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Studies on Glycolytic Enzymes: Glucose-6-Phosphatase, Phosphorylase and Phosphoglucomutase. Effect of Glucose Cycloacetoacetate (Hydrolysed). (29719)

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The activities of the hepatic glycolytic enzymes, glucose-6-phosphatase, phosphorylase and phosphoglucomutase have been found to be altered in disturbed carbohydrate metabolism. An increase in liver glucose-6-phosphatase activity has been reported in alloxan diabetic and fasting animals(1-5). A parallelism between liver glycogen and activity of phosphoglucomutase in physiologic and pathologic conditions was indicated by Weber and Cantero(6). An increase in hepatic phosphorylase in obese hyperglycemic mice(7) and a decrease in fasting rats have been observed (8).

Glucose cycloacetoacetate (GCA) has been found to have an ameliorating effect in alloxan diabetes; it cured alloxan diabetes in rabbits when given with estrogen and reduced blood glucose concentration when given alone (9). As it has also been reported to maintain glycogen synthesis and breakdown in equilibrium in the liver(10), it was considered interesting to study its effect on the activities of these hepatic enzymes in alloxan diabetic and fasting rats.

Materials and methods. Male albino rats weighing 100-150 g were divided into 5 groups as follows:

Group I—Normal fed rats; Group II—24 hr fasted rats; Group III—24 hr fasted rats injected GCA(h); Group IV—Alloxan diabetic rats; and Group V—Alloxan diabetic rats injected GCA(h).

The animals were fed a normal diet of the following composition:—wheat flour 60%, fats 10%, protein 20%, salt mixture 5%, yeast powder 5%, throughout the experimental period. Rats were made diabetic by subcutaneous injection of alloxan monohydrate (dose—130-150 mg/kg). Some rats died in 5 to 7 days. Those surviving and having a blood glucose level above 200 mg/100 ml were used in the experiment. GCA prepared by the method of West(11) as modified by Nath *et al*(12) was hydrolyzed with 2 N HCl for 20 minutes in a boiling water bath and extracted with ether to remove the resinous material formed. The aqueous solution was adjusted to pH 7.2 with 2 N NaOH. This was injected into the rats of Groups III and V intraperitoneally in 3 equal doses of 600 mg/kg at 10 a.m., 5 p.m. and again at 10 a.m. next morning. Two hours after the third injection, the rats were killed and their livers removed for determining the enzyme activities. The animals of Groups II and III were fasted for 24 hours before killing whereas the animals of other groups were fed.

The rats were stunned, decapitated and bled. The livers were quickly excised, pooled in a beaker standing on cracked ice, chilled for 5 minutes, blotted on filter paper and weighed. A 10% homogenate was prepared in ice cold water for glucose-6-phosphatase and phosphoglucomutase activity and in .1 N NaF for phosphorylase activity. Glucose-6-phosphatase activity was assayed according to the method of Swanson(13). Phosphorylase was assayed by measuring the rate of liberation of inorganic phosphate from glu-

TABLE I. Showing Body Weight, Liver Weight, Liver Glycogen and Liver Nitrogen.

Groups	Body wt, g	Liver wt, g	Liver/Body wt × 100	Liver glycogen, mg/g	Liver nitrogen, mg/g
I Normal	117 ± 16	6.05 ± .056	5.11 ± .072	21.1 ± 2.345	31 ± 2.19
II 24 hr fasted rats	110 ± 13.5	4.2 ± .056	3.83 ± .036	4.8 ± 1.09	36 ± 2.47
III *Idem*, inj GCA(H)	111 ± 12.2	4.92 ± .057	4.46 ± .028	12.4 ± 2.4	36 ± 4.33
IV Alloxan diabetic rats	118 ± 10.4	5.58 ± .038	4.7 ± .038	9.9 ± 2.098	34 ± 3.74
V *Idem*, inj GCA(H)	122 ± 14	5.7 ± .073	4.83 ± .073	12.7 ± 1.34	34 ± 4.58

cose-1-phosphate in the presence of 5′ AMP as described by Sutherland and Wosilait(14). Phosphoglucomutase activity was measured by conversion of the acid labile phosphorus of G.I.P into the acid stable phosphorus of G.6.P according to the method of Bodansky (15). Phosphorus was determined by the method of Fiske and Subbarow(16), glycogen by the method of Good *et al*(17), glucose by Nelson's method(18) and nitrogen by Kjeldahl's method(19).

