Glucose Uptake and Response to Insulin of the Isolated Rat Diaphragm

The Effect of Denervation

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It has been known for many years that exercise decreases the insulin requirements of diabetics and increases glucose uptake from the blood even in untreated diabetic subjects.[1] Ingle[2,3] demonstrated that severe work can cause a drop in blood sugar to extremely low levels in partially depancreatized diabetic rats receiving no insulin and that the effects of insulin and work are additive. Levine and his co-workers[4] investigated the effect of muscular work on the volume of distribution of non-metabolizable monosaccharides in eviscerated, nephrectomized dogs and showed that exercise promotes the cellular entry of some sugars as does insulin. The sugars which respond to exercise in this preparation are those that also respond to insulin. Huycke and Kruhoffer[5] studied the perfused hindlimb preparation of the cat and observed that muscular exercise increases the rate of disappearance of D-glucose and D-galactose from the extracellular space. Helmreich and Cori[6] observed that tetanic contraction of skeletal muscle causes accumulation of free glucose within the muscle cells of intact animals. These authors[7] also compared the rate of penetration of infused pentoses into resting and exercising gastrocnemius muscles of nephrectomized rats. They found that pentoses penetrate the intracellular space of working muscles at an accelerated rate and that the effect of exercise parallels that of insulin. These results were recently confirmed in the cat by Sachs and his colleagues[8] who also noted the additive effect of insulin and exercise.

These data demonstrate that exercise facilitates the entry of sugars into muscle cells. The mechanism of this action is essentially unknown. Levine and his co-workers[4] postulated that exercising muscle produces a humoral agent which accelerates the transfer of certain sugars across cell surface barriers. The work of Cori and others[5-9] suggests that the changes in permeability affect primarily the working muscles per se.

Park and Johnson[9] found rhythmically contracting muscles of the rat (heart, diaphragm) to be more responsive to insulin than the gastrocnemius, as measured by the accumulation of free glucose in muscle after infusion of glucose and insulin.

The isolated rat diaphragm is extensively used in bio-assays of insulin activity. As the diaphragm is a constantly working muscle we investigated the effect of antecedent paralysis in vivo on the subsequent glucose uptake in vitro and response to insulin of this tissue. We used diaphragms subjected to short-term denervation (twelve hours to eight days) in order to avoid using muscles with gross atrophy.

MATERIAL AND METHOD

Male Wistar rats between 75 and 150 gm. body weight were used. In individual experiments the body weights of rats were as similar as possible, generally within 10 gm. All animals were kept in the laboratory for at least five days prior to the experiment and maintained on Purina rat pellets and water ad libitum. They were fasted for sixteen hours prior to the experiments, except in one study, which will be indicated in the text.

Operative procedures. Right or left unilateral phrenicotomies were carried out under light ether anesthesia. In early experiments the phrenic nerve was exposed through a thoracotomy incision. A cervical approach was chosen in subsequent studies. Identical results were obtained with both technics. In each operation the nerve was isolated and identified by stimulation with faradic current prior to severing it with scissors. The complete paralysis of one hemidiaphragm was verified visually through a laparotomy incision before excising the diaphragm for study. In sham operated controls the phrenic nerve was exposed through a thoracotomy but not cut.

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The animals were in good physical condition after operation and prior to the experiments.

The determination of glucose uptake and response to insulin in vitro. The technic described by Vallance-Owen and Hurlock[13] for measurement of plasma insulin activity was used. Rats were killed by a blow on the head. The hemidiaphragms were excised separately and placed in chilled buffer solution (10 to 15° C.) for ten minutes. The diaphragms were blotted gently and placed in Warburg vessels containing 2 ml. of incubation medium, with or without insulin. The vessels were gassed for five minutes with a mixture of 95 per cent O_2 and 5 per cent CO_2, stoppered, and incubated for ninety minutes at 37° C. in a Warburg apparatus (80 cycles/min.). Incubation medium: Gey and Gey[14] balanced salt solution ($NaHCO_3$ buffer) plus glucose (300 mg. per cent). Insulin:* Five times recrystallized zinc insulin (Lilly), 0.001 U/ml. medium. At the end of the incubation period glucose in the medium was determined by the method of King.[15] The diaphragms were dried in an oven for two hours at 110° C. The glucose uptake was calculated in milligrams/gram diaphragm (dry weight).

