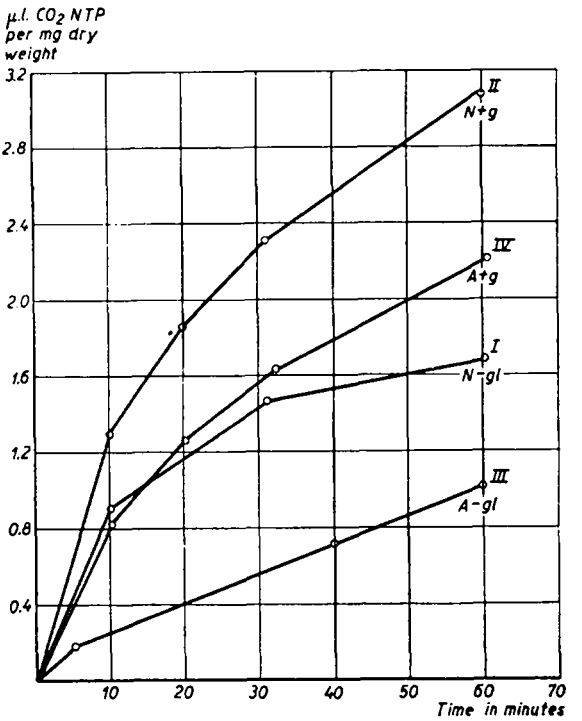

Fig. 1. Glycolysis of rat diaphragm under the influence of injected adrenaline. Curve I: normal blank; Curve II: normal + glucose; Curve III: adrenaline injected blank; Curve IV: adrenaline injected + glucose

TABLE I

ANAEROBIC GLYCOLYSIS OF RAT DIAPHRAGM AFTER INJECTION OF ADRENALINE

Exp. No.	Control rats			Rats injected with adrenaline 1 mg/kg subcutaneously		
	Blanks	In presence of 2 mg glucose per ml	Difference due to glucose utilization	Blanks	In presence of 2 mg glucose per ml	Difference due to glucose utilization
1	1.06	3.00	1.94	1.06	2.40	1.34
2	0.64	2.32	1.68	0.60	1.92	1.32
3	1.00	3.36	2.36	0.76	1.58	0.82
4	1.34	2.38	1.04	0.80	1.68	0.88
5	0.68	1.52	0.84	0.94	1.60	0.66
6	0.78	2.40	1.62	0.70	1.74	1.04
7	0.92	2.08	1.16	0.92	1.34	0.42
8	1.26	3.00	1.74	0.96	1.60	0.64
9	0.76	1.48	0.72	0.56	1.84	1.28
10	0.84	1.90	1.06	0.10	1.46	1.36
Mean:	0.92	2.34	1.41	0.74	1.71	0.97

The inhibition of glucose utilization in the treated animals is 31 %. The figures represent $Q^{N}_{CO_2}$.

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there is still a clear-cut difference between the glycolysis of diaphragm from animals treated with adrenaline compared with the controls. The inhibition achieved by the adrenaline is approx. 30-36%. The spread in the results is considerable but highly significant differences were obtained when the results within one experiment (using littermates at the same day under the same conditions) were compared. Every figure represents the mean glycolysis of 2 diaphragms.

Table II summarizes a series of experiments of the same type as those in Table I, but here no blanks were determined because it had been found that no significant difference exists between blanks of normal and treated diaphragm, so that they could be dispensed with for the calculation of the absolute difference in glucose utilization between diaphragms of treated and untreated animals. For the exact calculation of the percentage inhibition of glucose utilization the blanks should be known. In the case of Table II the blanks from Table I have been applied. If this is done the percentage inhibition calculated amounts to 36%. When the inhibition is calculated in relation to the overall glycolysis (including blank glycolysis) it is found to be 31%.

TABLE II

ANAEROBIC GLYCOLYSIS OF RAT DIAPHRAGM AFTER INJECTION OF ADRENALINE

Exp. No.	Control rats	Rats injected with adrenaline 1 mg/kg
11	3.6	2.2
12	2.04	1.96
13	2.10	2.2
14	2.72	1.44
15	1.90	1.4
16	1.84	1.3
17	3.26	1.84
18	2.76	1.74
Mean:	2.52	1.76

The difference between controls and treated animals = 0.76 = 31% of the overall glycolysis in the controls.

