Tissue and Serum Aldolase of Rats with Primary Hepatoma. (25087)

A. S. MULAY
Lab. of Pathology, Nat. Cancer Inst., N. I. H., Bethesda, Md.

Aldolase, one of 5 enzymes involved in glycolysis, is normally present in tissues, cells, and blood serum of rats. It catalyses the reversible cleavage of one mole of fructose-1,6-diphosphate into one mole each of glyceraldehyde phosphate and dihydroxyacetone phosphate. Meyerhof and Lohman characterized this enzyme(1), and reported its wide distribution in animal tissues. Warburg and Christian (2) showed that aldolase and triose isomerase were consistently elevated in blood-serum of rats bearing large Jensen sarcomas. Sibley and Lehninger(3) found elevated aldolase values in blood-serum of patients suffering from various neoplastic diseases. They also showed that surgical removal of the tumor or its therapeutic regression lowered serum aldolase values to near normal levels. This paper deals with aldolase levels of tissues and serums of rats bearing a primary hepatoma and a transplanted sarcoma, with emphasis on relative aldolase level of serums from blood entering and leaving tumor tissue.

Materials and methods. Rats of Osborne-Mendel strain were used. Number of animals in each case is given in Table I. A primary hepatoma and a transplanted sarcoma were the tumors used. *A primary hepatoma.* Male and female rats 3 to 4 months old were fed a semisynthetic diet containing 0.075% para-dimethylaminobenzene - 1 - azo - 2 - naphthalene for 230 days and then a laboratory diet of Purina chow pellets. Induction of these tumors and their histopathology have been described(4). Rats had large (30 to 100 g) hepatomas. The hepatoma tissue was selected by its glistening pinkish gray color and firm texture. Necrotic areas of the hepatoma were selected by the soft texture and dull brownish gray color. Non-tumorous liver tissue was taken from areas without any pale gray tumor nodules, as determined by examination with hand lens. *A transplantable sarcoma.* Pieces of spindle cell sarcoma(5) were implanted with a trochar, under the skin of right flank of male rats about 3 months old. Animals with transplants 6 cm or more in diameter and 80 g or more in weight were included in the sarcoma experiments. Method of induction of this sarcoma and histopathology of the primary tumor and transplants have been described(5). Sarcoma tissue was selected by its pearly gray color and firm texture. Necrotic areas of sarcoma were selected by the dark brown color and soft spongy texture. *Controls* included healthy, male and female rats 4 to 12 months old, weighing 150 to 400 g. Liver tissue and blood samples from hepatic vein, inferior vena cava and heart were obtained for aldolase determinations. Blood samples from hepatoma-rats were collected from the portal vein where it enters the liver, and the inferior vena cava where the hepatic veins empty into it, and those from sarcoma-rats were collected from a tumor vein and the heart. All blood samples were obtained under ether anesthesia. Serum was separated soon after clot was formed and stored in cold until analyzed. Serum samples with any trace of hemolysis were discarded. For tissue assays, rats were killed and bled, and the organs removed and placed on ice. All tissue pieces for aldolase determination were selected on the basis of gross appearance. This gross diagnosis was later confirmed by microscopic examination of adjacent piece of tissue. Two thin slices, similar in appearance, were removed from each piece of tissue. One slice was processed for microscopic examination and the second was weighed, ground in all glass homogenizer, and diluted to appropriate volume with ice-cold water. All assays were performed as soon as possible after collection of samples. Aldolase was determined by Sibley and Lehninger method(6). In all assays of tissue extracts, 0.5 ml of 5% egg albumin was added to the incubation mixture as a protective agent. A unit of aldolase is defined as amount of enzyme in unit weight (1 g) or unit volume (1 ml) of sample which can split 1 μl of fructose-1, 6-diphosphate substrate, at pH 8.6, in one hour at 38°C.