Binding of iodine^{131}-labeled insulin by diaphragms in vitro. Hemidiaphragms were incubated in balanced salt solution[14] plus glucose (300 mg. per cent) and I^{131} insulin (Abbott Laboratories). The insulin concentration varied between 0.001 and 0.004 U. of insulin and the specific activity between 0.16 and 0.27 μc/ml. medium.

The hemidiaphragms were placed in tared Warburg vessels containing 2 ml. of medium. The vessels were reweighed prior to incubation to determine the wet weight of the tissue.

After incubation each hemidiaphragm was washed in four changes of 25 to 50 ml. saline for a total period of sixteen minutes, as described by Stadie.[16] This procedure adequately eliminates all radioactive material that is not firmly bound to the tissue.

The hemidiaphragms were blotted and digested individually in 1 ml. 30 per cent KOH solution for thirty minutes at 100° C., neutralized with I N H_2SO_4, and diluted to 10 ml. with distilled water. Two milliliter aliquots of these samples and of the medium were counted in a scintillation wellcounter. The total counts corresponding to each hemidiaphragm were calculated as counts per gram wet weight and divided by the counts contained in 1 ml. medium prior to incubation. Results were expressed as the ratio of concentration of bound insulin to that of the medium.

Glycogen determinations. Under light ether anesthesia rats were exsanguinated through the aorta, the diaphragm frozen in situ,[1]* excised, and placed in iced saline.

The hemidiaphragms were trimmed, blotted, and transferred into tared Wassermann tubes containing 1 ml. of 30 per cent KOH, and the wet weight determined by difference. Glycogen was measured by a slight modification of the technic of Good and his associates.[17] Values are expressed as milligrams of glucose per gram diaphragm (wet weight).

Presentation of data. Mean values and standard errors are indicated in tables. The number of hemidiaphragms used in determining each mean is shown in brackets.

RESULTS

The effect of paralysis on the glucose uptake in vitro of hemidiaphragms. Results obtained at different time intervals after unilateral phrenicotomy are tabulated in table 1. "Basal glucose uptake" refers to the amount of glucose (milligrams/gram dry weight) consumed by the hemidiaphragm during ninety minutes incubation in the absence of insulin. "Glucose uptake with insulin" refers to the glucose uptake in the presence of 0.001 U. insulin per milliliter medium. "Insulin effect" is the increment in glucose uptake in the presence of insulin, obtained by subtracting the mean basal glucose uptake of each group from the corresponding mean glucose uptake with insulin.

Each value in table 1 represents pooled results of three or more experiments. In each experiment the operated animals were divided into two groups. The hemidiaphragms of one group served for the measurement of basal glucose uptake, and the other group was used for glucose uptake with insulin. One hemidiaphragm of each rat served as control of its paralyzed or sham-operated counterpart as each pair was incubated with or without insulin.

The data indicate clearly that, when incubated with insulin, the glucose uptake of nonparalyzed hemidiaphragms increased approximately 70 per cent over the basal glucose uptake. Paralyzed hemidiaphragms responded to the same amount of insulin with a much smaller increase in glucose uptake. Twelve to twenty hours after unilateral phrenicotomy there was a statistically significant difference ($p<0.01$) between the basal glucose uptake and glucose uptake with insulin of paralyzed hemidiaphragms. However, the insulin effect was less than half of that observed in nonparalyzed hemi-

*Insulin was obtained through the courtesy of Dr. F. B. Peck, Sr., from Lilly Research Laboratories, Indianapolis, Indiana.

*Fluro-Ethyl Spray, Gebauer Chemical Company.

