Effect of Antitumor Antibiotics and Antimetabolites on Rat Diaphragm Carbohydrate Metabolism

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Rat hemidiaphragms have been incubated with antitumor antibiotics and antimetabolites in a phosphate–saline medium containing 120 mg. per cent glucose and the changes in oxygen uptake, hexose utilization, and glycogen turnover ascertained. Aminopterin (0.40 mg.), triethylenemelamine (0.40 mg.), and 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (50 mcg.) caused a decrease in glycogen content; the latter two as well as chlorambucil and 8-azaguanine, both screened down to 10 mcg., depressed glucose utilization. Of the antibiotics, glycogenolysis occurred in the presence of tubercidin (0.50 mg.), antimycin D (0.75 mg.), streptonigrin (50 mcg.), and antimycin A (0.25 mg., suspension). Muscle glucose uptake was depressed in the presence of more physiologically significant levels of puromycin, tubercidin, streptonigrin, duazomycins A and B, and actinogan and with antimycin A (0.25 mg.); tylosin was effective in this regard at 1.00 mg. Diaphragm Q_{O_2} was depressed by 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (50 mcg.), 8-azaadenine (0.25 mg.), and 0.50 mg. each of streptonigrin, E73 base, glutinosin, psicofuranine, and actinogan and was elevated by porfiromycin (0.50 mg.).

IN RECENT studies by this laboratory, the effect of a variety of antibiotics was ascertained on the isolated rat diaphragm (1). Of these, albamycin decreased both glucose uptake and glycogen content at concentrations to 30 mcg. and at even lower levels, hexose utilization was depressed, as was also the case with chloramphenicol and kanamycin. In the present investigation, these criteria were applied to antitumor antimetabolites and antibiotics. The agents, tylosin and capreomycin, were also included as well as a cytotoxic sterol, kethoxal-bis(thiosemicarbazone), and 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide. The last compound is a streptomycin antagonist, inhibiting NADH and succinate oxidation by mitochondria (2–4).

EXPERIMENTAL

Antitumor antibiotics were generously supplied by several companies, the sources and code designations of which appear in Table I. The antimetabolites of high purity originated from commercial sources.[1] The agents were dissolved in 0.85% saline and for acids and water-insoluble aza-derivatives, solution was first effected with aqueous NaOH and then further diluted to the desired concentration with saline; all mixtures were prepared fresh prior to use. For the incubation of the hemidiaphragms, the medium of Stadie and Zapp (11) was used double strength (final concentration: 0.04 *M* Na_2HPO_4, 0.005 *M* $MgCl_2.5H_2O$, and 0.08 *M* NaCl, pH 6.8–7.0; the glucose level was 120 mg. %).

The removal of hemidiaphragms from male Holtzman rats weighing 135–165 Gm., starved for 24 hr., and their processing as well as the incubation and analytical procedures were identical with those described in previous reports (12–14). By the method of paired hemidiaphragms, one tissue was incubated with 1 ml. double strength medium + 1 ml. saline–drug solution, and the other hemidiaphragm, with 1 ml. medium + 1.0 ml. saline (control); the requisite flasks without muscle were also employed in each run. Incubation was carried out under oxygen in a Warburg apparatus at 37° for 1 hr., after which time the rinsed hemidiaphragms were hydrolyzed with 30% KOH and analyzed for glycogen (15–17); glucose in the incubation fluids was determined following dilution and deproteinization (18, 19).

RESULTS AND DISCUSSION

Average differences in Q_{O_2}, glucose uptake, and glycogen content for hemidiaphragms incubated with antimetabolites and antibiotics together with the standard errors and the Fisher *t* values in the comparisons appear in Table I. Since no experimental design was followed, a statistical method was applied to the bulk data; differences in excess of $\bar{R} \pm 2.5\ \bar{R}$, $\bar{R}$ being the average range, were discarded (20). As small to slight amounts of glutinosin (0.50 mg.), porfiromycin (0.50 mg.), streptimidone (0.50 mg.), and actinomycin D (0.75 mg.) and even larger portions of chlorambucil (0.50 mg.), tubercidin, antimycin A, and 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (0.050 mg.) remained undissolved, fine suspensions were used in each instance.

