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Blood Plasma Adenosine Deaminase in Rats with Fibrosarcoma. (27907)

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Adenosine is deaminated in the animal organism and converted to inosine which by phosphorolysis produces hypoxanthine. When xanthine oxidase is present hypoxanthine is oxydized to xanthine and uric acid. The deamination of adenosine is catalyzed by the enzyme adenosine deaminase as follows:

$$\text{Adenosine} + H_2O \rightarrow \text{Inosine} + NH_3.$$

Adenosine deaminase is largely distributed in animal tissues chiefly in the intestine, muscles, kidneys and liver. In blood almost all the enzymatic activity is confined to the erythrocytes, blood plasma being a poor source of this enzyme. In pathological cases high values have been reported. Straub *et al.* (1) and more recently Letnansky and Seelich(2) have shown in cancer patients an elevated adenosine deaminase of blood plasma correlated to the degree of the disease. However, the increase in enzyme activity seems not to be specific as pointed out by Schwartz and Bodansky(3). Such other pathological conditions as miliary tuberculosis and hepatitis also presented high values for plasma adenosine deaminase.

The present study deals with the adenosine deaminase of the plasma of rats bearing a tumor induced experimentally. As far as we know there is a lack of references dealing with plasma adenosine deaminase in experimental neoplasia.

Material and methods. The tumor was originally induced in a male albino Wistar rat by one of us (L.A.A.) by subcutaneous injection of 1,2,5,6-dibenzanthracene(4) and was maintained monthly with subcutaneous implantation of 2-3 cu mm of tissue by means of a trocar. The tumor has developed well in all the animals during 2 years of regular transplants. Histological examination indicated it to be a fibrosarcoma.

Plasma adenosine deaminase was determined in male rats averaging 169 g in weight with tumor weighing 38 g (22% of total body weight). Fig. 1 shows the size of the tumor.

The rats were used 30 days after transference of the tumor. Normal rats of the same age and sex and maintained at the same balanced diet were used as controls (average body weight 164 g).

Blood was withdrawn by heart puncture after slight ether anesthesia and then collected in heparinized tubes. The plasma was immediately separated by centrifugation, hemolyzed plasma being discarded.

The enzyme determinations were performed by the methods of Koehler and Benz(5) and Kalckar(6). In the first method the ammonia released was estimated by Nessleriza-

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FIG. 1. Rat after 30 days of the implantation showing size of the tumor after dissection.

tion after 60 minutes of incubation of plasma with the substrate in a McIlwain buffer pH 6.8. The color was read in a Klett-Summerson photocolorimeter equipped with a 42 filter. One unit is as 1 μg ammonia nitrogen liberated by 1 ml of blood plasma in 60 minutes at 37°C.

All the samples were also tested by an adaptation to plasma of the method of Kalckar, which is based on the decrease of the extinction coefficient of adenosine at 265 mμ ($-\Delta E_{265}$) measured spectrophotometrically. The inosine produced by deamination gives no absorption at this wave length.

Plasma was incubated at 37° for 60 minutes with the substrate (4×10^{-5}M adenosine) deproteinized with 12% perchloric acid clarified with celite and centrifuged. The clear supernatant was properly diluted (40 times) and the absorption measured in the ultraviolet at 265 mμ. Another sample of plasma not incubated was deproteinized immediately and treated as for the incubated sample. The difference of the extinction coefficient between the readings of the incubated and non-incubated plasma indicates the adenosine deaminase activity of the sample (amount of plasma used was 0.2 ml).

One unit is equivalent to the concentration of the enzyme which deaminates 1 μg of adenosine in 60 minutes and 37°C at pH 7.2 per 1 ml of plasma.

Results. A comparison of the levels obtained in normal rats and in rats with fibrosarcoma is shown in Fig. 2. The left side of Fig. 2 represents values obtained by the colorimetric method of Koehler and Benz and the right side those performed with the spectrophotometric method. There was a good agreement between the methods, permitting the conclusion that adenosine deaminase increases in rats bearing transplantable tumors. The results are highly significant statistically. More detailed data are summarized in Table I.