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TABLE 1

Effect of denervation on the glucose uptake in vitro of rat hemidiaphragms, with and without added insulin

Group	Time after operation	Basal glucose uptake		Glucose uptake with insulin		Insulin effect	
		Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed
Sham operated controls	16 hours to 5 days	39.4 ±2.2 (16)	40.0* ±2.4 (16)	70.6 ±2.2 (20)	70.8* ±2.0 (20)	+31.2	+30.8*
Unilateral phrenicotomy	12-20 hours	42.2 ±2.6 (8)	48.4 ±2.8 (8)	72.8 ±2.0 (10)	58.8 ±1.4 (10)	+30.6	+10.8
	40-72 hours	37.6 ±2.2 (8)	38.8 ±3.0 (8)	63.6 ±3.4 (15)	40.6 ±2.6 (15)	+26.0	+1.8
	5-8 days	48.0 ±6.6 (8)	43.2 ±4.2 (8)	74.4 ±4.0 (10)	41.4 ±2.0 (10)	+26.0	—1.8

Glucose uptakes expressed as glucose milligrams/gram hemidiaphragm (dry weight).
Basal=no insulin added to medium. Insulin=0.001 U/ml. medium. *=sham operated, not paralyzed.

diaphragms. Forty hours to eight days after phrenicotomy there was no statistically significant difference between the mean basal glucose uptake and glucose uptake with insulin of paralyzed hemidiaphragms. Without insulin in the medium, paralyzed and nonparalyzed hemidiaphragms consumed the same amount of glucose; with insulin, the glucose uptake of nonparalyzed hemidiaphragms was always significantly higher ($p<0.01$). In sham-operated controls no significant difference between the two hemidiaphragms was observed with or without insulin.

Figure 1 illustrates the quantitative aspect of the decreased response to insulin of paralyzed hemidiaphragms. The ordinates represent increments in glucose uptake above basal in the presence of insulin, while the insulin concentrations are indicated in the abscissae. The dots represent the mean increments in glucose uptake of hemidiaphragms of nonoperated rats, when incubated in the presence of either 100 or 1,000 micro-units of insulin. (These data were obtained while standardizing the insulin assay method. The technic and incubation medium were identical with those used subsequently for unilaterally denervated diaphragms.) Each dot represents thirty hemidiaphragms; the horizontal lines indicate the standard errors of the means. The cross-hatched bar represents the mean response of nonparalyzed hemidiaphragms of operated rats incubated with 1,000 micro-units of insulin and the open bars the response of paralyzed hemidiaphragms to the same amount of insulin.

It can be observed that the sensitivity of the insulin assay method used is sufficient to detect amounts of insulin smaller than 100 micro-units/ml. In the presence of 1,000 micro-units of insulin per milliliter, the increment in glucose uptake of paralyzed hemidiaphragms was significantly less than the response of control hemidiaphragms to 100 micro-units.

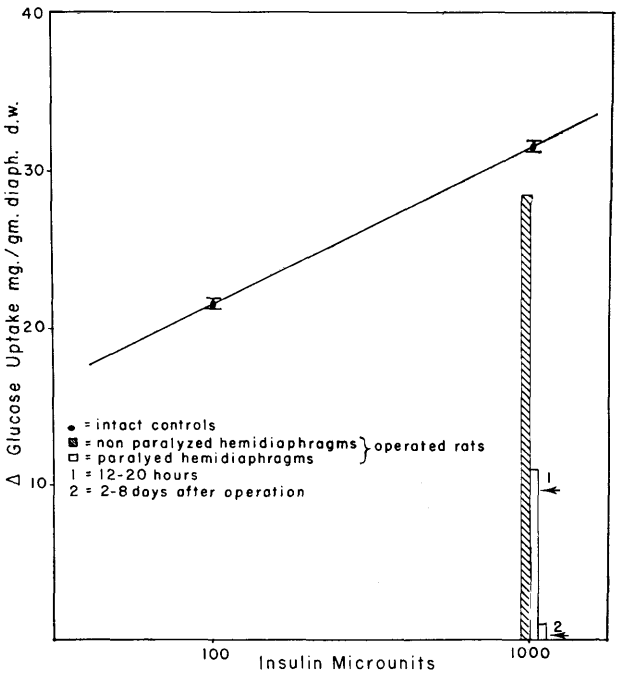

FIG. 1. Increments of glucose uptake above basal by paralyzed and control hemidiaphragms incubated with insulin.

