EFFECT OF CARCINOGENS ON RAT DIAPHRAGM CARBOHYDRATE METABOLISM

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ABSTRACT. — In the presence of high levels of the hydrocarbons diphenyl and acenaphthene or the potent carcinogen, 7, 12-dimethyl[a]benzanthracene, average differences in the oxygen and glucose uptakes and glycogen content of the rat diaphragm were not significantly altered. Incubation was carried out in a phosphate-saline medium containing 120 mg% glucose. The hepatocarcinogen, p-dimethylaminoazobenzene (1.00 mg), caused only a minor decrease in glucose utilization and N-2-fluorenylacetamide (1.00 mg) and β-naphthylamine (0.50 mg) were responsible for depressions in glycogen content; the amine at a level of 0.050 mg was without effect.

As the role of carcinogenic hydrocarbons and hepatocarcinogens in muscle metabolism is relatively unknown, a study was undertaken of the effect of such agents on the oxygen uptake, glucose utilization and glycogen turn-over of the isolated rat diaphragm.

MATERIALS AND METHODS

Eastman Kodak Company was the source of the high purity agents except for acenaphthene which was obtained from Gesellschaft für Teerverwertung, Germany. The phosphate-saline medium of Stadie and Zapp (1947) employed in the incubation of hemidiaphragms, was prepared double strength; the final glucose concentration was 120 mg%. Due to their low solubility in aqueous solution, the various agents were dissolved in 1,2-propanediol, the volume added per Warburg flask being 0.20 ml; warming was required to dissolve some of them.

Male rats ranging 130-165 gm in weight at the time of experimentation were purchased from Holtzman Rat Company, Madison, Wisconsin and starved for 24 hr. They were sacrificed by swift decapitation, incised and the hemidiaphragms removed, rapidly chilled in saline and after trimming and weighing, placed in the Warburg flasks. By the present method of paired hemidiaphragms, one tissue was incubated with 1.0 ml double strength phosphate-saline medium, 0.80 ml physiological saline and 0.20 ml of propylene glycol (control) and the corresponding hemidiaphragm, with 1.0 ml medium, 0.80 ml saline and 0.20 ml of the solution containing hydrocarbon or carcinogen (treatment). Control flasks without tissue were also employed concurrently. Incubation was carried out with pure oxygen at 37.5° C for 1 hr. Glycogen was determined following alkaline hydrolysis of the hemidiaphragms and the glucose in the incubated fluids ascertained after dilution and deproteinization. The procedures for the analytical determinations as well as the preparation of tissues and the requisite calculations have been presented earlier in greater detail (Gershbein *et al.*, 1964; Gershbein, 1965).

RESULTS AND DISCUSSION

Average differences in oxygen and glucose uptakes and glycogen content for the tissues incubated with 1.00 mg each of diphenyl, acenaphthene and 7,12-dimethyl-[a]benzanthracene, p-dimethylaminoazobenzene or butter yellow, N-2-fluorenylacetamide and 0.05 and 0.50 mg β-naphthylamine appear in Table 1. Significance of the respective differences was ascertained by the Fisher t-test.

As with the noncarcinogenic hydrocarbons, diphenyl and acenaphthene, dimethylbenzanthracene at a comparable weight level did not affect the respective differences. A small depression in glucose uptake was noted with p-dimethylaminoazobenzene to the exclusion of any effect on the Q_{O_2} and glucose utilization. However, marked glycogen inhibition occurred with both the fluorenylacetamide (1.0 mg) and β-naphthylamine (0.50 mg), the latter also depressing the oxygen uptake at the 1% level of probability. The naphthylamine proved ineffective when the level was reduced to 50 μg.

Although pharmacological dosages were screened, these findings are of importance in that they further delineate differences among the carcinogen types, in this instance,

TABLE 1. Average differences in Q_{O_2}, Glucose Uptake and Glycogen Content of Diaphragms Incubated with Hydrocarbons and Carcinogens[a,b]

Agent (mg)	Mean $Q0_2$ Difference		Mean Glucose Difference[c]		Mean Glucose Difference	
	μl/mg wet tissue/hr	t	μg/mg wet tissue/hr	t	μg/mg wet tissue/hr	t
7,12-Dimethyl[a]benzanthracene (1.00)	0.03 ± 0.044 (9)	0.60	−0.02 ± 0.139 (10)	0.13	0.10 ± 0.057 (12)	1.69
Diphenyl (1.00)	0.17 ± 0.254 (16)	0.66	−0.59 ± 0.555 (15)	1.06	0.07 ± 0.050 (17)	1.46
Acenaphthene (1.00)	0.00 ± 0.036 (10)	0.04	−0.11 ± 0.525 (11)	0.22	0.03 ± 0.053 (11)	0.54
N-2-Fluorenylacetamide (1.00)	0.08 ± 0.069 (17)	1.10	1.47 ± 0.864 (15)	1.70	0.14 ± 0.045 (17)	3.00*
p-Dimethylaminoazobenzene (1.00)	0.05 ± 0.062 (17)	0.79	1.81 ± 0.846 (16)	2.14*	−0.03 ± 0.032 (17)	0.83
β-Naphthylamine (0.050)	−0.01 ± 0.041 (11)	0.24	0.43 ± 0.225 (9)	1.92	0.04 ± 0.056 (11)	0.77
β-Naphthylamine (0.50)	0.17 ± 0.041 (20)	4.14**	0.53 ± 0.310 (16)	1.69	0.25 ± 0.045 (19)	5.60**

[a] The means (± S.E.) are deduced from the specific number of paired hemidiaphragms given in the parentheses.
[b] A positive mean difference indicates a decrease in response in the presence of the test agent.
[c] The extent of glucose utilization was based on the final concentration of the respective media incubated without diaphragm.
** $P < 0.01$.
* $P < 0.05$.

by the criterion of rat diaphragm metabolism. Preincubation of hemidiaphragms in media containing such agents as well as the determination of the combined effect of carcinogen and insulin on the isolated rat diaphragm would be of great interest, experiments of which are not included in the present report.

Literature Cited

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