The figures represent $Q_{CO_2}^{N_2}$.

Inhibition of glucose utilization (using the blank values of Table I) = 36% of the glucose utilization in the controls.

In a few experiments the lactic acid production was followed chemically and compared with the manometric results. There was good agreement. The inhibition of glycolysis after adrenaline injection was also clearly reflected in the results of the chemical estimations. Lactic acid was estimated by the method of LE PAGE (UMBREIT *et al.*, 1946).

Phosphate fractions in diaphragm

Earlier in this paper it was shown that a certain amount of acid production occurs in the absence of glucose particularly in the first 20 min. This phenomenon was assumed to be due to lactic acid production from some intrinsic substrate presumably either glycogen or a carbohydrate phosphate. It seemed interesting for the interpretation of the glycolysis curves obtained to try and get an idea about the presence of intrinsic substrates by direct estimation of phosphate fractions and glycogen. Comparison of figures from phosphorylated intermediates in diaphragms from normal and adrenaline

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injected animals might reveal such accumulation. Phosphorylated intermediates were estimated by carrying out phosphate hydrolysis curves, inorganic (incl. creatine phosphate) and total phosphate estimations. Results are given in Table IIIA, B and C.

TABLE IIIA

PHOSPHATE FRACTIONS OF NORMAL AND ADRENALINE INJECTED ANIMALS

Normal animals	Inorganic + creatine phosphate P	7 min P	15 min P	3 h P	Total P
1	46.1	68.6	73.6	73.5	102.2
2	48.8	67.5	72.8	—	92.5
3	32.2	46	48.2	64	69
Adrenaline treated animals	**Inorganic + creatine phosphate P**	**7 min P**	**15 min P**	**3 h P**	**Total P**
1	51.4	69	70	80	103
2	56.6	71.3	72.1	79	91.7
3	42.3	52.3	55.3	59.7	81.6
4	51.8	73.3	72	86	98

The figures express phosphate in mg/100 g diaphragm.
7′ P, 15′ P etc. figures represent the total of inorganic P estimable after the corresponding periods of hydrolysis at 100° C.

TABLE IIIB

PHOSPHATE FRACTIONS IN DIAPHRAGM OF NORMAL AND ADRENALINE INJECTED ANIMALS

Normal animals	Inorganic + creatine phosphate P	7 min P	15 min P	3 h P	Total P
1	45	67	72	72	100
2	53	73	79	—	100
3	47	67	70	93	100
Adrenaline treated animals	**Inorganic + creatine phosphate P**	**7 min P**	**15 min P**	**3 h P**	**Total P**
1	50	67	67	77	100
2	62	77	77	86	100
3	51	63	67	73	100
4	53	74	72	87	100

The figures express percentages of total phosphate content in diaphragm.

From Table IIIA it follows that no significant difference exists between treated animals and controls as regards the absolute amounts of the various phosphate fractions. From Table IIIB it may be concluded that the distribution of the phosphate fractions has not changed either after injection of adrenaline*.

* When from the figures of Table IIIA differences between the various fractions are calculated (Table IIIC) there seems to be a difference between the P 15–P 7 fractions from normal and adrenaline treated animals, which cannot be interpreted.

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TABLE IIIC

TABLE OF DIFFERENCES OF VARIOUS PHOSPHATE FRACTIONS (CALCULATED FROM TABLE IIIA)

Normal animals	P 7–P o	P 15–P 7	P 180–P 15	Pt–P 180
1	22.5	5.0	0.1	28.7
2	18.7	5.3		
3	13.8	2.2	15.8	5.0
Adrenaline treated animals	**P 7–P o**	**P 15–P 7**	**P 180–P 15**	**Pt–P 180**
1	17.6	1.0	1.0	23.0
2	14.7	0.8	6.9	12.7
3	10.0	3.0	4.4	21.9
4	21.5	—1.3	14.0	12.0

The figures represent mg P/100 g diaphragm.