The mean differences were not statistically significant for the following, the values in parentheses denoting the highest levels (mg.) screened: methotrexate (0.40), thio-tepa (0.40), 6-mercaptopurine (0.25), cyclophosphamide (0.40), 5-fluorouracil (1.00), 8-azaxanthine (0.25), 6-azathymine (0.25), 6-azauracil (0.25), 7-azatryptophan (0.050), 4-azaleucine·2HCl (1.00), cycloleucine (2.00), carzinophilin (400 u.), cycloserine (1.00), mitomycin C (0.10), kethoxal-bis(thiosemicarbazone) (0.50), the cytotoxic steroid (0.050), capreomycin disulfate (1.00), actino-

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[1] Thio-tepa, triethylenemelamine (trademark, TEM), aminopterin, and methotrexate were obtained from Lederle Laboratories; the antiviral and antitumor agent, kethoxal-bis(thiosemicarbazone) [(5); derivative of β-ethoxy-α-ketobutraldehyde], the cytotoxic sterol, γ-lactone of 16-β-hydroxy-3,11-dioxopregna-4,17(20)-dien-21-oic acid (6) from the Upjohn Co.; cyclophosphamide (trademark, Cytoxan) from Mead Johnson Laboratories; capreomycin disulfate (750 mcg./mg. solids) and tylosin from Eli Lilly & Co.; chlorambucil (trademark, Leukeran) from Burroughs Wellcome & Co.; 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, 8-azaadenine, and 8-azaguanine in addition to the antibiotic, antimycin A, from Sigma Chemical Co.; 5-fluorouracil from Roche Laboratories and 6-mercaptopurine, 6-azathymine, 6-azauracil, 7-azatryptophan, cycloserine (D-4-amino-3-isoxazolidone), the antitumor agent and methionine antagonist, cycloleucine [1-aminocyclopentanecarboxylic acid; (7–9)]; and a competitive antagonist of leucine utilization in some bacteria, 4-azaleucine hydrochloride [2-amino-3-dimethylaminopropanoic acid · 2HCl; (10)] from Nutritional Biochemicals Corp. The latter was also the source of the antibiotics, puromycin hydrochloride, carzinophilin, and mitomycin C.

TABLE I.—AVERAGE DIFFERENCES IN OXYGEN UPTAKE, GLUCOSE UTILIZATION, AND GLYCOGEN CONTENT OF HEMIDIAPHRAGMS INCUBATED WITH ANTITUMOR AGENTS[a,b]

