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Effects of Insulin and Diet on the Metabolism of L(+)-Lactate and Glucose by the Perfused Rat Heart

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The rat-heart preparation of Bleehe & Fisher (1954) has been used in the present investigation to study the effects of diet and insulin on the metabolism of glucose and L(+)-lactate by the beating heart when added singly and in combination to the perfusate. This preparation permits more precise measurements of cardiac metabolism under specified conditions than is possible in the isolated heart-lung preparation perfused with blood or in the intact animal. A particular advantage of the isolated-heart preparation is that maximum rates of utilization at high substrate concentrations and the competition between substrates may be measured without interference from unknown humoral factors.

METHODS

The perfusion apparatus and experimental techniques were described by Williamson & Krebs (1961). Male rats (Wistar strain) weighing between 220 and 280 g. were used. 'Well-fed' animals had unrestricted access to water and stock laboratory diet (Short & Parkes, 1949; Bruce, 1950), containing approximately 55% of carbohydrate. Starved rats were without food for 24 hr. Other rats were maintained on a high-carbohydrate diet for a week before use, and had unrestricted access to water. The high-carbohydrate diet contained approximately 67% of carbohydrate, and was composed of a paste of 200 g. of casein, 700 g. of sucrose, 50 g. of McCollum's salt mixture no. 185

(McCollum, 1917) and 100 g. of water. The initial volume of perfusate in aerobic experiments was either 17 or 20 ml. and samples (1 ml.) were removed for analysis after 15 and 75 min. In anaerobic experiments the initial volume of perfusate was increased to 50–70 ml. This ensured that the lactic acid formed by glycolysis did not overcome the buffering capacity of the bicarbonate of the perfusion fluid.

Crystalline L(+)-lactic acid was prepared by the method of Krebs (1961) and neutralized with N-NaOH. Glucose, glycogen, lactate and the oxygen consumption were measured by the methods used by Williamson & Krebs (1961).

RESULTS

Rate of uptake of L(+)-lactate. The time-course of the uptake of L(+)-lactate by the perfused heart from a fed rat was determined by measuring the concentration of lactate in the perfusion medium after 15 min., and at subsequent 30 min. intervals up to a total time of perfusion of 105 min. During this time the concentration of lactate in the perfusate fell from 5.0 to 1.4 mM, but the rate of removal of lactate remained approximately linear, and was of the order of 260 μ moles/g. dry wt./hr. (Table 1).

Effect of starvation on the metabolism of L(+)-lactate. The mean rate of lactate uptake was measured over the period between 15 and 75 min. of perfusion, and the mean glycogen content of hearts was obtained after 15 and 75 min. of perfusion. The initial concentration of lactate in the

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perfusate was 5 mM. Hearts from starved rats removed 267 μ moles of lactate/g. dry wt./hr. from the perfusate whereas those from fed rats removed only 131 μ moles/g. dry wt./hr. (Table 2). The amount of oxygen needed to oxidize 267 μ moles of lactate completely is only half the observed oxygen uptake (Table 6). It was thought that the utilization of lactate may be increased in hearts from rats fed on a special high-carbohydrate diet. This was not the case, however, since the lactate uptake of hearts from rats fed on such a diet was 241 μ moles/g. dry wt./hr. (Table 2), which was not significantly different from the value of 267 μ moles/g. dry wt./hr. obtained with fed rats. There was no change of glycogen during perfusion with lactate in hearts from fed rats, but almost a third of the glycogen present in hearts from starved rats after 15 min. of perfusion had disappeared after 75 min. Despite the increased glycogenolysis in hearts from starved rats the net disappearance of lactate and glycogen, expressed as glucose equivalents (last column, Table 2), was reduced by starvation from 133 to 100 μ moles/g. dry wt./hr.

Effect of insulin on the metabolism of L(+)-lactate. Insulin had no effect on the lactate uptake or the glycogen content of hearts from either fed or starved rats (Table 2).

Effect of starvation on the metabolism of glucose in the absence of insulin. When hearts from fed rats were perfused with glucose at an initial concentration of 5 mM, the glucose uptake was 72 μ moles/g. dry wt./hr. and there was no lactate formation.