Glycogen estimations

Glycogen was estimated in the diaphragm of normal and adrenaline treated animals.

The method used was the PFLÜGER method as modified by SOMOGYI (GOOD, CRAMER AND SOMOGYI, 1933). Reduction after final hydrolysis was measured by the ceric sulphate method (MILLER AND VAN SLYKE, 1936). A group of 8 animals starved for a period of approx. 20 hours was used: 4 were injected with adrenaline, 4 served as controls. The animals were killed and the diaphragms taken out. The pooled diaphragms of every group of 4 were mixed and divided into 4 portions; every portion contained approximately equal amounts of each contributing diaphragm. The 4 portions of each pool, namely pool A (normal diaphragm) and pool B (diaphragm from treated animals) were dealt with as follows: the first 2 portions of each pool were placed directly into 30 % KOH for glycogen estimations. The 2 remaining portions of each pool were incubated anaerobically as in the glycolysis experiments, one portion serving as a blank, the other in presence of glucose; after the incubation glycogen was estimated.

The results of a typical experiment are summarized in Table IV.

TABLE IV

GLYCOGEN BREAKDOWN IN DIAPHRAGM OF ANIMALS INJECTED WITH ADRENALINE AND CONTROLS DURING ANAEROBIC GLYCOLYSIS

Normal diaphragm			Diaphragm from adrenaline injected animals		
Initial	After incubation with glucose	After incubation without glucose	Initial	After incubation with glucose	After incubation without glucose
0.20 0.22	0.15	0.12	0.19 0.19	0.17	0.18

The figures represent % glycogen.

The table shows:

1. That there is no essential difference in initial glycogen level between the diaphragms of treated animals and controls, and that a low glycogen level cannot therefore be connected with the lowered glycolysis in diaphragms from adrenaline treated animals.

2. That the diaphragms of normal animals used 0.9 g of glycogen/100 g of muscle in the absence of glucose but only 0.6 g in the presence of glucose.

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3. The diaphragm of adrenaline treated animals used practically no glycogen when incubated either with or without glucose.

Experiments with other substrates and tissues

In a few experiments carried out on diaphragm in order to assess in what stage the anaerobic glycolysis was affected, hexose-6-phosphate and hexose diphosphate were used as substrates. No utilization took place of hexose-6-phosphate, whereas hexose diphosphate was utilized. The failure of diaphragm to utilize hexose monophosphate confirmed similar results of DIXON AND NEEDHAM (Personal Communication) on rat skin glycolysis. The method of determination of the anaerobic glycolysis in presence of these phosphates was the same as that employed with glucose. The substrate concentration was M/100.

In two experiments no difference was found in the utilization of hexose diphosphate in diaphragm of adrenaline injected animals and controls, indicating an effect of adrenaline prior to the action of zymohexase. The number of experiments is far too small to draw definite conclusions.

In 3 experiments the glycolysis of rat heart muscle slices was investigated. On this tissue the blank glycolysis in the absence of glucose is practically nil, whereas in the presence of glucose there is a considerable glycolysis. It was highly interesting to find that in all three experiments the glycolysis of the heart of the rats injected with adrenaline was considerably *higher* than of the controls.

Normal controls	Adrenaline treated animals
Q_{CO_2}	Q_{CO_2}
10.4	16.7
8.8	10.0
11.3	14.1

The inhibition of glucose utilization seen in other muscle (diaphragm) after adrenaline seems in the heart to be prevented if not reversed. This state of affairs is highly favourable to meet the particular stress which is exerted on the heart after adrenaline administration.

Effect of the addition of adrenaline on the anaerobic glycolysis of diaphragm in vitro

Adrenaline in a dose of 50–100 μg was added from a KEILIN tube to normal diaphragm glycolysing in BARCROFT manometers under the usual conditions. Fig. 2 shows that no effect whatsoever is observed on the rate of glycolysis by the addition of adrenaline (at the arrow). It is therefore impossible to show any direct effect of adrenaline on the utilization of glucose by rat diaphragm under the conditions of the experiment.

