Multiple Enzyme Determinations in Sera and Livers of Tumor Bearing Hamsters.* (31479)

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Enzyme changes in experimental tumor bearing animals and cancer patients have been the subject of many reports since Warburg's original work, "Metabolism of tumours"(1). Subsequent reports by a number of investigators were concerned with enzymes of the glycolytic pathway(2,3,4), since it had been suggested that excessive glycolysis of tumor tissue might be reflected by changes of glycolytic enzymes in serum(1,2). Attention has been directed primarily to lactic dehydrogenase and the transaminases. Wroblewski and coworkers have observed correlations between plasma LDH activities and tumor mass, growth inhibition and regression(5,6).

Abnormal glutamic oxalacetic transaminase values have been found mainly in cancer patients with metastasis to the liver or myocardium(7,8). Elevations of glycolytic enzymes such as phosphohexose isomerase and isocitric dehydrogenase in the circulation have also been observed in diverse neoplastic diseases(3,8,9,10,11). Alkaline and acid phosphatase have been most frequently found elevated in leukemia and in association with tumors of the prostate gland, bone, liver and/or metastatic involvements of these tissues(8,12,13).

Since similar enzyme changes occur in many diverse pathological lesions, determinations of single enzymes are of limited value as a specific aid in cancer diagnosis.

On the other hand, multiple enzyme determinations have been shown to be valuable for typing and localizing metastatic involvements (8,9). Enzyme *changes in the liver,* for example, were studied in various liver diseases including hepatoma and metastasizing tumors (7,14,15). Little is known, however, concerning the enzyme activities in liver tissue of animals or patients bearing tumors with no metastatic involvements of the liver.

The purpose of this study was 2-fold. First, changes of glycolytic and oxidative enzymes in serum of Syrian hamsters *(Mesocricetus auratus)* bearing adenovirus type 12 induced tumors were compared with serum enzyme values of adenovirus type 5 infected as well as with uninoculated control hamsters. Adenovirus type 12 is known to produce a high incidence of undifferentiated sarcomas in newborn hamsters(16) whereas adenovirus type 5 is poorly oncogenic (if at all). Second, an effort was made to determine whether enzyme changes in serum represent a specific symptom due to release from the tumor tissue itself or is a part of the "acute syndrome" in Hauss's sense(14). To accomplish this, enzyme contents in livers of hamsters bearing non metastatic tumors were compared with that of livers of uninoculated hamsters.

The enzymes chosen for this study were lactic dehydrogenase (LDH), glutamic oxalacetic transaminase (GOT), isocitric dehydrogenase (ICD), phosphohexose isomerase (PHI), alkaline and acid phosphatase.

Materials and methods. Virus: Adenovirus type 12 (strain Hui) grown in HeLa and KB cells were used for inoculation of baboon kidney tissue cell cultures. The baboon kidney cell line was employed for virus titrations and stock preparations instead of the KB cell for reasons which are described by Smith(17). Virus stock was neutralized with specific antisera and was free of PPLO. Adenovirus type 5 virus stocks were prepared in KB cells and also tested with type specific antiserum. Virus titrations in KB cells indicated titers of $10^{-3.5}$ and $10^{-2.5}$ TCD_{50} per 0.1 ml for types 12 and 5 respectively.

Hamster inoculation: Newborn Syrian hamsters were inoculated intraperitoneally with .05 cc undiluted virus suspensions within 24 hours post partum. At certain time intervals

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TABLE I. Serum Enzyme Changes in Syrian Hamster Bearing Adenovirus Type 12 Induced Tumors, in Adenovirus Type 5 (Nononcogenic) Inoculated and in Uninoculated Hamsters.

Inoculum	Age of animals (wk)	No. of animals	Size of tumors	LDH*	PHI*	GOT*	ICD*	Alk. Ph.*	Ac. Ph.*
Av 12	3	12	—	160	206	83	656	7.00	3.04
Av 12	4	6	.5–2 cm	290	234	60	270	7.00	2.50
Av 12	5	5	1 –3 "	550	170	108	836	3.60	3.35
Av 12	6	3	1 –4 "	1260	206	172	1300	1.62	2.10
Av 5	2	15	—	380	114	64	380	12.60	4.80
Av 5	4	10	—	500	149	125	336	8.30	2.70
Av 5	5	7	—	110	61	87	300	4.96	2.45
Av 5	6	5	—	—†	—†	—†	—†	—†	—†
Control	3	10	—	190	170	72	328	11.28	2.85
	4	5	—	555	170	87	530	9.80	2.35
	5	4	—	—†	—†	—†	—†	—†	—†
	6	7	—	410	155	78	464	4.10	1.55