Starvation reduced the glucose uptake to 42 μ moles/g. dry wt./hr., and lactate was formed at the rate of 35 μ moles/g. dry wt./hr. (Table 3). These values are about a quarter of those reported by Shipp, Opie & Challoner (1961), who used perfused hearts from starved rats. With fed rats there was no change in the glycogen content of the heart during perfusion, but with starved rats there was an appreciable glycogenolysis (Table 3; cf. Table 2). Despite the increased glycogen breakdown in hearts from starved rats, the net disappearance of glucose, after allowance for glycogen changes and lactate formation, was reduced by starvation from 72 to 45 μ moles/g. dry wt./hr. (last column, Table 3).

Effect of starvation and high-carbohydrate diet on the metabolism of glucose in the presence of insulin. In accordance with the original observations of Bleehen & Fisher (1954), insulin at a concentration of 2 milliunits/ml. (2 mU/ml.) increased the glucose uptake of hearts from fed rats from 72 to 266 μ moles/g. dry wt./hr. The glucose uptake of hearts from starved rats was increased to a similar value by insulin, and this produced a sixfold stimulation of glucose uptake by insulin with starved rats, compared with a fourfold stimulation with fed rats (Table 3).

Starvation increased lactate formation from glucose in the presence of insulin from 114 to 281 μ moles/g. dry wt./hr. This represents an increase in the proportion of glucose converted into lactate from 21 to 50 % of the glucose uptake.

Hearts from fed rats perfused in the presence of

Table 1. *Rate of removal of lactate from the perfusion medium*

The heart (dry wt. 148 mg.) from a well-fed rat was perfused with 20 ml. of sodium L(+)-lactate (initial concentration 5 mM). Samples (1 ml.) were removed at the times stated and diluted to 50 ml. for analysis.

Time (min.)	...	...	...	...	...	0	15	45	75	105
Concn. of lactate (mM)						5.00	4.62	3.60	2.48	1.42
Lactate removed (μ moles)						—	7.6	27.0	47.2	65.2
Lactate removed over consecutive 30 min. intervals (μ moles/g. dry wt./hr.)						—	—	262	272	244

Table 2. *Effects of insulin and diet on lactate utilization of the perfused rat heart*

Rat hearts were perfused with 20 ml. of sodium L(+)-lactate (initial concentration 5 mM). Samples (1 ml.) were removed for analysis after 15 and 75 min. S.E.M. are given with numbers of experiments in parentheses. Glycogen is expressed as glucose equivalents.

Glycogen is expressed as glucose equivalents.		Metabolic changes (μ moles/g. dry wt./hr.)				
Nutritional state	Insulin (mU/ml.)	Lactate uptake (A)	Glycogen content		Difference in glycogen content (15–75 min.) (B)	Net disappearance of lactate plus glycogen (A/2 – B)
			15 min.	75 min.		
Well fed	0	267 $\pm$ 17 (8)	53 $\pm$ 4 (4)	58 $\pm$ 7 (6)	+ 5 $\pm$ 9	133
	2	286 $\pm$ 26 (3)	56 $\pm$ 7 (4)	49 $\pm$ 5 (3)	– 7 $\pm$ 11	143
Starved for 24 hr.	0	131 $\pm$ 16 (4)	119 $\pm$ 11 (4)	84 $\pm$ 5 (4)	– 35 $\pm$ 12	100
	2	131 $\pm$ 5 (4)	114 $\pm$ 12 (8)	83 $\pm$ 6 (3)	– 31 $\pm$ 21	96
High-carbohydrate diet	0	241 $\pm$ 11 (4)	56 $\pm$ 1 (5)	37 $\pm$ 2 (4)	– 19 $\pm$ 2	139

Table 3. *Effects of insulin and diet on glucose utilization of the perfused rat heart*

Rat hearts were perfused with 20 ml. of 5 mM-glucose. Samples (1 ml.) were removed for analysis after 15 and 75 min. S.E.M. are given with numbers of experiments in parentheses. Glycogen is expressed as glucose equivalents.