DISCUSSION

The most significant conclusion that can be drawn from the experiments described is that the peripheral utilization of glucose is decreased after injection of adrenaline (1 mg/kg) by 30% approximately as compared with the controls. This decrease is tentatively taken to be the results of an impaired hexokinase reaction which may be due to inhibition of the enzyme itself or to insufficiency of other factors determining the rate

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of the reaction (*e.g.*, ATP-level). The possibility of a decrease of lactic acid production after adrenaline treatment due to inhibition beyond the hexokinase reaction has not yet been excluded in these experiments. It will be expounded in a following paper on muscle extracts.

The fact that adrenaline added *in vitro* to the diaphragm had no inhibitory effect suggested an indirect action, the possible nature of which is dealt with in a separate paper.

The phosphate and glycogen estimations show that the differences found in the glycolysis of diaphragm of adrenaline treated animals and controls are not due to different quantities of intrinsic metabolite present before incubation.

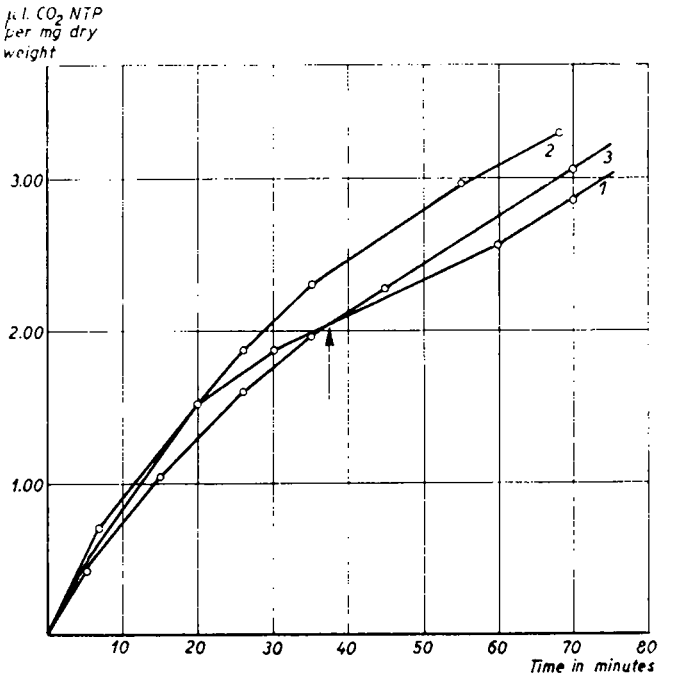

Fig. 2. Glycolysis of rat diaphragm under influence of adrenaline *in vitro*. In curve No. 1 adrenaline was added at the arrow in a dose of 50 μg/ml. In curve No. 2 adrenaline was added at zero time in a dose of 100 μg/ml. In curve No. 3 no adrenaline was added.

The absence in the phosphate experiments of increased accumulation of hexose-6-phosphate after adrenaline as found by CORI (1931) in the gastrocnemius, is probably due to the blurring out of this effect by the massive breakdown of glycogen into hexose phosphate in treated animals as well as in controls during the killing when no anaesthesia is used, demonstrated by the same author.

Neither can differences in the utilization of intrinsic substrates be held responsible for the marked differences observed in the glycolysis of diaphragms from adrenaline treated animals compared with the controls in the presence of glucose. This conclusion emanates from the experiments indicating that in the absence of added substrate, the presence of these intrinsic substances is not able to account for anything but a slight insignificant difference between the glycolysis of diaphragms of both groups of animals. The conclusion is based on the assumption that the breakdown of intrinsic substrate

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and its inhibition after adrenaline is not larger in the presence than it is in the absence of added glucose. This assumption is certainly valid as far as glycogen is concerned since the difference in the rate of breakdown of this substance between diaphragms of treated and untreated animals has been shown to be even smaller in presence than it is in the absence of glucose.

On the strength of these arguments it appears that the inhibition of glycolysis after adrenaline in the experiments described in this paper is not dependent on differences in pre-existing quantities of intrinsic substrate but affects only the metabolism of added glucose.