Relation between morphological changes and the decreased response to insulin of denervated hemidiaphragms. The dry weights of paralyzed hemidiaphragms excised at different time intervals after operation were compared with hemidiaphragms of intact controls and

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sham-operated rats. Rats between 75 and 134 gm. body weight were included in the study. There was no significant difference between the mean body weights of different groups.

Observing the data listed in table 2 it is evident that no changes in dry weight of paralyzed hemidiaphragms occurred prior to forty-eight hours after operation. On the third day they increased slightly and five to eight days after phrenicotomy the paralyzed hemidiaphragms were significantly heavier ($p<0.01$).

The wet weight of hemidiaphragms was not determined in the above discussed experiments. However, in the course of other studies on paralyzed hemidiaphragms the tissues were weighed immediately after excising them. Comparing the weights of paralyzed and nonparalyzed hemidiaphragms of forty-four rats, between 89 and 139 gm. body weight, three to four days after unilateral phrenicotomy, it was found that the mean wet weight of paralyzed hemidiaphragms was 123 ± 2.6 mg. while the mean weight of their nonparalyzed pairs was 96.7 ± 2.5 mg.

The nitrogen content of diaphragms of ten unilaterally phrenicotomized rats three to five days after operation was also determined and related to the wet weight of the tissues. The mean nitrogen content of paralyzed hemidiaphragms was 2.3 ± 0.1 mg. per cent and that of their nonparalyzed counterpart was 2.2 ± 0.1 mg. per cent. These findings indicate that the observed increase in dry weight is paralleled by a simultaneous increase in wet weight.

Routine histological examination of sections of paralyzed hemidiaphragms, obtained three to five days after operation, did not reveal any striking changes, except the presence of degenerated nerve fibers.

In table 3 data from table 1 are used, but the glucose uptakes are expressed as milligrams of glucose consumed by the hemidiaphragms, without reference to the weight of the tissues. These data are presented to show the absolute glucose uptakes of the hemidiaphragms, irrespectively of eventual changes in their weight. The results obtained are the same as in table 1. There is no difference in basal glucose uptake between paralyzed and nonparalyzed hemidiaphragms. The response to insulin decreases as soon as twelve hours after denervation and is minimal between forty hours and eight days. It is evident that the decreased response to insulin precedes any changes in the weight of paralyzed hemidiaphragms by at least two days, and is independent of the weight of the muscle.

Effect of parenteral insulin on the glucose uptake of paralyzed hemidiaphragms. Unilateral denervation of the diaphragm was carried out in sixteen rats three to four days prior to these studies. Half of the animals underwent left and half right phrenicotomy. On the day of experiment the animals were divided into two groups. The first group was injected intraperitoneally with 0.1 U. of insulin in 0.5 ml. saline, while the second group received saline only. The animals were sacrificed thirty to forty-two minutes after the injection. The two hemidiaphragms were excised separately and handled as described for the preceding experiments. All hemidiaphragms were incubated for ninety minutes in balanced salt solution containing 300 mg. per cent glucose.

Results are summarized in table 4. They demonstrate that the glucose uptake of hemidiaphragms of rats which had not received insulin was not altered by previous denervation. The hemidiaphragms of rats injected with insulin took up more glucose than the controls. However, the response to insulin of paralyzed hemidiaphragms was significantly smaller ($p<0.05$) than that of their nonparalyzed counterparts.