Nutritional state	Insulin (mU/ml.)	Metabolic changes (μ moles/g. dry wt./hr.)					
		Glucose uptake (A)	Lactate formation (B)	Glycogen content		Difference in glycogen content (15-75 min.) (C)	Net disappearance of glucose plus glycogen ($A - \frac{1}{2}B - C$)
				15 min.	75 min.		
Well fed	0	72 $\pm$ 8 (5)	0 (5)	77 $\pm$ 6 (4)	72 $\pm$ 5 (5)	- 5 $\pm$ 8	72
	2	266 $\pm$ 7 (6)	114 $\pm$ 14 (6)	94 $\pm$ 6 (5)	140 $\pm$ 9 (6)	+ 46 $\pm$ 11	164
Starved for 24 hr.	0	42 $\pm$ 5 (4)	35 $\pm$ 8 (4)	106 $\pm$ 9 (4)	86 $\pm$ 7 (6)	- 20 $\pm$ 11	45
	2	286 $\pm$ 14 (5)	281 $\pm$ 26 (4)	129 $\pm$ 5 (4)	126 $\pm$ 8 (4)	- 3 $\pm$ 10	146
High-carbohydrate diet	2	250 $\pm$ 14 (4)	111 $\pm$ 12 (4)	76 $\pm$ 6 (5)	120 $\pm$ 4 (4)	+ 44 $\pm$ 7	151

glucose and insulin showed a net glycogen synthesis of 45 μ moles/g. dry wt./hr. (Table 3, cf. Fisher & Lindsay, 1956). When hearts from starved rats were perfused under similar conditions there was no net glycogen change. The apparent lack of an insulin effect on the glycogen content of hearts from starved rats was probably due to an increased glycogen breakdown rather than to an impairment of glycogen synthesis, since glycogen synthesis occurred if lactate was also present in the medium (Table 5).

The high-carbohydrate diet compared with normal feeding had no effect on the glucose uptake, lactate formation or glycogen synthesis of hearts perfused in the presence of glucose and insulin (Table 3).

The disappearance of glucose not accounted for as glycogen or lactate (and presumably oxidized) was reduced by starvation from 164 to 146 μ moles/g. dry wt./hr. Insulin produced a two- to three-fold stimulation of glucose oxidation in hearts from both fed and starved rats.

Anaerobic uptake of glucose. In the absence of insulin hearts perfused with medium containing 5 mM-glucose took up 10 times as much glucose under anaerobic as under aerobic conditions (cf. Tables 3 and 4). The anaerobic rate of glucose uptake could be still further increased either by increasing the glucose concentration to 10 mM, or by insulin. Hearts perfused anaerobically, unlike those perfused aerobically, did not respond to insulin at low insulin concentrations. An insulin concentration of 2 mU/ml., which produced a maximum stimulation of glucose uptake aerobically (Bleehen & Fisher, 1954), was ineffective anaerobically (Table 4). Ten times this concentration of insulin increased the glucose uptake when the initial glucose concentration was 5 mM. With an initial glucose concentration of 10 mM, the glucose uptake was twice that at 5 mM, but could not be further increased by insulin. The maximum

Table 4. *Effects of glucose concentration and insulin on anaerobic uptake of glucose by the perfused rat heart*

Hearts from well-fed rats were perfused with 50-70 ml. of medium equilibrated with CO₂ + N₂ (5:95, v/v).

Initial glucose concn. (mM)	Insulin concn. (mU/ml.)	Glucose uptake (μ moles/g. dry wt./hr.)	
		15-45 min.	45-75 min.
5	0	756	532
5	2	742	550
5	20	1172	920
10	0	1400	1288
10	2	1404	1380
10	20	1440	1360

anaerobic rate of glucose uptake in the presence of insulin was of the order of 1400 μ moles/g. dry wt./hr., which is more than twice the value obtained by Morgan, Henderson, Regen & Park (1961). These authors also found that hearts perfused under anaerobic conditions normally stopped beating after 2-3 min.

In the present experiments, when hearts were perfused anaerobically with 5 mM-glucose, the heart rate fell rapidly during the first few minutes, but settled to a slow steady rate of 30-80 beats/min., which was not increased by insulin. With 10 mM-glucose, the heart rate was usually above 100 beats/min., and again was not affected by insulin. After 45 min. of perfusion, the heart rate tended to fall, and this was associated with a decreased glucose uptake from 45 to 75 min. compared with that from 15 to 45 min. (Table 4). In a few experiments the formation of lactate by hearts perfused anaerobically with glucose was measured; it was sufficient to account quantitatively for the glucose disappearance.