Agent (mg.; Code No.)[c,d]	Mean Difference in Q_{O_2} μl./mg. Wet Tissue/hr.	t	Mean Glucose Difference[e] mcg./mg. Wet Tissue/hr.	t	Mean Glycogen Difference mcg./mg. Wet Tissue/hr.	t
Drugs						
Sodium aminopterin (0.40)	0.05 ± 0.057 (22)	0.81	0.11 ± 0.346 (19)	0.33	0.13 ± 0.060 (22)	2.13[f]
Triethylenemelamine (0.010)	0.00 ± 0.074 (20)	0.01	0.52 ± 0.330 (17)	1.58	0.02 ± 0.097 (20)	0.21
Triethylenemelamine (0.50)	0.02 ± 0.022 (47)	0.81	0.54 ± 0.196 (35)	2.73[g]	0.10 ± 0.044 (46)	2.39
Chlorambucil (0.010)	0.04 ± 0.026 (11)	1.50	1.71 ± 0.387 (12)	4.40[g]	0.00 ± 0.086 (11)	0.01
Chlorambucil (0.10)	0.02 ± 0.043 (24)	0.44	1.59 ± 0.272 (26)	5.80[g]	−0.04 ± 0.087 (24)	0.42
8-Azaguanine (0.010)	0.05 ± 0.056 (21)	0.89	0.86 ± 0.304 (22)	2.83[g]	−0.13 ± 0.063 (23)	2.02
8-Azaguanine (0.050)	−0.06 ± 0.053 (17)		1.38 ± 0.470 (18)	2.90[g]		
8-Azaguanine (0.25)	0.03 ± 0.026 (27)	1.30	1.50 ± 0.346 (28)	4.33[g]	−0.13 ± 0.101 (27)	1.24
8-Azaadenine (0.25)	0.07 ± 0.033 (24)	2.10[f]	0.16 ± 0.424 (23)	0.38	0.01 ± 0.041 (19)	0.23
7-Azatryptophan (0.25)	0.07 ± 0.047 (12)	1.57	0.52 ± 0.189 (11)	2.76[h]	0.04 ± 0.078 (11)	1.50
2-Heptyl-4-hydroxyquinoline-*N*-oxide (0.010)	−0.01 ± 0.044 (12)	0.20	1.11 ± 0.294 (12)	3.76[g]	−0.15 ± 0.271 (11)	0.55
2-Heptyl-4-hydroxyquinoline-*N*-oxide (0.050)	0.38 ± 0.081 (12)	4.70[g]			1.35 ± 0.200 (12)	6.70
Antibiotics						
Puromycin · 2HCl (0.020)	−0.03 ± 0.032 (16)	0.94	0.95 ± 0.374 (15)	2.55	−0.13 ± 0.084 (16)	1.52
Puromycin · 2HCl (0.25)	0.05 ± 0.066 (23)	0.70	1.08 ± 0.346 (23)	3.10[g]	0.02 ± 0.071 (22)	0.30
Tylosin (0.10; EL, 820316)	0.11 ± 0.091 (11)	1.20	−0.16 ± 0.489 (11)	0.33	0.16 ± 0.143 (11)	1.10
Tylosin (1.00)	0.07 ± 0.036 (39)	1.97	0.61 ± 0.212 (32)	2.85[g]	0.07 ± 0.053 (36)	1.40
Actinogan (5.0×10^{-4}; BL, K937)	0.01 ± 0.082 (11)	0.11	1.35 ± 0.520 (12)	2.61[g]	−0.06 ± 0.069 (11)	0.51
Actinogan (0.0010)	0.06 ± 0.052 (32)	1.18	1.33 ± 0.280 (25)	4.75[g]	0.08 ± 0.085 (32)	0.99
Actinogan (0.050)	0.03 ± 0.043 (25)	0.80	1.03 ± 0.224 (23)	4.60[g]	−0.07 ± 0.173 (24)	0.40
Actinogan (0.50)	0.06 ± 0.026(24)	2.21[f]	1.15 ± 0.346 (13)	3.33[g]	0.16 ± 0.080 (23)	2.00
Porfiromycin (0.50; U-14743)	−0.21 ± 0.074 (16)	2.87[g]	0.48 ± 0.050 (15)	1.10	0.08 ± 0.084 (13)	0.90
Tubercidin (0.050; U-10071)	0.02 ± 0.041 (24)	0.45	0.77 ± 0.255 (27)	3.00[g]	0.01 ± 0.094 (24)	0.16
Tubercidin (0.50)	−0.01 ± 0.032 (13)	0.46	0.47 ± 0.208 (14)	2.60[f]	0.10 ± 0.032 (13)	2.85[h]
Psicofuranine (0.50; U-9586)	0.32 ± 0.051 (12)	6.19[g]	0.88 ± 0.728 (8)	1.20	−0.05 ± 0.107 (12)	0.49
E73 base (0.50; PF, 2679-40-3)	0.17 ± 0.067 (9)	2.60[f]	1.16 ± 0.714 (12)	1.60	0.06 ± 0.152 (12)	0.40
Glutinosin (0.50; PF, 5910-272-8)	0.14 ± 0.040 (12)	3.60[g]	−0.35 ± 0.180 (11)	1.90	0.02 ± 0.128 (12)	0.20
Streptonigrin (0.010; PF, 1027-196-1)	−0.01 ± 0.033 (12)	0.30	0.68 ± 0.430 (11)	1.60	0.29 ± 0.140 (12)	2.10
Streptonigrin (0.050)	−0.15 ± 0.081 (11)	1.90	0.59 ± 0.910 (10)	0.60	0.46 ± 0.185 (11)	2.50[f]
Streptonigrin (0.50)	0.38 ± 0.078 (12)	4.90[g]	1.10 ± 0.360 (12)	3.10[g]	0.36 ± 0.129 (11)	2.80[h]
Mithramycin (0.010; PF, 5523-261-1A)	−0.01 ± 0.026 (10)	0.20	0.58 ± 0.208 (12)	2.80[h]		
Mithramycin (0.50)	0.10 ± 0.060 (18)	1.70	1.61 ± 0.360 (18)	4.50[g]	0.11 ± 0.110 (18)	0.99
Duazomycin A (0.010; PF, 5912-215-2)	−0.01 ± 0.032 (12)	0.40	1.23 ± 0.479 (12)	2.56[f]	−0.03 ± 0.047 (11)	0.54
Duazomycin A (0.50)	0.03 ± 0.031 (24)	1.10	0.60 ± 0.220 (24)	2.70[h]	0.02 ± 0.075 (24)	0.20
Duazomycin B (0.050; PF, 5727-249-3)			1.83 ± 0.424 (10)	4.30[g]		
Duazomycin B (0.50)	0.04 ± 0.024 (28)	1.80	1.05 ± 0.220 (26)	4.80[g]	−0.15 ± 0.085 (27)	1.70
Antimycin A (0.25; suspension)	0.09 ± 0.052 (29)	1.65	1.20 ± 0.361 (30)	3.32[g]	0.29 ± 0.073 (30)	3.98[g]