Results and discussion. Table I represents data showing body weight, liver weight, liver/body weight, liver glycogen and nitrogen. Enzyme activities observed are shown in Tables II, III and IV.

TABLE II. Glucose-6-phosphatase Activity.

Groups	Specific activity*	Total activity†
I Normal	7.8 ±.049	39.83 ± 3.52
II 24 hr fasted rats	14.12 ±.098	54.17 ± 3.79
III *Idem*, inj GCA(H)	9.8 ±.086	43.5 ± 4.04
IV Diabetic	12.48 ±.0734	58.65 ± 3.24
V *Idem*, inj GCA(H)	8.5 ±.08	41.05 ± 3.6

* Micromoles of inorganic P liberated from G-6-P per min by enzyme derived from 1 g of liver.

† Total activity =

$$\frac{\text{Specific activity} \times \text{Total wt of liver}}{\text{Body wt of rat}} \times 100.$$

Glucose-6-phosphatase activity observed is shown in Table II. A rise both in specific and total activity of glucose-6-phosphatase was found in fasted and alloxan diabetic rats. GCA(h) decreased this activity to normal values. The effect of GCA on lowering the hepatic glucose-6-phosphatase activity of alloxan diabetic rats may account for its hypoglycemic action in alloxan diabetes as reported earlier from this laboratory(20). Insulin and the sulfonylureas which are hypoglycemic also decrease the lower glucose-6-phosphatase activity of the diabetic animals (21).

Liver phosphorylase activity (Table III) was found to be decreased in fasting and diabetic rats; the specific as well as the total activity being lowered. Lower phosphorylase activity in fasting animals has also been observed by Sorensen(8). A decrease in phosphorylase activity was also noticed in partially or totally depancreatized rats by Ardy and Montini(22). GCA(h) injection did not affect phosphorylase activity though hepatic glycogen was increased. According to Leloir and Cardini(23) glycogen synthesis in liver takes place by UDPG pathway, and phosphorylase only regulates the breakdown of liver glycogen. When liver glycogen stores are low, GCA(h) may increase its synthesis by stimulating the UDPG pathway and thus helping to maintain glycogen synthesis, breakdown in equilibrium in liver as observed previously(10). Phosphoglucomutase activity (Table IV) per unit wet weight or per mg

TABLE III. Phosphorylase Activity.

Groups	Units/g/liver*	Units/g liver protein	Units/total liver
I Normal	11.63 ± 1.06	62.03 ± 4.97	69.78 ± 5.701
II 24 hr fasted rats	8.19 ± .099	36.58 ± 3.86	34.53 ± 4.07
III *Idem*, inj GCA(H)	8.9 ± .076	39.7 ± 3.19	43.7 ± 3.44
IV Diabetic	9.43 ± .064	44.05 ± 3.1	52.6 ± 3.63
V *Idem*, inj GCA(H)	8.86 ± .0343	41.47 ± 1.723	50.3 ± 1.93

* mg of inorganic P liberated/15 min.

TABLE IV. Phosphoglucomutase Activity.