Competition for metabolism between glucose and L(+)-lactate in the absence of insulin. Hearts perfused with medium containing initially 5 mM-glucose and 5 mM-L(+)-lactate removed glucose

and lactate from the medium at approximately the same rates as when each substrate was present alone. Unlike hearts from fed rats perfused with either glucose or lactate separately, similar hearts perfused with glucose and lactate in combination showed a net synthesis of glycogen. This was approximately equal to the glucose uptake. The disappearance of glucose and lactate expressed as glucose equivalents after correction for the glycogen change was $114 \mu\text{moles/g. dry wt./hr.}$ with fed rats (Table 5). This figure gives a measure of the combined oxidation of glucose and lactate. The corresponding values with perfusates containing lactate alone and glucose alone were 133 and $72 \mu\text{moles/g. dry wt./hr.}$ respectively (Tables 2 and 3), showing that glucose and lactate oxidation were not additive when both substrates were present together in the medium.

Starvation had no effect on glucose uptake, but lactate uptake was more than halved (Table 5). The glycogen content of hearts from starved rats did not change during perfusion, presumably because the glycogenolysis, observed in hearts from starved rats perfused with glucose or lactate alone, was counteracted by an increased rate of glycogen synthesis when glucose and lactate were present together. As with fed rats, glucose and lactate oxidation were not additive. Starvation reduced the net disappearance of lactate and glucose from 114 to $88 \mu\text{moles/g. dry wt./hr.}$

Competition for metabolism between glucose and L(+)-lactate in the presence of insulin. The glucose uptake was increased approximately fourfold by insulin with 5 mM-glucose and 5 mM-lactate present initially in the perfusate, but the lactate uptake was entirely suppressed, and was even reversed by the formation of additional lactate from glucose.

The additional presence of lactate reduced the glucose uptake of hearts from fed rats from 266 to $200 \mu\text{moles/g. dry wt./hr.}$ Lactate formation was reduced from 114 to $39 \mu\text{moles/g. dry wt./hr.}$, and

glycogen synthesis from glucose was increased from 45 to $57 \mu\text{moles/g. dry wt./hr.}$ (Tables 3 and 5). The disappearance of lactate and glucose after correction for the glycogen change was $123 \mu\text{moles/g. dry wt./hr.}$ when hearts from fed rats were perfused with glucose, lactate and insulin (Table 5), compared with $164 \mu\text{moles/g. dry wt./hr.}$ for perfusates containing glucose and insulin (Table 3), and $143 \mu\text{moles/g. dry wt./hr.}$ for perfusates containing lactate and insulin (Table 2). Surprisingly, insulin did not appreciably increase the net disappearance of glucose and lactate when both substrates were present in combination in the perfusate (Table 5).

When hearts from starved rats were used, the additional presence of lactate reduced the glucose uptake from 286 to $224 \mu\text{moles/g. dry wt./hr.}$, whereas the lactate formation was reduced from 281 to $242 \mu\text{moles/g. dry wt./hr.}$, and glycogen synthesis was increased from zero to $29 \mu\text{moles/g. dry wt./hr.}$ Hearts from starved rats had a high rate of glycogenolysis when perfused with glucose or lactate alone, which was just balanced by glycogen synthesis when the perfusate contained glucose and insulin. Glycogen synthesis only exceeded glycogen breakdown when the perfusate contained high concentrations of lactate in addition to glucose and insulin. The disappearance of glucose after correction for glycogen and lactate changes was reduced by starvation from 123 to $74 \mu\text{moles/g. dry wt./hr.}$

Oxygen consumption. Data on the oxygen consumption are included in Table 6. Values for the oxygen consumption of hearts perfused with glucose are taken from Fisher & Williamson (1961). The mean oxygen uptake of hearts perfused with lactate was $1550 \mu\text{moles/g. dry wt./hr.}$, which is slightly lower than the mean value of $1770 \mu\text{moles/g. dry wt./hr.}$ obtained by Fisher & Williamson for hearts perfused with glucose. Starvation or insulin had no effect.