The results of the glycogen estimations suggest that the slight difference found in glycolysis experiments between the blanks of treated animals and controls in the first 20 minutes of incubation may be due to a more intense glycogen breakdown in the normal animals as compared to those treated with adrenaline*. The presence of glucose seems to inhibit the breakdown of glycogen during anaerobic incubation in diaphragm from the controls. It seems surprising that glycogen should be broken down better in the diaphragm from control animals than from the treated ones. This effect, however, is not constant and may easily be spurious because the number of experiments so far done is too small to allow a conclusion involving the comparison of phenomena in *different* mixtures of diaphragm (that is composed of tissue slices from *two* groups of animals). The inhibition of glycogen breakdown by glucose in the controls was established by comparison of tissue mixtures of identical composition (that is mixtures composed of tissue slices from one and the same group of animals) on three occasions and is therefore considered to be real. It has been demonstrated previously by other authors (SOSKIN, 1939).

It may be that some tissues containing hexokinase may not react to adrenaline according to the usual pattern. The few experiments on the glycolysis of heart slices recorded show that in the heart the hexokinase is not inhibited after adrenaline injection. Obviously the strain on the heart in its hyperactive state after adrenaline is enormous and it may well be that here a mechanism is operating preventing the usual inhibition of hexokinase in order to secure a steady flow of glucose into the organ. Such a discrepancy between the responses to adrenaline by heart muscle compared to other muscle is by no means unique. It will be remembered that, whereas adrenaline usually has a constricting effect on the arterial system in the body, it has a tendency to dilate the coronary arteries of the heart. Constriction would have led to poor feeding of the hyperactive organ which would have created a highly unfavourable condition. A mechanism is introduced which secures a steady flow of the necessary requirements to the active organ.

The physiological significance of the inhibition of the hexokinase reaction under conditions of emergency accompanied by release of adrenaline, is still obscure. A possible course of events is the following: When the body is called upon for quick emergency action, adrenaline is produced and as a result of this the breakdown of glycogen in the muscle is increased. This mechanism for providing energy produces more ATP per glucose residue than the hexokinase reaction. The latter reaction would interfere with the former in as far as it uses ATP for its initial phosphorylation. This interference is prevented by inhibition of hexokinase by adrenaline (indirectly) and glycogen breakdown can proceed undisturbed.

* An accompanying manometric experiment showed a good agreement between glycogen disappearance and lactic acid production.

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An alternative possibility would be that after injection of adrenaline ATP is used for reactions closely related to mechanical muscle contraction. Less ATP would then be available for the hexokinase reaction. The so called *increased* glycogen breakdown may in this conception very well consist of a *decrease* in aerobic glycogen-synthesis, also caused by the lack of available ATP.

Whatever may be the case, obviously the glycogen reserves would quickly get exhausted particularly in those organs which like the heart are very active after release of adrenaline. Possibly in such organs no inhibition of the hexokinase reaction takes place. Moreover the blood sugar concentration rises after a short while so that an *increased* glucose utilization in such organs can take place after an initial period under the influence of the now prevailing hyperglycaemia, whereas the initially *decreased* glucose utilization as a result of the inhibition of the hexokinase reaction in other muscles etc. will now be compensated for by the high level of blood sugar making replenishments of stores possible even in the presence of a still impaired hexokinase activity or ATP level. These replenishing processes occur after an initial period during which the hyperglycaemia develops.

SUMMARY

1. This work was undertaken with the object of investigating whether the rise in blood sugar after adrenaline may at least be partly due to inhibition of glucose uptake by the tissue: more explicitly whether the hexokinase reaction determining the rate of glucose uptake by the tissues from the blood was impaired directly or indirectly after injection of adrenaline.

2. There is a marked inhibition of the anaerobic glycolysis of diaphragms from rats which have been injected with adrenaline (1 mg/kg subcutaneously) 40–70 minutes previously. This inhibition is approximately 30–36%. The manometric result could be confirmed by chemical lactic acid estimations. The inhibition does not occur in the blank estimations in the absence of glucose.