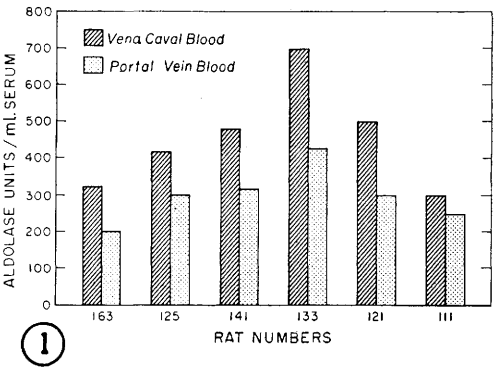
TABLE I. Serum and Tissue Aldolase Values of Normal and Tumor-Bearing Rats.

Tissue	No. of rats	Range*	Avg*
Normal control rats			
Liver	27	7,900–22,100	14,400
Portal vein serum	22	50–100	70
Vena caval "	22	"	"
Heart blood "	22	"	"
Hepatoma-bearing rats			
Hepatoma	40	8,900–20,200	12,400
Liver (non-tumorous)	30	6,900–15,400	9,600
Portal vein serum	18	200–700	375
Vena caval "	18	250–900	540
Hepatoma–necrotic	8	300–500	385
Sarcoma-bearing rats			
Sarcoma	28	5,600–10,300	7,700
Liver	28	6,800–12,400	9,100
Heart blood serum	15	140–250	176
Tumor vein "	15	130–260	194
Sarcoma–necrotic	10	150–220	178

* Units of aldolase/g of tissue or ml of serum.

Results. Serum Aldolase. Table I presents aldolase values for hepatoma and sarcoma, and for serum and liver of normal and tumor-bearing rats. In control rats, serum aldolase values of blood from portal vein, inferior vena cava and heart ranged from 50 to 100, average 70 units. Fluctuations in serum aldolase values showed no correlation with age, sex, or weight of rats studied. In rats bearing transplants of sarcoma, serum aldolase values for tumor-vein blood was significantly* higher than that for heart blood of the same rat. Average mean difference of these values was 18 with P value less than .0001. In a few rats of the hepatoma series killed before hepatomas were palpable, no significant changes were found in either serum or liver aldolase values from those in control rats. In rats bearing primary hepatomas, the average serum aldolase value of vena cava blood was 540 and that of portal vein blood was 375 units. Serum aldolase values for blood leaving the tumorous liver were consistently and significantly higher than values for blood entering the same liver. A comparison of representative serum aldolase values on individual rats is graphically shown in Fig. 1.

Tissue aldolase. Liver aldolase values for normal rats ranged from 7,900 to 22,100, average 14,400 units. The aldolase values for non-tumorous livers of tumor-bearing animals, as a rule, were lower than those for livers of normal animals (Table I). Only 11% of normal control rats, but 63% of rats bearing hepatomas and 79% of rats bearing sarcomas had liver aldolase values under 10,000 units. The range of liver aldolase values of rats bearing hepatomas was 6,900 to 15,400 units and that of rats bearing sarcomas was 6,800 to 12,400 units. Aldolase values for hepatomas ranged from 8,900 to 20,200 average 12,400 units. Aldolase values for necrotic areas of these tumors ranged from 300 to 500 units and were similar to serum aldolase values. As a rule, hepatoma aldolase values were higher than liver aldolase values of the same rat (Fig. 2). Aldolase values of sarcomas ranged from 5,600 to 10,300 with average of 7,700



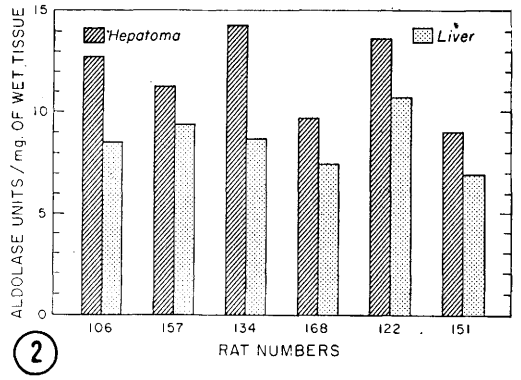


FIG. 1. Serum aldolase values for blood entering and leaving tumorous liver of 6 typical hepatoma-bearing rats.

FIG. 2. Comparison of aldolase values of hepatoma tissue with hepatic tissue (non-tumorous) of the same rat.