Table 5. *Effects of insulin and diet on the competition for utilization between glucose and lactate*

Rat hearts were perfused with 20 ml. of medium containing initially 5 mM-glucose and $5 \text{ mM-sodium L(+)-lactate}$. Samples (1 ml.) were removed for analysis after 15 and 75 min. Removal of lactate from the medium is denoted by a negative sign and formation by a positive sign.

S.E.M. are given with numbers of experiments in parentheses. Glycogen is expressed as glucose equivalents.

Nutritional state	Insulin (mU/ml.)	Metabolic changes ($\mu\text{moles/g. dry wt./hr.}$)					
		Glucose uptake (A)	Lactate uptake (-) or formation (+) (B)	Glycogen content		Difference in glycogen content (15-75 min.) (C)	Net disappearance of lactate and glucose ($A - \frac{1}{2}B - C$)
				15 min.	75 min.		
Well fed	0	55 ± 7 (6)	-223 ± 10 (6)	62 ± 3 (8)	115 ± 6 (8)	$+53 \pm 7$	114
	2	200 ± 13 (6)	$+39 \pm 10$ (4)	100 ± 8 (4)	157 ± 8 (7)	$+57 \pm 12$	123
Starved for 24 hr.	0	47 ± 5 (8)	-91 ± 23 (5)	117 ± 9 (5)	121 ± 5 (8)	$+4 \pm 9$	88
	2	224 ± 5 (7)	$+242 \pm 20$ (8)	132 ± 5 (5)	161 ± 11 (8)	$+29 \pm 15$	74

Table 6. *Effects of insulin and diet on the contribution of lactate, glucose and endogenous substrates to energy requirements of the perfused rat heart*

Results are from Tables 2, 3 and 5, and additional data on oxygen consumption.

Nutritional state	Substrate added (initial concn. 5 mm)	Insulin (2 mU/ml.)	Metabolic changes (μ moles/g. dry wt./hr.)			Fuel of respiration (%)					
			Glycogen		Oxygen uptake	Oxygen equivalent		Lactate	Glucose	Glycogen	Endogenous non-carbohy- drate
			Synthe- sized	Used		Lactate	Glucose plus glycogen				
Well fed	Lactate	0	0	0	1550	801	0	52	0	0	48
	Glucose		0	0	1700	0	432	0	24	0	76
	Glucose + lactate		53	0	1700	668	0	39	0	61	
	Lactate	+	0	0	1550	858	0	55	0	0	45
	Glucose		45	0	1700	0	984	0	57	0	43
	Glucose + lactate		57	0	1700	0	738	0	43	0	57
Starved for 24 hr.	Lactate	0	0	35	1550	393	210	25	0	13	62
	Glucose		0	20	1700	0	270	0	9	7	84
	Glucose + lactate		0	0	1700	273	258	16	15	0	69
	Lactate	+	0	31	1550	393	186	25	0	13	62
	Glucose		0	0	1700	0	876	0	51	0	49
	Glucose + lactate		29	0	1700	0	444	0	26	0	74
High-carbohydrate diet	Lactate	+	0	19	1550	834	114	54	0	7	39
	Glucose		44	0	1700	0	906	0	53	0	47

DISCUSSION

Contribution of glucose and lactate to the fuel of respiration

In order to compare the contributions made by glucose and lactate to the total fuel of respiration, the amount of oxygen necessary to oxidize completely the glucose and lactate removed from the medium and not converted into glycogen has been obtained by multiplying the net disappearance of glucose and lactate, shown in Tables 2, 3 and 5, by six and three respectively (Table 6). These values are termed oxygen equivalents, since glucose requires 6 and lactate 3 molecules of oxygen for complete oxidation. It has been assumed (*a*) that intermediate products of oxidation did not accumulate, and (*b*) that the oxygen uptake of hearts perfused with glucose and lactate in combination was the same as when glucose alone was present. This latter assumption is based on the observation that the oxygen uptake of the perfused rat heart is little affected by the nature of the substrate being oxidized (Fisher & Williamson, 1961).