[a] The means (±S.E.) are deduced on the basis of the specified number of paired hemidiaphragms given in the parentheses. [b] A positive mean difference indicates a decrease in response in the presence of the antimetabolite or antibiotic. [c] mg. of agent per Warburg flask in a fluid volume of 2.0 ml. [d] In the codings, BL, EL, MSD, PD, PF, and U refer to Bristol Laboratories, Eli Lilly & Co., Merck Sharp & Dohme, Parke, Davis & Co., Chas. Pfizer & Co. and the Upjohn Co., respectively. [e] The extent of glucose utilization was based on the final concentration of the respective media incubated without diaphragm. [f] $p < 0.05$. [g] $p < 0.01$. [h] $p < 0.02$.

bolin sulfate (0.50, Parke Davis), streptimidone (0.50, Parke Davis), 6-diazo-5-oxo-L-norleucine (0.50, Parke Davis), azaserine (1.00, Parke Davis), pactomycin (0.50, Upjohn), streptovitacin A (0.50, Upjohn), decoyinine (0.50, Upjohn), cycloheximide (0.50, Upjohn), actinomycin P_2 (0.050, Pfizer), roseolic acid (0.50, Pfizer), and netropsin sulfate (0.50, Pfizer).

Of the tumor antimetabolites, decreases in glycogen content occurred only with aminopterin (0.40 mg.), triethylenemelamine (0.40 mg.), and even more pronounced, with 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (0.050 mg.). The last two agents also caused depressions in glucose uptake as was also noted with chlorambucil (0.010 and 0.10 mg.) and 8-azaguanine screened down to levels of 10 mcg.; the streptomycin antagonist also markedly depressed oxygen uptake. In this regard, similar runs conducted with 8-azaxanthine, 8-azaadenine, and 6-azauracil, each at 0.25 mg., showed them to be essentially without effect, except for a minor decrease in Q_{O_2} with 8-azaadenine. At this dosage, 7-azatryptophan, an agent which can be incorporated into protein (21, 22), lowered glucose utilization, an effect which did not extend to 0.050 mg.

With the antibiotics, puromycin (0.020 and 0.25 mg.) and tubercidin (0.050 and 0.50 mg.), representing the nucleoside types, diminished glucose uptake and at the higher level of tubercidin (7-deaza-adenosine), the glycogen content underwent a definite decrease. In fact, of the antibiotics, a fall in glycogen content, in addition to tubercidin, was noted with the high dosage of actinomycin D (0.75 mg.), streptonigrin (0.050 and 0.50 mg.; almost borderline significance with 0.10 mg.), and antimycin A (0.25 mg. suspension). Glucose utilization was decreased in the presence of the last agent and streptonigrin at 0.50 mg., a level which also inhibited diaphragm respiration. It is of interest that the glutarimides, cycloheximide, streptovitacin A, and the related E73 base were without effect except that the decrease in Q_{O_2} was significant only with E73 (0.50 mg., $p < 0.05$). Respiration was also depressed with glutinosin (0.50 mg.) and psicofuranine (0.50 mg.) but was elevated at the 2% level of probability with porfiromycin (0.50 mg.). Tylosin proved a depressant of glucose uptake solely at the higher dosage (1.00 mg.).

The antitumor glycoprotein, actinogan (23), markedly depressed glucose utilization by rat diaphragm even at a level of 0.50 mcg. and with 0.50 mg., the Q_{O_2} difference was decreased at the 5% level of probability but the diminution in glycogen content was just short of statistical significance. The findings were somewhat similar with mithramycin and duazomycins A and B, each screened down to levels of 10, 10, and 50 mcg., respectively. However, Q_{O_2} and glycogen content differences were not affected at the highest dosage of each (0.50 mg.). It would be of interest to compare the effect of actinogan with peptinogan, a polypeptide fraction obtained from this antibiotic and which also possesses antitumor action (24), an experiment not included in this series.

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