EFFECT OF ANTIMETABOLITES, PYRIDINE DERIVATIVES, METALLIC SALTS AND INTERMEDIARY METABOLITES ON RAT DIAPHRAGM METABOLISM

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ABSTRACT.—Rat hemidiaphragms have been incubated with a variety of compounds, including antimetabolites, amino acids, pyridine derivatives, intermediary carbohydrate metabolites and inorganic salts in a saline-phosphate medium containing 120 mg glucose per 100 ml and the mean differences in oxygen and glucose uptakes and in glycogen content ascertained. Several of the agents depressed respiration and the glycogen content was diminished in the presence of guanidine•HCl (up to 3.00 mg), imidazole (0.50 mg) and dehydracetic acid (1.00 mg). Glucose uptake was depressed by high levels of sulfanilic acid, elaidic acid, oxalate, phenylpyruvate and citrate. Of a number of inorganic salts, only sodium fluoride at 2.4 μmole caused decreases in both oxygen uptake and glycogen turn-over and these depressions were no longer evident with 0.48 μmole.

The isolated rat diaphragm constitutes an invaluable tissue for the study of muscle carbohydrate metabolism. Among others, the diaphragm has been used to investigate insulin (Gemmill, 1940; Stadie and Zapp, 1947), sulfhydryl compounds Spencer and Gershbein, 1964), vitamins (Gershbein et al., 1961; Gershbein, 1965a) and thyroid hormones (Gershbein, 1965b). In the present investigation, the effect of growth factors, antimetabolites, various pyridine derivatives and analogs, amino acids and metallic ions on the respiration, the glucose uptake and the glycogen content of the rat diaphragm was ascertained. Several carboxylic acids and tricarboxylic acid cycle components were likewise screened. In this conjunction, the utilization of acetate and pyruvate by normal and diabetic rat diaphragms has been reported and except for aconitate, pyruvate metabolism was little influenced by oxaloacetate, α-ketoglutarate or succinate; the incorporation of pyruvate carbon into Krebs' cycle acids was also determined (Villee and Hastings, 1949; Foster and Villee, 1957). The uptake and partition of palmitate-1-C^{14} by diaphragm has been investigated by Shtacher and Shafrir (1963).

MATERIALS AND METHODS

All compounds were obtained from commercial sources and were of high or A.R. purity. Coramine (Ciba) was used in 25% aqueous solution. With the organic acids, either the salts as such or the acids neutralized with aqueous sodium hydroxide were diluted with saline. Incubation was carried out in the phosphate-saline medium of Stadie and Zapp (1947), the concentration of glucose being 120mg/100 ml.

Male Holtzman rats weighing 135-170 g were starved for 16 hr, sacrificed by decapitation and the hemidiaphragms were rapidly removed and introduced

into chilled saline. They were trimmed, blotted between filter paper and the weighed tissues placed in Warburg flasks containing the appropriate media. The latter contained 1.0 ml of double strength Stadie and Zapp medium and 1.0 ml saline as such (control) or 1.0 ml saline solution plus the given test compound (treatment). Control flasks without tissue were also included in each run. The flasks were gassed with pure oxygen and incubated at 37.5 C for 1 hr, after which time the supernatant fluids and the hemidiaphragms were analyzed for glucose and glycogen, respectively. The processing of the tissues and the analytical procedures employed in the method of paired hemidiaphragms have been described in detail in an earlier report (Gershbein, 1965b).

Results and Discussion

Mean differences in Q_{O_2}, glucose uptake and glycogen content of hemidiaphragms incubated with several antimetabolites, acids and metabolic intermediates are presented in Table 1. Oxygen uptake was significantly influenced by desoxypyridoxine•HCl (0.75 mg), isoascorbic acid (1.00 mg), α-methyl-DL-methionine (1.00 mg), 2, 6-diaminopurine sulfate (0.25 mg) and guanidine•HCl (up to 3.00 mg) to the exclusion of any effect on the glucose uptake or glycogen content except for decreases in glycogen turn-over with guanidine as was also the case with imidazole (0.50 mg). At high levels of sodium oxalate, elaidic acid and phenylpyruvate, both the glucose uptake and Q_{O_2} were decreased. With dehydracetic acid at 1.00 mg, the glycogen content and respiratory activity fell but the decreases did not occur at the lower concentration (0.10 mg).