With lactate as the sole substrate in the medium no more than 55% of the total respiration of hearts from fed rats could be accounted for by the oxidation of lactate. Since the uptake of lactate was independent of concentration at least over the range 1.4–5 mM, it is unlikely that this figure would be appreciably increased by a higher concentration of lactate in the perfusate. With an initial lactate concentration of 5 mM there was no net synthesis or utilization of glycogen by the heart. Insulin had no effect on the oxidation of either lactate or glycogen.

Glucose in the absence of insulin was oxidized to a small extent, and only contributed 24 % to the total fuel of respiration with fed rats. It is reasonable to suppose that, in the absence of insulin, glucose oxidation was limited by glucose availability, and, since neither lactate nor glycogen was formed by hearts from fed rats, all the glucose removed from the medium was probably oxidized. In the presence of insulin, however, glucose penetration was no longer rate-limiting (Morgan, Henderson, Regen & Park, 1961) and the glucose uptake exceeded the capacity of the heart to oxidize it, and lactate appeared in the medium and glycogen in the heart. Insulin increased the oxidation of glucose by the perfused rat hearts two- to three-fold. This observation differs from reports by Beloff-Chain *et al.* (1954) on the effect of insulin on the isolated rat diaphragm. These authors found that the extra glucose disappearing from the incubation medium in the presence of insulin was almost entirely converted into oligo- and poly-saccharides, and that the oxidation of glucose was not increased. This latter observation is contradictory to the findings of

Villee & Hastings (1949), who also used incubated rat diaphragm. With the perfused rat heart only about 20% of the glucose removed from the medium was converted into glycogen. The different behaviour of the two tissues may be due to the very different incubation conditions rather than to a different qualitative effect of insulin on heart and diaphragm.

When lactate and glucose were both added to the perfusate, the relative oxidation of each substrate by perfused hearts depended on the presence of insulin. However, insulin did not increase the combined oxidation of glucose and lactate (Table 6). In the absence of insulin, all the glucose removed from the perfusate was converted into glycogen, and lactate accounted for 39% of the total fuel of respiration with fed rats. In the presence of insulin, there was no net removal of lactate from the perfusate, and glucose accounted for 43% of the total fuel of respiration. These figures, when compared with values of 57% for the contribution of glucose to the total fuel of respiration with perfusates containing only glucose and insulin, and values of 52–55% for the contribution of lactate with perfusates containing only lactate, indicate that lactate inhibited the oxidation of glucose, and that glucose inhibited the oxidation of lactate.

The results with the three different perfusion media show that the perfused rat heart had a limited total capacity for the oxidation of lactate and glucose even in the presence of insulin. In other words glucose and lactate, unlike acetoacetate, were not able to suppress the oxidation of endogenous substrates (Williamson & Krebs, 1961). Pyruvate also suppressed the oxidation of endogenous fuel (Williamson, 1961). Lactate and glucose, unlike pyruvate and acetoacetate, require the participation of cytoplasmic diphosphopyridine nucleotide for their oxidation. Hence the oxidation of lactate and glucose may be limited by the rate of oxidation–reduction of this coenzyme, a step which may involve hydrogen transfer to intramitochondrial pyridine nucleotides.

Other workers, using different heart preparations, have observed a similar limitation of glucose and lactate oxidation. Brin & Olson (1952), using duck-ventricle slices, found that pyruvate was oxidized in preference to L(+)-lactate, and that at lactate concentrations below 5 mM the oxidation of lactate accounted for less than half the observed oxygen uptake. Heart slices did not behave metabolically in the same way as the perfused heart, however, as shown by the low oxygen consumption, and the fact that there was a considerable non-oxidative removal of lactate and pyruvate in the slices, especially at higher substrate concentrations (Miller & Olson, 1952). Investigations of myocardial metabolism in the intact lightly

anaesthetized dog, and in man, by means of cardiac catheterization of the coronary sinus, have shown that the utilization of lactate was a function of arterial concentration over the normal physiological range (0.2–2.4 mM) and that the total carbohydrate utilization accounted for considerably less than the total oxygen uptake (Goodale & Hackel, 1953; Bing *et al.* 1953; Allela, Williams, Bolene-Williams & Katz, 1956; Goodale, Olson & Hackel, 1959). Investigations with isolated blood-perfused dog hearts have shown that at higher arterial concentrations of lactate (up to 14 mM) the utilization of lactate became independent of concentration, and that the combined extraction of glucose and lactate was not sufficient to account for the oxygen consumption (McGinty, 1931; Evans, Grande & Hsu, 1934; Cavert & Johnson, 1956).