* The values are expressed as units/ml serum.
† Samples with recognizable hemolysis were not measured.

inoculated and uninoculated litters were exsanguinated by cardiac puncture under light ether anesthesia. The blood was centrifuged immediately at 4°C. Serum samples with recognizable hemolysis were discarded. Serum samples of all the animals from one litter were pooled, kept at —20°C and tested within a week of collection for enzyme activities. Livers were removed from the animals immediately after exsanguination. After rinsing in cold PBS, they were weighed and homogenized with a Ten-Broeck tissue grinder in cold PBS and made up to a 10% suspension. The homogenates were frozen and thawed 3 times and centrifuged at 12,000 × g for 15 minutes. The clear supernatant was used for enzyme assays and the values were expressed in units/g liver tissue. The entire procedure was carried out at 4°C.

Enzyme assays: Enzyme activities of LDH, GOT, ICD, PHI and alkaline and acid phosphatases were measured colorimetrically (18) in a Coleman Junior spectrophotometer. All reagents were obtained from Sigma Chemical Co.

Results. The values for each of the serum enzymes studied are shown in Table I. Each value represents the enzyme activities in the serum pools of the number of animals indicated in Table I. Inoculation of 24-hour-old hamster babies with adenovirus type 12 resulted in a 2-3-fold increase of LDH 5-6 weeks post inoculation. GOT and ICD serum changes also occurred about 5-6 weeks post infection during the final stage of the development of the virus induced sarcoma. The averages of the GOT and ICD values at this stage were 77% and 141% respectively above the averages of the uninoculated controls.

The PHI serum activities, however, appeared slightly elevated in adenovirus type 12 injected animals throughout the experiment. The values were generally 24% higher than those of the uninoculated animals.

Alkaline phosphatase serum levels showed decreasing values from the beginning to the end of the experiment in all 3 experimental groups. Acid phosphatase showed little or no measurable activity changes in the sera of all three animal groups.

The serum enzyme levels of LDH, ICD, alkaline and acid phosphatase of adenovirus type 5 infected animals remained essentially in the range of the uninoculated controls. PHI showed slight decreased activities. GOT levels remained comparable to the controls except for spontaneous high values 4 weeks after initial inoculations.

The liver enzyme values of adenovirus type 12 infected and uninfected hamsters are summarized in Tables II and III. Macroscopic and microscopic examinations of the livers revealed no metastasis. Most of the livers from inoculated animals showed cloudy swelling but no evidence of necrosis or malignant cell formation.‡

‡ The participation of Dr. C. S. Kim who reviewed the microscopic slides of the livers is appreciated.

No significant differences in enzyme activities in livers of tumor bearing hamsters when compared with uninoculated animals were observed for LDH, PHI, alkaline and acid phosphatase. GOT and ICD levels were highly increased in the livers of tumor bearing animals. Both enzymes showed statistically significant differences ($p > .001$) 42 days post inoculation when compared with the controls. GOT values were increased more in proportion to the tumor mass and reached the greatest rate of change 42 days post inoculation, when death among the animals started to occur. The values at this stage were 3-4 times greater than those of the uninoculated control group, while ICD values showed abnormally higher levels 21 days post inoculation and remained at an increased level.

TABLE II. PHI, GOT and ICD Activities in Livers of Adenovirus Type 12 Inoculated and Uninoculated Hamsters.

	Days after inoc.	Tumor size (cm)	PHI*	Mean	S_D†	P‡	GOT*	Mean	S_D†	P‡	ICD*	Mean‡	S_D†	P‡
Uninoc. control	21	—	1700				700				18,250			
	21	—	1750				800				19,250			
	21	—	1600				1200				14,000			
	21	—	1450				700				9,500			
				1625	292			850	294			15,250	8482	
						>.1				>.05				>.1
Av 12 inoc.	21	.75–2	1550				1800				24,500			
	21	.75–2	2050				2100				27,000			
	21	.75–2	1700				1400				24,400			
	21	.75–2	2400				1500				25,250			
				1925	621			1700	469			25,288	1580	
Uninoc. control	42	—	3000				1700				6,900			
	42	—	2000				1750				6,650			
	42	—	1250				950				8,950			
				2083	1393			1467	826			7,500	1917	
						>.1				>.001				>.001
Av 12 inoc.	42	2.54	2250				5750				25,800			
	42	.75	1550				4300				27,250			
	42	3.50	2600				5700				23,500			
				2133	943			5250	1425			25,517	3100	

* Values expressed as units/g liver tissue.
† S_D = standard deviation.
‡ P = significance of difference from controls.