The effect of paralysis on the binding of I^{131}-labeled insulin by rat hemidiaphragms. Stadie and his co-workers[16] demonstrated by using labeled insulin that one of the prerequisites for the action of insulin is its firm binding to muscle tissue.

Experiments were carried out to compare the binding of I^{131}-labeled insulin to paralyzed and nonparalyzed hemidiaphragms in the following situations: (a) during incubation with labeled insulin; (b) after in vivo injection.

In all experiments rats were studied three to four days after unilateral denervation of the diaphragm. Half of the animals underwent right and half left phrenicotomy. The nonparalyzed hemidiaphragm of each rat served as control for its paralyzed counterpart .

A. *In vitro experiments.* The pooled data of five independent experiments are summarized in table 5. Values are expressed as the ratio of concentration of bound insulin to that of the medium. Analysis of the data showed no significant difference in this ratio when the diaphragms were incubated with 0.001, 0.002, or 0.004 units of insulin per milliliter medium. The binding of insulin increased during the first hour of incubation, while there was no significant further increase during the next thirty minutes. At none of the three time intervals studied was a significant difference demonstrable between the I^{131} uptake of paralyzed and nonparalyzed hemidiaphragms.

B. *In vivo experiments*: Under light ether anesthesia I^{131}-labeled insulin diluted to 0.5 ml. with saline was injected into the inferior vena cava of rats (body weight 90 to 100 gm.). The dose of insulin administered was either 0.03 units or

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TABLE 2

Effect of denervation on the dry weight of hemidiaphragms

Group	Controls		Paralyzed hemidiaphragms			
	Nonoperated	Sham operated				
Time after operation	—	16 hours to 5 days	10-20 hours	40-48 hours	60-72 hours	5-8 days
Hemidiaphragms mg.	19.7 ±0.2 (36)	20.4 ±1.2 (30)	19.9 ±0.5 (44)	19.4 ±0.6 (26)	21.4 ±0.9 (16)	27.2 ±1.2 (22)

TABLE 3

Effect of denervation on the glucose uptake in vitro of rat hemidiaphragms, with and without added insulin

Time after unilateral phrenicotomy	Basal glucose uptake		Glucose uptake with insulin		Insulin effect	
	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed
12-20 hours	0.90 ±0.06 (8)	0.95 ±0.04 (8)	1.75 ±0.09 (10)	1.29 ±0.05 (10)	+0.85	+0.34
40-72 hours	0.92 ±0.07 (8)	0.91 ±0.04 (8)	1.49 ±0.07 (15)	0.92 ±0.09 (15)	+0.57	+0.01
5-8 days	1.11 ±0.15 (8)	1.12 ±0.12 (8)	1.82 ±0.10 (10)	1.25 ±0.08 (10)	+0.71	+0.13

Glucose uptakes expressed as milligrams of glucose consumed by each hemidiaphragm.
Basal=no insulin added to medium.
Insulin: 0.001 U/ml. medium.

TABLE 4

Effect of parenteral insulin on the subsequent glucose uptake in vitro of paralyzed hemidiaphragms

Group	No insulin		Parenteral insulin 0.1 U/rat	
Hemidiaphragm	Nonparalyzed	Paralyzed	Nonparalyzed	Paralyzed
Glucose uptake	34.0 ±3.0 (8)	37.4 ±3.2 (8)	77.2 ±4.6 (8)	57.8 ±6.2 (8)

Glucose uptake expressed as milligrams/gram (dry weight).

0.25 units per rat, with a specific activity of 2 or 6 μc. The animals were sacrificed thirty minutes after the injection, the hemidiaphragms excised, weighed, washed, and digested as in the earlier in vitro experiments. Aliquots of the digested samples and of the injected insulin solution were diluted and counted in a scintillation wellcounter.