3. When various phosphate fractions like inorganic P (incl. creatine P), 7 min P, 15 min P, 180 min P and Total P were determined in diaphragms from adrenaline injected rats and compared with normal controls, no significant differences could be demonstrated.

4. There is no essential difference in initial glycogen level between the diaphragms of treated animals and controls. The presence of glucose during anaerobic incubation seems to inhibit glycogenolysis in diaphragm from normal animals. It appears that quantitative differences in intrinsic substrate (glycogen, phosphorylated carbohydrate) cannot be responsible for the marked inhibition observed in the glycolysis of diaphragms from adrenaline treated animals compared with the controls in the presence of glucose. This inhibition only affects the metabolism of added glucose.

5. When hexose diphosphate is used as a substrate no inhibition of the anaerobic glycolysis of diaphragm from adrenaline treated animals is observed suggesting the inhibiting action towards glucose to occur prior to the zymohexase reaction in the glycolysis chain.

6. A direct inhibition of glucose utilization of diaphragm under influence of adrenaline added *in vitro*, cannot be demonstrated.

RÉSUMÉ

1. Le but de ce travail était de rechercher si l'augmentation du sucre sanguin par l'adrénaline est due, partiellement au moins, à l'inhibition de l'absorption du glucose par les tissus; ou, d'une façon plus précise, si l'action de l'hexokinase qui détermine la vitesse de l'absorption du glucose à partir du sang par les tissus, est plus ou moins directement bloquée après injection d'adrénaline.

2. Il se manifeste une inhibition marquée de la glycolyse anaérobie du diaphragme de rats ayant reçu de l'adrénaline par injection sous-cutanée (1 mg/kg) 40–70 minutes auparavant. Cette inhibition est de l'ordre de 30–36%. Les résultats obtenus par voie manométrique ont pu être confirmés par des dosages d'acide lactique. Cette inhibition ne se manifeste pas dans des expériences témoins en l'absence de glucose.

3. On ne constate aucune différence dans la teneur en diverses fractions de phosphates: P minéral (y compris P de la créatine) P hydrolysable en 7 min, en 15 min, en 180 min et P total dans les diaphragmes de rats normaux ou de rats ayant reçu de l'adrénaline.

4. Il n'existe aucune différence notable dans la teneur en glycogène initial des diaphragmes d'animaux traités ou non. La présence de glucose au cours de l'incubation anaérobie semble inhiber

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la glycogénolyse dans le diaphragme des animaux normaux. Il apparaît que des différences quantitatives dans la nature du substrat (glycogène, hydrates de carbone phosphorylés) ne peuvent provoquer l'inhibition importante que l'on observe dans la glycolyse du diaphragme d'animaux traités par l'adrénaline, par rapport à ce que donne le diaphragme des animaux témoins, en présence de glucose. Cette inhibition s'exerce uniquement sur le métabolisme du glucose ajouté.

5. Lorsque l'on utilise comme substrat de l'hexosediphosphate, on n'observe aucune inhibition de la glycolyse anaérobie du diaphragme des animaux traités à l'adrénaline. Ceci conduit à penser que l'action inhibitrice vis-à-vis du glucose s'exerce antérieurement à la réaction zymohexase dans la chaîne des réactions de la glycolyse.

6. Aucune inhibition directe de l'utilisation du glucose du diaphragme sous l'influence de l'adrénaline ajoutée *in vitro* n'a pu être observée.

ZUSAMMENFASSUNG

1. Die vorliegende Arbeit wurde mit der Absicht unternommen, um zu untersuchen ob die Zunahme des Blutzuckers durch Adrenalin wenigstens teilweise durch Hemmung der Glukoseaufnahme durch die Gewebe verursacht sein könnte; genauer ausgedrückt, ob die Hexokinasereaktion, die die Geschwindigkeit der Glukoseaufnahme aus dem Blut durch die Gewebe bestimmt, direkt oder indirekt nach Injektion von Adrenalin gestört wäre.