Liver phosphoglucomutase activity	Bases of expression	Normal	Fasting	Fasting inj GCA(H)	Diabetic	Diabetic inj GCA(H)
Per unit liver						
(i) Activity/wet wt	Units*/g wt × 100	239.5 ± 4.591	225.3 ± 4.704	214.3 ± 4.79	230.9 ± 1.996	214.3 ± 5.924
(ii) Specific activity	Units/mg N × 10^2	77.23 ± 1.378	62.55 ± 1.365	59.54 ± 1.239	67.56 ± .0682	63.03 ± 1.662
Per total liver						
(1) Total wet wt activity	Units/g wet wt × liver wt	114.95 ± 2.774	94.675 ± 2.292	105.065 ± 1.758	128.88 ± 1.246	122.16 ± 3.775
(2) Total specific activity	Units/mg N × liver wt × 10^2	467.99 ± 7.773	262.62 ± 5.602	293.03 ± 6.526	379.22 ± 2.569	360.22 ±10.3
Activity/100 g body wt						
Total available wet wt activity	Units/g wet wt × liver/body wt	122.428 ± 2.177	86.31 ± 2.28	96.78 ± 3.108	108.56 ± .09069	103.51 ± 3.184
Total available specific activity	Units/mg N × liver/body wt × 10^2	394.67 ± 6.557	239.5 ± 5.657	265.71 ± 5.762	318 ± .1413	308.3 ± 9.094

* mg acid labile P disappeared/10 min.

nitrogen was not significantly altered in fasting or diabetic rats. However, its activity per total liver or per 100 g body weight was greatly decreased. The results in fasting rats were in agreement with those of Weber and Cantero(6). GCA(h) was not found to affect phosphoglucomutase activity.

Summary. The effect of injection of GCA(h) on liver glycolytic enzymes, glucose-6-phosphatase, phosphorylase and phosphoglucomutase in fasting animals and alloxan diabetics has been studied. A rise in glucose-6-phosphatase was observed both in fasting and alloxan diabetes. GCA(h) has been found to lower the increased activity of glucose-6-phosphatase. The activity of the enzyme phosphorylase was decreased in both fasting and alloxan diabetes. GCA(h) has no significant effect. The activity of the enzyme phosphoglucomutase was also decreased in fasting and alloxan diabetic rats. GCA(h) does not have any effect on the activity of this enzyme.

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Sparing Effect of X-Rays for Mice Inoculated Intranasally with Egg-Adapted Influenza Virus, CAM Strain.* (29720)

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The final amount of pneumonia in mice inoculated with influenza virus depends on the strain of mice(1), degree of adaptation of the virus(2,3,4), and amount of virus in the inoculum(5,6). Viruses that are adapted to mice cause pneumonia after serial passage from mouse to mouse. Small doses of adapted viruses multiply to high titer in lungs and cause deaths, while equal amounts of unadapted virus may have little evident effect. Nevertheless, large doses of active unadapted virus can cause lesions in the lungs(6,7,8), by a direct toxic effect on cells apparently not dependent on multiplication of viruses within the cells.

The effect of ionizing radiation on 2 of these variables has been examined in other studies, but the total information is meager. Exposure of several stocks of albino and black agouti mice to X-rays before infection increased their susceptibility to type B influenza virus unequally(1). The radiation had the greater effect on the black agouti stocks which normally were more susceptible to the virus than the albino strains. In addition, X-rays and γ-rays increased virus proliferation in mice inoculated with type A and type A-prime strains(9,10). However, no observations on susceptibility to viral toxicity have been described.

The objective of the present study was to ascertain the effect of prior exposure to X-rays on the course of the pneumonia caused by the toxic effect of influenza virus in mice. Therefore, the virus employed was an egg-adapted line of CAM virus, an A-prime strain which causes pneumonia and even death although it is not adapted to mice(8). The findings indicate that pretreatment with a large sub-lethal dose of X-rays had a sparing effect on production of fatal pneumonia.

Materials and methods. A. Mice. Mice used in these experiments were CF-1 adult albino males weighing approximately 25 g. Irradiated and non-irradiated mice were inoculated intranasally with infected allantoic fluid containing egg-passaged CAM A-prime strain of influenza virus. The pneumotoxic effect of the virus was ascertained in terms of the risk of developing lung lesions and the risk of dying. All experiments were performed during summer months.

Animals dying before the end of an experiment were autopsied as soon as they were discovered in order to establish the cause of death. The occurrence of influenzal lung lesions at autopsy was accepted as presumptive evidence of death due to toxic pneumonitis regardless of the extent of pneumonia.

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