Results (table 6) are expressed as micro-units of insulin bound per gram hemidiaphragm (wet weight). Table 6 demonstrates that there was no decrease in the insulin binding capacity of paralyzed hemidiaphragms. On the contrary, paralyzed hemidiaphragms took up significantly ($p<0.02$) more labeled insulin than their nonparalyzed pairs. This may be due to differences in blood flow between paralyzed and nonparalyzed muscles. Correlating the data on glucose uptake (table 4) and insulin binding (table 6) of denervated hemidiaphragms after the injection of insulin, it is of interest to note that although the paralyzed hemidiaphragms bound approximately 50 per cent more insulin than their nonparalyzed pairs, the insulin effect on glucose uptake was less than half of that observed in nonparalyzed muscles. This emphasizes the decreased response to insulin of paralyzed muscles.

The effect of denervation on the glycogen content of rat hemidiaphragms. It is generally accepted that muscular contractions accelerate glycogenolysis in muscle cells. On the other hand, insulin is known to stimulate

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TABLE 5

In vitro binding of I^{131} insulin

Incubation minutes	Ratio of I^{131} insulin bound by hemidiaphragms	
	Nonparalyzed	Paralyzed
30	0.59 ±0.04 (9)	0.67 ±0.04 (9)
60	1.09 ±0.07 (12)	1.25 ±0.11 (12)
90	1.29 ±0.14 (11)	1.25 ±0.07 (11)

I^{131} insulin binding expressed as ratio of counts/gram tissue (wet weight) to counts/milliliter medium.
Insulin concentration: 0.001-0.004 U insulin/milliliter medium in different experiments.

TABLE 6

Binding of parenteral I^{131} insulin

I^{131} insulin microunits injected per rat	I^{131} insulin bound by hemidiaphragms	
	Nonparalyzed	Paralyzed
30	0.16 ±0.02 (9)	0.24 ±0.02 (9)
250	1.37 ±0.05 (9)	1.75 ±0.12 (9)

Bound I^{131} insulin expressed in microunits/gram hemidiaphragm (wet weight).

the synthesis of glycogen in these tissues.

The preceding experiments have shown that denervation diminishes the ability of muscle to respond with an increased glucose uptake to the presence of insulin. Thus the glycogen content of paralyzed hemidiaphragms would be influenced by at least two factors: (a) the lack of exercise decreases glycogenolysis; and (b) the diminished response to insulin should slow down the synthesis of new glycogen.

Glycogen determinations were carried out on hemidiaphragms of nonoperated controls and of operated rats two to four days after right or left phrenicotomy. The animals were either fed ad libitum or fasted for sixteen hours prior to their sacrifice. Table 7 demonstrates the results obtained.

Comparing the glycogen content of hemidiaphragms of fasted, operated rats it is evident that the paralyzed hemidiaphragms contained more glycogen than their nonparalyzed pairs ($p<0.01$). The diaphragms of fasted nonoperated controls contained less glycogen than the paralyzed hemidiaphragms ($p<0.01$) but more ($p<0.01$) than the nonparalyzed hemidiaphragms of operated fasted rats.

Feeding increased the glycogen content of nonparalyzed hemidiaphragms by more than 200 per cent, while the increase observed in paralyzed hemidiaphragms was only 32 per cent of the fasting value. Consequently, in rats fed ad libitum the glycogen content of paralyzed hemidiaphragms was significantly lower ($p<0.01$) than that of their nonparalyzed pairs.

These results can be interpreted in the light of the above mentioned two factors, glycogen breakdown and synthesis. In the fasting state little glucose is available for synthesis of muscle glycogen. As the diaphragm is a rhythmically contracting muscle, glycogen breakdown continues at a high rate. In these circumstances, paralyzed hemidiaphragms contain more glycogen than their working pairs or the diaphragms of nonoperated controls. In rats fed ad libitum the rate of glycogen synthesis and the muscle glycogen content increase. Since this increase is much smaller in paralyzed than in nonparalyzed hemidiaphragms, it is probable that the ability of paralyzed hemidiaphragms to synthesize glycogen is impaired. This correlates well with the decreased response to insulin of these muscles. To establish cause and effect relationship further experiments are necessary.