* All significant differences had a P value of less than .0001. I am grateful to Mr. N. Mantel of the Biometrics Branch for statistical analysis of figures.

units. About 70% of sarcomas had values from 6,000 to 9,000 units. Necrotic areas of tumor tissue had aldolase values of 150 to 220 units and were similar to serum aldolase values of the respective animals.

Discussion. This study confirms previous reports of high serum aldolase levels in rats bearing large tumors. Tumors used were carcinogen-induced primary hepatomas and transplanted sarcomas from carcinogen-induced tumors originating in this laboratory. Serum aldolase values of hepatoma-bearing rats were 5 to 8 times and those of sarcoma-bearing rats 2 to 3 times as high as corresponding values for control rats.

In rats on a carcinogenic diet, serum aldolase values were not significantly higher than those for control rats, before the hepatomas were readily palpable, and there was no significant difference between portal vein and vena cava blood values. In rats bearing primary hepatomas, the serum aldolase value of blood leaving tumor area was 20 to 65% higher than that of blood entering this area. Serum aldolase on rats with transplanted sarcoma showed similar but smaller differences between tumor-vein and heart blood. Sibley and co-workers(7) reported similar differences in serum aldolase values on blood from tumor-vein and that from the heart.

The level of serum aldolase showed a positive relationship to level of tumor aldolase. Hepatoma-bearing rats with high hepatoma aldolase levels (average 12,400 units) had high serum aldolase levels (average 375 and 540 units); while sarcoma-bearing rats with lower sarcoma aldolase levels (average, 7,700 units) had lower serum aldolase levels (average 176 and 194 units). Necrotic areas of tumors had aldolase values of the same order as serum values of animals bearing those tumors. There was no apparent relationship between amount of tumor necrosis and level of serum aldolase.

Aldolase, a normal constituent of animal tissues and cells, is constantly released into the blood stream. It is continuously removed from the blood stream(7) either by excretion or destruction. Thus level of serum aldolase is a dynamic balance between rates of its entry into and exit from the blood stream. Aronson and Volk(8) reported a rise in serum aldolase in dogs subjected to sectioning of femoral nerve or removal of entire cauda equina root system. This showed that the rate of release of aldolase by non-neoplastic tissue can be increased under certain conditions. Tumor slices, in absence of glucose in media, liberated significantly more aldolase under aerobic conditions than liver slices under similar circumstances(7). Hiatt(9) produced evidence of complete conversion of intraperitoneally injected glucose to lactate by Ehrlich ascites tumor cells, before it reached the blood stream. Horecker and Hiatt(10) attribute this unrestrained glycolysis by tumor cell to its rapid growth and consequent rapid conversion of adenosine triphosphate to adenosine diphosphate.

From above observations, it is clear that given the proper conditions, neoplastic as well as non-neoplastic cells, accelerate release of aldolase into blood stream. Thus, it is suggested that the relatively rapid growth pattern of neoplastic tissue creates conditions conducive to high glycolytic rate. This induces local cellular hypoglycemia, which accelerates the rate of aldolase release. Accelerated release of aldolase from tumor tissue may account for higher serum adolase levels in animals bearing tumors. However, the possibility of accelerated release of aldolase from other sources is not ruled out.

Summary. 1) Tissue and serum aldolase of normal and tumor-bearing (primary hepatoma and transplanted sarcoma) Osborne-Mendel rats were studied. Blood for aldolase assay was drawn from portal vein and inferior vena cava of hepatoma-bearing and control rats, from tumor vein and heart of sarcoma-bearing rats and also from heart of control rats. 2) Control rats had an average serum aldolase value of 70 units each for portal vein, vena cava and heart blood. Serum aldolase values of sarcoma-bearing rats were about 3 times and those of hepatoma-bearing rats about 8 times the values for control rats. These values averaged 375 units for blood entering tumorous liver and 540 units for blood leaving it. Serum aldolase values for heart blood averaged 176 while that for sarcoma-vein blood of the same rats averaged 194

units. Average aldolase value for normal liver, hepatoma, hepatoma-free liver, sarcoma and sarcoma-rat liver were 14,400, 12,400, 9,600, 7,700 and 9,100 units, respectively. Liver aldolase levels for tumor-bearing rats were about 35% lower than those for control rats. Aldolase values for necrotic areas of tumors were similar to serum aldolase values for respective rats.