2. Eine deutliche Hemmung der anaeroben Glykolyse des Zwerchfells trat bei Ratten auf, die 40–70 Minuten vorher Adrenalininjektionen (1 mg/kg subkutan) erhalten hatten. Diese Hemmung beträgt ungefähr 30–36%. Die manometrischen Resultate konnten durch chemische Milchsäurebestimmungen bestätigt werden. Die Hemmung tritt bei Blindproben bei Abwesenheit von Glukose nicht auf.

3. Bei der Bestimmung verschiedener Phosphatfraktionen wie anorganisches Ph (incl. Kreatinphosphat), Phosphat nach Hydrolyse während 7, 15 oder 180 Min, und Gesamtphosphat in den Zwerchfellen von Ratten, die Adrenalininjektionen erhalten hatten, konnten bei Vergleich mit normalen Kontrollen keine bedeutenden Unterschiede nachgewiesen werden.

4. Zwischen dem Glykogengehalt des Diaphragmas behandelter und normaler Tiere besteht im Beginn kein Unterschied von Bedeutung. Die Anwesenheit von Glukose während der anaeroben Inkubation scheint die Glykogenolyse im Zwerchfell normaler Tiere zu hemmen. Es hat den Anschein, dass die deutliche Hemmung, die bei Vergleich der Glykolyse des Zwerchfells von Tieren, die mit Adrenalin behandelt wurden, mit Kontrolltieren bei Anwesenheit von Glukose wahrgenommen wird, nicht durch quantitative Unterschiede der inneren Substrate, (Glykogen, phosphorylierte Kohlenhydrate) verursacht wird. Die Hemmung betrifft nur den Stoffwechsel zugefügter Glukose.

5. Wenn Hexosediphosphat als Substrat verwendet wird, wird keine Hemmung der Zwerchfellglykolyse bei Tieren, die mit Adrenalin behandelt wurden, wahrgenommen. Dies führt zu der Annahme, dass die hemmende Wirkung gegenüber Glukose vor der Zymohexasereaktion in der Reaktionskette der Glykolyse auftritt.

6. Eine direkte Hemmung des Glukoseverbrauchs des Zwerchfells unter Einfluss von *in vitro* zugefügtem Adrenalin kann nicht nachgewiesen werden.

REFERENCES

J. W. CONN, E. S. CONN, AND M. W. JOHNSTON, *J. Nutrition*, Supp. 19 (1940) 16.
C. F. CORI AND G. T. CORI, *J. Biol. Chem.*, 79 (1928a) 309.
C. F. CORI AND G. T. CORI, *id.*, 79 (1928b) 343.
C. F. CORI AND G. T. CORI, *id.*, 79 (1928c) 321.
C. F. CORI AND G. T. CORI, *id.*, 94 (1931) 581.
F. C. COURTICE, *Proc. Roy. Soc. sB*, 127 (1939) 41.
D. B. DILL, R. E. JOHNSON, AND C. DALY, *Am. J. Med. Sci.*, 198 (1939) 702.
M. DIXON AND D. M. NEEDHAM, *Personal Communication.*
GOOD, CRAMER, AND M. SOMOGYI, *J. Biol. Chem.*, 100 (1933) 485.
H. P. HIMSWORTH AND D. B. MCN. SCOTT, *J. Physiol.*, 93 (1938) 159.
H. R. B. JONKERS, *Thesis*, Leyden (1945).
E. LUNDSGAARD, *Bull. Johns Hopkins' Hosp.*, 63 (1938) Nov. 1.
MILLER AND V. SLYKE, *J. Biol. Chem.*, 114 (1936) 583.
S. SOSKIN, *Am. J. Physiol.*, 81 (1927) 382.
S. SOSKIN, R. LEVINE, AND M. TAUBENHAUS, *Proc. Soc. Exptl. Biol. Med.*, 42 (1939) 689.
G. A. LE PAGE, W. W. UMBREIT, R. H. BURRIS, AND J. F. STAUFFERS, *Manometric Methods, Burgess Pub. Cy. Minneapolis, Minn.*
M. WIECHMANN, *Deut. Arch. klin. Med.*, 154 (1927) 296.

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