TABLE 7

The effect of denervation on the glycogen content of hemidiaphragms

Group	Hours fasted	Glycogen content of hemidiaphragms	
		Nonparalyzed	Paralyzed
Controls	16	1.5 ±0.07 (22)	—
Unilateral phrenicotomy	16	1.2 ±0.09 (19)	2.0 ±0.12 (19)
	0	3.7 ±0.17 (24)	2.6 ±0.20 (24)

Glycogen expressed as glucose milligrams/gram (wet weight).

DISCUSSION

There is ample evidence that exercise promotes the entry of glucose and certain nonmetabolizable sugars into muscle cells.[2-9] The work of Hines and his colleagues[10-12] indicates that the absence of exercise also interferes with the carbohydrate metabolism of muscles. These authors showed that (a) the glycogen content of immobilized or denervated rat gastrocnemii decreases; (b) they do not respond with an increase in glycogen to the administration of glucose and insulin; and (c) periodical electrical stimulation prevents the decrease in glycogen content.

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The diminished response to insulin of paralyzed hemidiaphragms could be due either to lack of activity or denervation per se. The first hypothesis seems more likely as the decreased insulin sensitivity can be easily demonstrated twelve hours after denervation, when degenerative changes have not yet occurred in muscle. Preliminary experiments conducted in this laboratory indicate that incubation with acetylcholine (5 μg./ml. medium) does not restore the response to insulin of paralyzed hemidiaphragms, but that electrical stimulation in vivo restores their subsequent sensitivity to insulin in vitro. Studies in vivo[7] showed that stimulated muscles maintain their increased permeability to pentoses for thirty minutes after the cessation of muscle work.

This finding is in agreement with the assumption that the changes in glucose uptake in vitro described in our study may be due to the previous state of activity or inactivity of the hemidiaphragms.

No difference in basal glucose uptake was observed in our experiments between paralyzed and nonparalyzed diaphragms, while it has been repeatedly described[2-8] that exercise enhances the permeability of muscle to sugars in the absence of insulin. Comparison is difficult as the experimental conditions of these studies[2-8] differed in many respects from ours. However, the following factors deserve consideration:

1. In each of the mentioned studies[2-8] it was noted that in order to obtain an increase in the permeability to sugars without insulin the muscle has to carry out a considerable amount of work. Gastrocnemii were stimulated (three to five times per second) lifting a weight which represented 10 to 25 per cent of the animals' body weight. The amount of work performed by these preparations must have been far in excess of that carried out by a hemidiaphragm while contracting under physiological conditions. It is possible that the first mechanism by which muscle adapts to the increased caloric requirements of moderate exercise is by increasing its responsiveness to insulin, and only under severe stress, when this mechanism proves insufficient, does the "basal" permeability to glucose increase.

2. Several authors used intact,[5,6,8] alloxan diabetic,[7] or partially depancreatized[2,3] animals in their studies, which presumably had small amounts of circulating insulin. It is possible that the observed effect of exercise on muscle permeability was due to the increased responsiveness of working muscles to endogenous insulin.

3. Since glucose was used as substrate in this study, the results cannot be freely compared with those obtained in experiments with pentoses and D-galactose.[4,6,8] It should be considered that different mechanisms may regulate the permeability of working muscles to glucose and to nonmetabolizable sugars.

This study is in accord with the observations[5-8] that exercise enhances the permeability to sugar of muscles by a local action on the working muscle per se. It is possible, however, that in addition exercising muscles produce a humoral factor, as suggested by Levine.[4]

The difference in the response to insulin between paralyzed and nonparalyzed hemidiaphragms was not due to decreased binding of insulin by denervated muscles as shown in experiments with I^{131}-labeled insulin in vivo and in vitro.