Discussion. Many investigators believe that excessive glycolysis and other metabolic derangements of tumor cells are reflected in serum enzyme activity changes(1,2,3,4,11). Certainly, damage, autolysis or necrosis of tumor tissue contribute to the passage of many of these metabolic enzymes into the circulation. The increased IDH and PHI activities, which were found in the serum of hamsters bearing adenovirus type 12 induced tumors, are in accordance with the data of other investigators reporting similar increases in various neoplastic diseases(5,8,19,20,21).

Also increased serum activities of oxidative enzymes such as GOT and ICD were found associated with carcinoma with or without metastatic involvements of the liver(7,8,9, 11,20). The findings of increased GOT and

TABLE III. LDH, Alkaline and Acid Phosphatase Activities in Livers of Adenovirus Type 12 Inoculated and Uninoculated Hamsters.

	Days after inoc.	Tumor size (cm)	LDH*	Mean	S_D†	P‡	Alkaline phosph.*	Mean	S_D†	P‡	Acid phosph.*	Mean	S_D†	P‡
Uninoc. control	21	—	11,600				.05				11.1			
	21	—	18,000				.00				9.2			
	21	—	13,300				.10				7.4			
	21	—	9,300				.11				9.4			
				13,050	5954			.07	.09			9.3	3.0	
						>.1				>.1				>.1
Av 12 inoc.	21	.75–2	13,300				.02				6.1			
	21	.75–2	11,400				.18				10.3			
	21	.75–2	9,800				.01				8.7			
	21	.75–2	16,200				.24				9.9			
				12,675	5057			.11	.16			8.8	3.6	
Uninoc. control	42	—	11,400				.56				11.2			
	42	—	10,500				.77				11.4			
	42	—	13,400				.10				11.2			
				11,767	990			.48	.57			11.3	.34	
						>.1				>.1				>.1
Av 12 inoc.	42	2.54	13,050				.00				14.5			
	42	.75	9,650				.00				14.9			
	42	3.50	14,650				.20				20.0			
				12,450	4937			.07	.14			16.5	4.3	

* Values expressed as units/g liver tissue.
† S_D = standard deviation.
‡ P = significance of difference from controls.

ICD serum activities led subsequently to studies of the same enzymes in the liver of tumor bearing hamsters.

Microscopic examination of these livers revealed no malignant cells and no other lesions except for the presence of cloudy swelling. The marked increase of GOT and ICD in the liver tissue, therefore, led to the opinion that variations of serum enzyme activities in neoplastic diseases cannot be interpreted solely as consequences of enzyme "leakage" from the tumor tissue itself. Thus, in particular GOT and ICD serum enzyme activity measurements seem to be a valuable indicator not only for metastatic involvements of the liver, but also for any type of metabolic changes of the liver.

It is of interest that Hauss and Leppelmann (14) found enzyme variations in the histologically normal liver following myocardial infarction.

Serum alkaline phosphatase decreases in proportion to the age of the animals. This effect is understandable since a difference of about 3.2 Sigma units/ml serum has been found between children and adults(16). The slightly greater degree of decrease of this enzyme in tumor bearing animals than in controls is not statistically significant.

The elevation patterns of enzymes by neoplastic diseases therefore are probably dependent upon several factors: release of the enzymes from the tumor tissue into the blood, concentration of the enzymes in the tumor tissue, release of enzymes from distant organs, especially the liver, in the course of metabolic derangements and the rate of removal of these elevated enzymes from the circulation.

Summary. Enzyme activity changes of LDH, GOT, ICD, PHI, alkaline and acid phosphatase in the serum and livers of Syrian hamsters bearing adenovirus type 12 induced tumors have been studied. Five to six weeks post inoculation the serum values for LDH, GOT and ICD were 154%, 77% and 141% respectively above the means of the uninoculated controls. PHI serum levels were slightly increased from the beginning until termination of the experiment 6 weeks post inoculation. The values during this period were 24% (mean) higher than those of the uninoculated controls. Serum enzyme activities of adenovirus type 5 infected animals remained essentially unchanged, except for slightly decreased PHI levels. The enzyme content of GOT and ICD in the livers of adenovirus type 12 injected hamsters, which revealed no evidence of malignant cells by microscopic examination, were 3-4 times greater than controls. Variations in enzyme activities in the serum have been discussed in the light of the different sources of enzymes released into the blood and in relation to finding the enzyme content in the livers.

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