With the tricarboxylic acid cycle intermediates, respiration of hemidiaphragms was in the direction of stimulation, the increases being definite with α-ketoglutarate, succinate and oxaloacetate but not significant with citrate although the latter agent depressed glucose uptake. In contrast to phenylpyruvate which plays a prominent role in phenylketonuria, pyruvic acid at a high level was without any real effect on the diaphragm. The general antagonistic action of malonate was reflected by an inhibition of respiration.

For the majority of agents screened, the mean differences engendered in Q_{O_2}, glucose utilization and glycogen turn-over were not significant. The compounds together with the respective levels in mg, are listed below:

Adenosine (0.25)
4-Aminobutyric acid (1.00)
2-Amino-4-methylpyrimidine (0.35)
2-Amino-3-phenylbutanoic acid
L-Arginine•HCl (3.00)
Benzimidazole (0.25)
Caffeine (0.50)
Canavanine sulfate (0.50)
Coramine (0.50)
Desthiobiotin (0.35)
Dihydroxyacetone (1.00)
3.4-Dihydro-2H-pyran-2-carboxylate
DL-Ethionine (3.00)
Fumaric acid (1.00)
6-Hydroxynicotinic acid (0.50)
Indole (0.25)
3-Indoleacetic acid (0.50)
Isonicotinic acid (0.75)
Methanesulfonylcholine chloride (0.50)
Nicotinuric acid (0.50)
Oxythiamine·HCl (0.50)
Phosphoenol-pyruvic acid (cyclohexyl ammonium salt; (0.50))
Phosphorylcholine chloride (1.00)
α-Picolinic acid·HCl (0.35)
Pyridine-3-acetic acid (0.50)
Pyridine-3-sulfonic acid (0.50)
DL-Serine (3:00)
Sodium acetate (3.00)
Sodium cholate (0.75)
Sodium cyclohexylsulfamate (succaryl sodium; (3.50))
Sodium formate (0.50)
Sodium isethionate (0.50)
Sodium pantoyltaurine (0.50)
Sodium propionate (3.00)
Tartaric acid (2.00)
β-2-Thienylalanine (0.50)
Uracil (0.25)

TABLE I. Mean Differences in Diaphragm Oxygen Uptake, Glucose Utilization and Glycogen Content in the Presence of the Test Agents[a].