Paralyzed hemidiaphragms were found to have less capacity to increase their glycogen content after feeding, and the glycogen content of denervated hemidiaphragms was lower than that of their working pair in fed animals. These observations agree with studies by Hines and his co-workers[10-12] using denervated rat gastrocnemii and were interpreted to be due to impaired glycogen synthesis. However, these authors also observed that paralyzed gastrocnemii contain less glycogen than controls in fasted animals, which did not occur in the case of the paralyzed hemidiaphragm. This apparent discrepancy can be interpreted by considering that contrary to the gastrocnemius the diaphragm is a continuously working muscle. In control (working) diaphragms of fasted rats the rate of glycogenolysis may exceed the rate of synthesis, resulting in a glycogen content less than that of denervated (inactive) hemidiaphragms.

SUMMARY

The glucose uptake in vitro and responsiveness to insulin of rat diaphragms were studied twelve to 200 hours after unilateral denervation.

Intact and paralyzed hemidiaphragms were incubated in buffer containing 300 mg. per cent glucose with or without added insulin. The amount of glucose consumed during ninety minutes' incubation was related to the tissues' dry weight.

The glucose uptake of paralyzed and control hemidiaphragms was identical in the absence of insulin. When insulin (0.001 U. per milliliter) was added to the medium, the glucose uptake of paralyzed hemidiaphragms increased less than that of controls. Dose response studies indicated that the insulin sensitivity decreased to one tenth of normal twelve hours after denervation. Similarly, after injecting rats in vivo with insulin, paralyzed hemidiaphragms when subsequently assayed in vitro consumed less glucose than controls.

No impairment of binding of insulin labeled with I^{131} was observed in denervated hemidiaphragms.

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After feeding, the glycogen content increased 30 per cent in denervated hemidiaphragms and 200 per cent in controls.

The insulin binding capacity of paralyzed hemidiaphragms is unimpaired while their metabolic response to the hormone is decreased, due to inactivity or denervation. The decreased response to insulin preceded detectable morphological changes in muscle. It is suggested that increased insulin responsiveness may represent a mechanism of adaptation by which muscles obtain the necessary sources of energy during exercise.

SUMMARIO IN INTERLINGUA

Le Acceptation De Glucosa E Le Responsa A Insulina Per Isolate Diaphragmas De Rattos

Le acceptation de glucosa in vitro e le responsivitate a insulina per diaphragmas de rattos esseva studiate ab dece-duo usque a duo centos horas post le effectuation de disnervation unilateral. Intacte e paralysate hemidiaphragmas esseva incubate in un solution tampon que contineva 300 mg. pro cento de glucosa con o sin le addition de insulina. Le quantitate de glucosa consumite durante novanta minutas de incubation esseva relationate al peso sic del diaphragma.

Le acceptation de glucosa per paralysate e intacte hemidiaphragmas esseva identic in le absentia de insulina. Quando insulina esseva addite al medio (in un concentration de 0,001 unitates per millilitro), le acceptation de glucosa per paralysate hemidiaphragmas esseva minus marcatemente augmentate que illo observate in le caso del hemidiaphragmas de controlo. Studios del responsa a varie dosages indicava que le sensibilitate a insulina decresceva a un decimo del valor normal intra dece-duo horas post le disnervation. Similemente, post le injection de insulina in rattos in vivo, paralysate hemidiaphragmas, subsequentemente investigate in vitro, consumeva minus glucosa que hemidiaphragmas de controlo.

Nulle reducite ligation de insulina a I^{131} esseva observate in hemidiaphragmas disnervate.

Post alimentation, le contento de glycogeno cresceva per 30 pro cento in hemidiaphragmas disnervate e per 200 pro cento in hemidiaphragmas de controlo.

Le capacitate de ligar insulina remane intacte in hemidiaphragmas paralysate durante que lor responsa metabolic a ille hormon es reducite in consequentia de inactivitate o de disnervation. Le reducite responsa a insulina precedeva detegibile alterationes morphologic in le musculos. Es formulate le opinion que le augmentate responsivitate a insulina representa possibilemente un mechanismo de adaptation per que le musculos acquire le energia necessari in exercitio.

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