It is interesting to recall that in 2 rats after 15 days of the transplant, with tumors

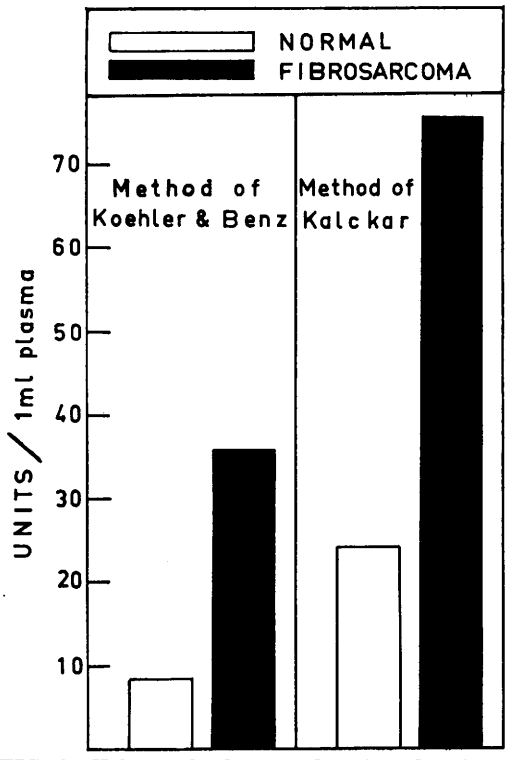

FIG. 2. Values of plasma adenosine deaminase for normal rats and for rats with fibrosarcoma.

TABLE I. Blood Plasma Adenosine Deaminase of Normal Rats and of Rats with Fibrosarcoma.*

	Method of Koehler & Benz(5)		Method of Kalckar(6)	
	Controls	Fibrosarcoma	Controls	Fibrosarcoma
No. of rats	8	9	8	9
Range in units per 1 ml	3.9 — 12.8	20.6 — 46.7	11 — 38	27 — 150
$\bar{x} \pm S_{\bar{x}}$† units/1 ml	7.9 ± 1.0	34.4 ± 3.1	23 ± 4	74 ± 12
Significance of the differences	$t = 8.030$	$P < .001$	$t = 4.048$	$P < .01$

* Values expressed in units/1 ml.
† $\bar{x}$ = arithmetic mean.

$S_{\bar{x}}$ = Standard deviation of mean = $\pm \sqrt{\frac{(x - \bar{x})^2}{n\,(n-1)}}$

representing 5% of body weight, the enzyme showed normal values. These observations indicate that the increase of adenosine deaminase in plasma is not a precocious occurrence.

Summary. The levels of plasma adenosine deaminase were studied in rats with a transplantable fibrosarcoma and compared with normal rats. High values were found in the plasma of fibrosarcoma rats, the enzyme being estimated by two methods based on the ammonia liberated and measured colorimetrically, and on the decrease of the adenosine determined spectrophotometrically.

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Effect of BCG Infection on Leukemia and Polyoma in Mice and Hamsters.* (27908)

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Phagocytic activity and antibody production appear to be involved in the host response to neoplastic processes. Tubercle bacilli of the BCG strain have a stimulating effect on both functions and could thus exert an influence on neoplasms. BCG action was studied in several forms of tumors and found to be useful against some(1,2,3,4). This paper describes experiments in which the effect of BCG was investigated on transplanted and spontaneous leukemia in mice and on polyoma in hamsters.

Transplanted leukemia. Methods. Two experiments were carried out with transplanted leukemia. In the first, the transplanted tissue happened to be very active, killing controls in 15 days, and consequently the differences between BCG-treated mice and controls were not too conspicuous and significant. Therefore, only the second experiment will be reported in detail.

The experimental arrangement was essen-

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