Effects of starvation

The principal effects of starvation on the metabolism of glucose and lactate by perfused hearts were (1) a lowered lactate uptake, (2) a lowered glucose uptake, and (3) an increased rate of aerobic glycolysis. Hearts from starved rats invariably had a higher initial glycogen content than those from fed rats, but showed a markedly greater rate of glycogenolysis during perfusion. Despite the higher rate of glycogenolysis, starvation reduced the total carbohydrate oxidation, whether the perfusate contained glucose alone, lactate alone or glucose and lactate in combination (Table 6). These observations are in agreement with those of Goodale & Hackel (1953), who found that starvation reduced the extraction of glucose, lactate and pyruvate by the dog myocardium *in vivo*, despite near-normal arterial concentrations of these substances. Similar results in man were obtained by Goodale, Olson & Hackel (1959). Thus starvation promotes the oxidation of endogenous non-carbohydrates, which are probably largely derived from the fat stores. The oxidation of exogenous fatty acids by isolated rat diaphragm was increased by starvation (Neptune, Sudduth, Fash & Foreman, 1959), and so it is uncertain whether the primary effect of starvation is to inhibit carbohydrate oxidation or to promote fat or fatty acid oxidation. Similar differences in the relative oxidation of glucose and fatty acids to those observed after starvation have been obtained with isolated diaphragm from rats fed with a high-fat diet (Tepperman, Tepperman & Schulman, 1956; Wertheimer & Ben-Tor, 1952).

The changes in the metabolism of carbohydrate and fat by muscle tissues from starved animals bear some resemblance to the alterations in the metabolism of these tissues in the diabetic state. Evans, Grande, Hsu, Lee & Mulder (1935), using isolated hearts from diabetic dogs, and Goodale,

Olson & Hackel (1959) and Ungar, Gilbert, Siegel, Blain & Bing (1955) in human subjects with diabetes mellitus, have observed a decreased myocardial utilization of lactate and glucose. Morgan, Cadenas, Regen & Park (1961) have shown that the glucose uptake of perfused hearts from diabetic rats, both in the presence and absence of insulin, was lower than that of hearts from normal rats; Hall (1960) observed a decreased lactate uptake in incubated strips of skeletal, diaphragm and heart muscle from diabetic rats. The indication is that the metabolic changes occurring during starvation and diabetes may affect the oxidation of carbohydrate and fat of skeletal and cardiac muscle by a similar mechanism. However, it is not clear whether the factors controlling the balance between carbohydrate and fat metabolism are exerted by changes in enzyme amount or by the relative availability of cofactors (Field, 1959).

SUMMARY

1. The removal of L(+)-lactate and glucose, the formation of lactate, the consumption of oxygen and the changes in glycogen content were measured in the perfused rat-heart preparation described by Bleehen & Fisher (1954). L(+)-Lactate, glucose and insulin, alone and in combinations, were added to the perfusate, and hearts from well-fed and 24-hr.-starved rats were studied.

2. Lactate when added as sole substrate (5 mM) contributed 52% to the respiration of hearts from fed rats, and glucose (5 mM) contributed 24%. Insulin was without effect on lactate oxidation but increased the contribution of glucose to 57%.

3. Lactate decreased the oxidation of glucose in the presence or in the absence of insulin, and stimulated glycogen synthesis from glucose.

4. Glucose in the absence of insulin decreased lactate oxidation by 25%, but in the presence of insulin lactate oxidation was completely suppressed.

5. Starvation increased the rate of glycogenolysis, but decreased the total oxidation of lactate, glucose and glycogen, whether glucose, lactate and insulin were added singly or in combinations to the perfusate.

6. The maximum anaerobic rate of glucose uptake was about five times the maximum insulin-induced rate of glucose uptake aerobically.

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