Compound (mg)	Mean Q_{O_2} Change		Mean Glucose Difference[b]		Mean Glucose Difference	
	μl/mg wet tissue/hr	t	μg/mg wet tissue/hr	t	μg/mg wet tissue/hr	t
Desoxypyridoxine·HCl (0.75)	0.22 ± 0.049 (12)	4.48**	0.73 ± 0.457 (15)	1.61	0.13 ± 0.087 (15)	1.45
Isoascorbic acid (1.00)	0.14 ± 0.052 (16)	2.63*	0.26 ± 0.230 (11)	1.11	0.01 ± 0.114 (16)	0.09
Imidazole (0.50)	0.02 ± 0.031 (26)	0.62	0.30 ± 0.616 (21)	0.48	0.21 ± 0.067 (26)	3.07**
L-3-Aminotyrosine·HCl (1.00)	0.25 ± 0.067 (11)	3.79**	0.26 ± 0.360 (13)	0.72	0.14 ± 0.104 (12)	1.30
α-Methyl-DL-methionine (1.00)	0.10 ± 0.040 (11)	2.42*	0.11 ± 0.370 (9)	0.31	−0.03 ± 0.050 (11)	0.38
Guanidine·HCl (3.00)	0.22 ± 0.070 (23)	3.10**	.....	.....	0.30 ± 0.069 (20)	4.30**
2,6-Diaminopurine sulfate (0.25)	−0.20 ± 0.020 (11)	9.85**	1.99 ± 1.327 (12)	1.04	0.04 ± 0.081 (11)	0.32
Dehydracetic acid (0.10)	−0.18 ± 0.139 (12)	1.30	0.00 ± 0.447 (11)	0.01	0.22 ± 0.140 (12)	1.60
Dehydracetic acid (1.00)	0.36 ± 0.098 (12)	3.67**	0.15 ± 0.447 (11)	0.34	0.25 ± 0.072 (12)	3.44**
Sulfanilic acid (1.00)	0.10 ± 0.070 (11)	1.40	1.01 ± 0.290 (10)	3.50**	−0.06 ± 0.059 (11)	1.00
Oxalic acid (1.40)	0.13 ± 0.051 (10)	2.50*	0.85 ± 0.330 (12)	2.60*	−0.02 ± 0.230 (11)	0.10
Elaidic acid (0.75)	0.49 ± 0.114 (12)	4.25**	1.36 ± 0.118 (12)	11.57**	0.02 ± 0.058 (10)	0.37
Phenylpyruvic acid (0.90)	0.25 ± 0.082 (14)	2.98**	1.57 ± 0.731 (11)	2.15*	0.12 ± 0.108 (13)	1.09
α-Ketoglutaric acid (1.00)	−0.11 ± 0.047 (13)	2.41*	0.16 ± 0.390 (12)	0.40	0.11 ± 0.079 (14)	1.41
Succinic acid (1.00)	−0.11 ± 0.037 (13)	2.83*	1.00 ± 0.632 (14)	1.58	0.07 ± 0.134 (14)	0.51
Citric acid (0.75)	−0.01 ± 0.017 (8)	0.17	0.82 ± 0.311 (18)	2.65*	0.08 ± 0.100 (17)	0.76
Pyruvic acid (0.80)	0.06 ± 0.064 (21)	0.98	0.92 ± 0.600 (21)	1.53	0.05 ± 0.046 (22)	1.02
Oxaloacetic acid (1.00)	0.17 ± 0.045 (25)	3.85**	0.13 ± 0.556 (22)	0.22	0.11 ± 0.078 (26)	1.45
Malonic acid (1.20)	0.18 ± 0.061 (23)	2.87*	0.65 ± 0.380 (18)	1.67	0.14 ± 0.087 (18)	1.58

[a] The means (± S.E.) are deduced from the number of paired hemidiaphragms specified in the parentheses.
[b] A negative mean value is indicative of an increase in response in the presence of test compound.
[c] Based on the final concentration of the respective media incubated with tissue.
** $P < 0.01$.
* $P < 0.05$.

Serotonin creatinine sulfate (1.00)
Tryptamine·HCl (1.00)
Tyramine·HCl (1.00)

Glucose utilization was not ascertained with the last three compounds.

The effects of a number of inorganic ions were also investigated in relation to diaphragm oxygen uptake and glycogen. Of these compounds, sodium fluoride at 0.10 mg (2.4 μmole) depressed both. The results were negative with fluoride at 0.48 μmole as well as with the following, the concentrations being expressed as micromoles: sodium tetraborate (10.0), cesium chloride (1.5), lithium chloride (11.9), stannous chloride (1.6), aluminum chloride (2.3), didymium chloride (2.0), lanthanum chloride (2.0), rubidium chloride (4.1), strontium chloride (3.2), barium chloride (1.2), zinc chloride (1.8), sodium iodide (1.7), sodium thiocyanate (6.2), and sodium cyanide (2.0). It might be pointed out that Shaw and Stadie (1957) observed a reduction in lactic acid formation from glucose by the diaphragm in the presence of fluoride, a known inhibitor of glycolysis.

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