ADENASE ACTIVITY IN ANIMAL TISSUES

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Adenase activity was found in various rat organs by means of a sensitive radioactive method. An increase in the specific activity of the enzyme was shown in rapidly growing tissues (embryonic tissue, regenerating liver, some transplanted tumors). Changes in adenase activity in the liver and blood of animals with tumors compared with normal are not connected with the presence of an inhibitor or activator in the tumor tissue.

KEY WORDS: adenase; embryonic tissue; regeneration of the liver; transplanted tumors.

Information on the distribution and properties of adenase (adenine aminohydrolase, E.C. 3.5.4.2) in the tissues and fluids of higher animals is very limited and contradictory [1-3]. However, the enzymic deamination of adenine has been discovered in Escherichia coli [12], Azotobacter vinelandii [7, 10], and other microorganisms [6]. Extracts from the tissues of some lower animals (Anodonta, Crustacea, Insecta) contain adenine-deaminase but do not contain enzymes capable of deaminating adenosine or adenylic acid [4,8]. The question of whether adenase is or is not present in mammalian tissues thus remains open.

Adenase activity was determined in the present investigation in various organs of rats, in transplanted tumors, in regenerating rat liver, and in embryonic tissue.

EXPERIMENTAL METHOD

Adenase activity was determined in hepatoma-27, sarcomas M-1 and M-45, in the liver and blood of tumor-bearing animals (rats weighing 150-180 g), and in embryonic and regenerating tissues. During the investigation of embryonic tissue, a tissue homogenate of whole mouse embryo (line CBA) at the 13th day of development and the liver of an 18-day embryo were used for the determination of adenase activity. After decapitation of the animals, the test tissues were homogenized at 0-4° in the ratio of 1:3 in 0.05 M phosphate buffer, pH 7.0, until a homogeneous mince was obtained. The experimental samples (0.35 ml) contained: 0.05 ml adenine-8-C^{14} (specific radioactivity 45 mCi/mmole), 0.1 ml 0.05 M phosphate buffer, pH 7.0, 0.1 ml homogenate, and 0.2 mg unlabeled adenine in 0.1 ml. The samples were incubated for 60 min at 37°C. The reaction was stopped by the addition of 2 N perchloric acid (0.1 ml). After removal of the protein residue the supernatant was neutralized with 2 N KOH solution, cooled, and the resulting precipitate was removed on the centrifuge. The hypoxanthine formed was separated from adenine by ascending chromatography on paper, using a mixture of isopropanol–water–25% ammonia solution (85:15:1.5). The chromatogram was examined in the ultrachemiscope and the radioactivity of spots corresponding to the reference substances applied was determined in a liquid scintillation counter. The specific activity of the enzyme was expressed in nanomoles hypoxanthine formed per milligram protein of the homogenate [11]. The mean results of 2-4 experiments are given in Table 1.

EXPERIMENTAL RESULTS

After incubation for 1 h in the presence of adenine-8-C^{14} the label was incorporated into hypoxanthine, indicating that the tissues studied contained adenase. The highest adenase activity was observed in the liver, muscles, and spleen of the rats; relatively lower activity was present in the kidneys and brain. A very low level of activity was found in the heart and blood of the rats.

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TABLE 1. Adenase Activity in Various Organs and Tissues

Organ, tissue	Hypoxanthine formation (nmoles)	Protein (mg)	Specific activity (nmoles substrate/ mg protein/h)
Liver	4,4	5,6	0,78
Muscles	3,0	4,1	0,73
Spleen	1,6	3,6	0,44
Kidneys	1,1	4,4	0,24
Brain	0,65	3,1	0,21
Heart	0,60	4,5	0,13
Blood	2,0	16,8	0,11
Embryo at 13th day of develop.	2,4	2,5	0,96
Embryo at 18th day of develop., liver	1,6	3,0	0,53
Hepatoma-27	2,7	3,8	0,71
Liver of affected rat	2,2	4,4	0,5
Blood of affected rat	1,4	18,3	0,07
Sarcoma M-1	2,9	2,4	1,2
Liver of affectd rat	5,5	4,1	1,2
Blood of affected rat	0,95	17,4	0,05
Sarcoma M-45	3,05	2,6	1,17
Liver of affected rat	5,95	5,3	1,12
Blood of affected rat	0,85	26,4	0,032
Regenerating liver:			
16 h after operation	7,0	4,5	1,55
30 h after operation	5,0	5,2	0,96
40 h after operation	3,4	4,5	0,75

It will be clear from Table 1 that adenase was present in mouse embryonic tissues in the early stages of development, but the specific activity of the enzyme in the embryonic liver falls after birth. Adenase activity was found in transplanted hepatoma-27 and higher activity was detected in sarcomatous tissue. The specific adenase activity in the liver and blood of rats with transplanted hepatoma (Table 1) was one-third less than its normal activity, but this was evidently not the result of a direct inhibitory effect of the products of tumor metabolism on enzyme activity. In experiments in vitro the tumor homogenate or homogenate from the liver of an affected rat, if added to normal liver homogenate, did not lower the adenase activity. This applied equally to experiments carried out with the blood of an affected rat, which was added to the blood of a normal animal. The increased specific adenase activity in the sarcoma, and also in the liver of the tumor-bearing animals, was unconnected with the presence of activator in the tumor tissue but was probably accounted for by a disturbance of the system responsible for adenase synthesis.

The specific adenase activity in the regenerating and rapidly growing tissue 16 h after partial hepatectomy [9] was twice as high as in the liver of control animals. The adenase activity 30 days after the operation was lower and it reached the control level 40 h after the operation.

The experiments thus showed that adenase is present also in mammalian tissues and that the synthesis of this enzyme is increased in rapidly growing tissues (embryonic tissue, regenerating liver, and certain transplanted tumors of rats).

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