1. Meyerhof, O., Lohmann, K., *Biochem. Z.*, 1934, v271, 89.

2. Warburg, O., Christian, W., *ibid.*, 1943, v314, 399.

3. Sibley, J. A., Lehninger, A. D., *J. Nat. Cancer Inst.*, 1949, v9, 303.

4. Mulay, A. S., Congdon, C. C., *ibid.*, 1953, v14, 571.

5. Mulay, A. S., O'Gara, R. W., *ibid.*, 1957, v18, 843.

6. Sibley, J. A., Lehninger, A. L., *J. Biol. Chem.*, 1949, v117, 859.

7. Sibley, J. A., Fleisher, G. A., Higgins, G. M., *Cancer Research*, 1955, v15, 306.

8. Aronson, S. M., Volk, B. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 360.

9. Hiatt, H. H., *Cancer Research*, 1957, v17, 240.

10. Horecker, B. L., Hiatt, H. H., *New Eng. J. Med.*, 1958, v258, 177, 225.

Received June 1, 1959. P.S.E.B.M., 1959, v101.

6-Methylmercaptopurine: Identification as Metabolite of 6-Mercaptopurine *in vivo* and Its Activity *in vitro*.*† (25088)

EDWARD J. SARCIONE AND LEON STUTZMAN (Introduced by R. Tarail)
Roswell Park Memorial Inst., Buffalo

6-Mercaptopurine possesses definite antitumor activity in animals(3) and is widely used clinically in leukemia(4). Previous studies demonstrated the thiol methylation of thiouracil(5) and its iodo analogue *in vivo* (6). It appeared of interest to determine whether 6-mercaptopurine can also undergo thiol methylation *in vivo* and to quantitate the extent of this reaction. 6-Mercaptopurine can inhibit incorporation of C^{14}-precursors into nucleic acid fractions of tumor cells *in vivo*(7) and *in vitro*(8). To determine whether thiol-methylation interferes with or increases this action, the inhibitory effects of 6-methylmercaptopurine (6-MeMP) and 6-mercaptopurine (6-MP) on incorporation of formate $-C^{14}$ and glycine $-C^{14}$ into nucleic acid and protein fractions of ascites tumor cells were compared.

Methods. 6-MP-S^{35}, unlabeled 6-MeMP, and 6-MeMP-S^{35} used were prepared by methods of Elion *et al.*(9,10) and their purity determined chromatographically prior to use. *Identification and quantitation of 6-MeMP in urine.* Sprague-Dawley rats weighing 250-300 g were injected subcutaneously with 48.5 mg of either unlabeled 6-MP or 6-MP-S^{35} (0.62 mc/mM) dissolved in slightly alkaline aqueous solution. The animals were placed in individual metabolism cages and urine was collected for 24 hours under toluene. Urine from animals injected with unlabeled 6-MP was lyophilized to dryness and extracted with absolute ethanol. Aliquots of alcohol extracts were then chromatographed on Whatman No. 40 paper, alongside authentic 6-MeMP, by the descending method. 6-MeMP was located by its vivid yellow fluorescence under ultraviolet light (Mineralite lamp). R_f values of authentic and suspected 6-MeMP corresponded exactly when these fractions were eluted and rechromatographed in 3 different solvent systems. The solvent systems used were n-butanol:acetic acid:water (35.5:9.6:16.0); n-butanol saturated with 2N NH_4OH; and 70% ethanol. R_f values obtained were 0.84, 0.52 and 0.81 respectively. The yellow fluorescent areas were eluted from the paper with 0.1 N HCl and their absorption spectra were compared in a Beckman U.V. spectrophotometer. Spectra of authentic and sus-

* Supported by research grant from Nat. Inst. Health, P.H.S.

† Preliminary reports have been presented(1,2).