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Reduced Liver Glucose-6-Phosphatase in Human Leukemia.* (29750)

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During experiments concerned with rat liver glucose-6-phosphatase it became of interest to study human liver glucose-6-phosphatase. Specimens of human liver obtained during post-mortem examinations were homogenized and assayed immediately. It was noted that each of the livers from children with acute lymphatic leukemia was very deficient in this enzyme activity. Some of the mechanisms responsible for this reduction in activity were investigated and form the basis of this report.

Methods. At time of autopsy the patient's age, diagnosis, drug therapy, and hours since death were recorded. A 10 g specimen of liver was placed immediately in 0.12 M KCl at 0°C, and was kept at 0°C during subsequent steps. A 10% homogenate was prepared using a glass-teflon homogenizer at 1000 rpm for 2 to 3 minutes. The homogenate was centrifuged at 600 × *g* for 10 minutes and the residue was discarded. The supernatant was centrifuged in a Servall refrigerated centrifuge at 37000 × *g* for 30 minutes and the residue was suspended in 0.12 M KCl at a concentration 5 to 10 mg protein per ml. It had been previously demonstrated that rat liver glucose-6-phosphatase activity was purified 3-fold in this residue, when compared to liver homogenate. The activity of the 37000 × *g* residue was determined by the method of Swanson(1), at pH 6.5 in malate buffer. Protein was determined by Lowry's(2) method.

The effects of protein synthesis inhibitors on enzyme activity were determined in two ways. First, 6-mercaptopurine, methotrexate (4-amino-N-methylpteroyl glutamic acid), and cortisol were added during the assay of enzyme activity. Second, 6-mercaptopurine (1.0 mg/150 g rat/day), and methotrexate (0.1 mg/150 g rat/day) were fed in 2 groups of rats with their regular Purina Laboratory Chow diet, and other rats received 100 μg actinomycin D by intraperitoneal injection daily for 4 days. After several days the rats were all weighed, their livers were weighed, and liver homogenates were assayed for glucose-6-phosphatase activity.

Results. Table I summarizes the human liver glucose-6-phosphatase data. It can be seen that enzyme activity was not clearly related to age, hours elapsing between death and post-mortem examination or drug therapy. The livers were generally larger than the normal weight for the patients' ages. Enzyme activity is expressed in μg P/mg protein/hour, and since the reduction of enzyme activity is extreme (4- to 10-fold) it is not possible to account for this reduction by dilution of normal tissue by leukemic cells. The patient with neuroblastoma had diffuse liver metastases and a normal enzyme activity.

It was noted that 6-mercaptopurine (0.1 mg/ml), methotrexate (0.02 mg/ml) and cortisone (50 μg%) in the reaction mixture

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TABLE I. Human Liver Glucose-6-Phosphatase.

	Age	Hr post mortem	Drugs	Diagnosis	G liver wt (normal for age)	Glucose-6-phosphatase activity (μg P/mg/hr)
1.	1 mo	1	—	Cong heart disease	170 (127)	275
2.	15 day	14	—	Cong anomalies	75 (123)	171
3.	6 yr	4	chloramphenicol penicillin	Meningitis	820 (680)	95
4.	5 day	20	chloramphenicol penicillin	"	88 (66)	91
5.	4 mo	3	—	Mongolism & cong heart disease	123 (127)	351
6.	5 yr	18	prednisone	Cong heart disease, pneumonia	—	206
7.	7 day	7	—	Cong heart disease	120 (78)	273
8.	11 yr	26	—	Cystic fibrosis	769 (866)	115
9.	5 wk	9	—	Cong heart disease	120 (130)	291
10.	3 yr	15	—	Neuroblastoma	722 (470)	140
11.	6 "	4	6-mercaptopurine methotrexate	Leukemia	1010 (712)	28
12.	3 "	13	vincristine chloramphenicol	"	790 (418)	29
13.	6 "	15	oxalone	"	1762 (708)	24
14.	8 "	8	—	"	—	21

TABLE II. Rat Liver Glucose-6-Phosphatase.

Group (No. animals)	Days	Body wt (% of control)	Liver wt (% of control)	Glucose-6-phosphatase activity, μg P/mg/hr (% of control)
6-mercaptopurine (2)	0	96	—	—
methotrexate (2)	0	95	—	—
6-mercaptopurine (2)	7	100	100	139
methotrexate (2)	7	101	107	134
6-mercaptopurine (2)	13	91	86	102
methotrexate (2)	13	83	93	118
Actinomycin D (2)	0	101	—	—
Idem (2)	4	80	60	92

gave 80%, 103% and 100% of the activity of simultaneous control assays in duplicate experiments. Table II summarizes data obtained when the drugs were administered to the animals according to the above dosage schedules. Administration of methotrexate, 6-mercaptopurine and actinomycin D to rats for 13, 13 and 4 days respectively, did not alter the glucose-6-phosphatase activity of the liver homogenate. The doses selected for administration were large enough to significantly impair growth of the animal.

Discussion. The significance of the low liver glucose-6-phosphatase activity in the patients with acute lymphatic leukemia is unknown. The data suggest that it is probably not a result of therapy with agents used to combat the leukemic process. Hypoglycemia in patients with leukemia, especially during fasting is rarely encountered, possibly because it is rarely suspected. Cortisol is known to induce rat liver glucose-6-phosphatase(3), and is also useful as an anti-leukemic drug.

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