CATALASE ACTIVITY OF LIVER AND KIDNEY IN FROGS WITH SPONTANEOUS RENAL CARCINOMA*

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There is now abundant evidence that tumors of cold blooded vertebrates are essentially similar in morphology and behavior to the corresponding types of tumors in birds and mammals, including man (1, 2). Little, however, is known about their chemical activities. A promising approach to investigations in this field is the study of enzymatic properties of tumors. For these purposes the kidney carcinoma of the leopard frog (*Rana pipiens*) is excellent material; it occurs as a "spontaneous" tumor in frogs living under natural conditions (3); it is readily available and attains a size adequate for analysis; the frogs can be maintained over a wide range of environmental temperature, the effects of which upon enzymatic processes can thus be determined.

The present study deals with the enzyme catalase, whose properties have been thoroughly investigated in cancer of warm blooded animals, chiefly mice and rats (4). In mammals two main changes in catalase activity with respect to cancer have been found. First, the catalase activity of any kind of neoplastic tissue is uniformly low. Second, any kind of cancer no matter where located, if sufficiently large, has systemic effects on enzyme systems, the most sensitive indicator of which is diminution of catalase activity of the liver. The results of our experiments will be presented in the following order: (*a*) Comparison between catalase activity of normal frog kidneys and of kidney tumor; (*b*) catalase activity of normal or non-tumorous portions of kidneys from tumor-bearing frogs; (*c*) comparison between liver catalase activity of normal and of tumor-bearing frogs; (*d*) correlation between catalase activity of tumors and of livers from the tumor-bearing frogs; (*e*) effect of environmental temperature upon catalase activity; (*f*) effect of intracoelomic injection of homogenates of kidney tumor and of kidney upon liver catalase.

Material and Methods

Adult normal frogs of both sexes and frogs with palpable renal tumors were kept on thick pads of wet cotton in individual aquarium jars. They were housed in thermostatically controlled rooms, the temperature of which for most series was 18°C.; in one series two additional temperatures, 8°C. and 26.5°C., were used.

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The frogs were sacrificed by pithing, and tumor, liver, and kidney removed immedi ately. Sections from several "healthy appearing" portions of the tissues were placed in phosphate buffer at pH 6.8 at 3°C. for catalase assay, as previously described (5). Briefly stated, the tissues were separately homogenized, and their total nitrogen content determined by a micro Kjeldahl method. For assay of catalase activity we used the method of Jolles as modified by Sumner and Somers (6); the free iodine evolved was determined by titration with sodium thiosulfate.

All catalase activity values ("K") were expressed in terms of amounts of tissue equivalent to 0.03 mg. N. Slices closely adjacent to the portions removed for assay were fixed in formalin and prepared for microscopic study. Nearly all the tumors were bilateral and large, and measured 10 to 15 mm. in greatest diameter.

Since blood is known to have high catalase activity, in pilot experiments the pithed frogs were thoroughly perfused through the abdominal vein with amphibian Ringer's solution. Comparison of catalase values from such frogs with assays obtained from non-perfused frogs disclosed no significant differences.

RESULTS

Histologic examination of the areas selected from the tumors, kidneys, and livers showed neither necrotic areas nor any inflammatory reaction. The catalase assays were made therefore on "healthy" parts of tumors and on normal kidneys and livers.

Catalase Activity of Normal Kidneys and of Kidney Tumors

Representative values of catalase activity of both normal kidneys and of bilateral tumors are demonstrated by random experiments in Table I. All assays were made on 4 slices taken from different portions of the two kidneys or tumors. The values vary somewhat from frog to frog and in the 2 kidneys, and a similar variation occurs in bilateral tumors. There is, however, much less fluctuation in any one kidney or tumor. The activity values obtained from the kidneys of 24 normal frogs and from the tumors of an equal number of frogs are summarized in Fig. 1. For the normal kidneys the values are more scattered than are the values for tumors. The mean of catalase activity of normal kidneys is 0.138 (standard error of mean = 0.009). In sharp contrast, the average catalase activity of the kidney tumors is only 0.018 (standard error of mean = 0.003). In other words, catalase activity of tumors is diminished to 13 per cent of the normal. Even more striking is the fact that there is no overlapping between the lowest activity value of the normal kidneys and the highest value of the tumors.

These experiments make it evident that an amphibian cancer has essentially the same uniformly low value of catalase activity that is characteristic of cancers in warm blooded animals (4).

Catalase Activity of Normal Parts of Tumor-Bearing Kidneys

We now come to the question: Does the renal cancer affect catalase activity of remaining normal portions of the kidney? Information on this question was

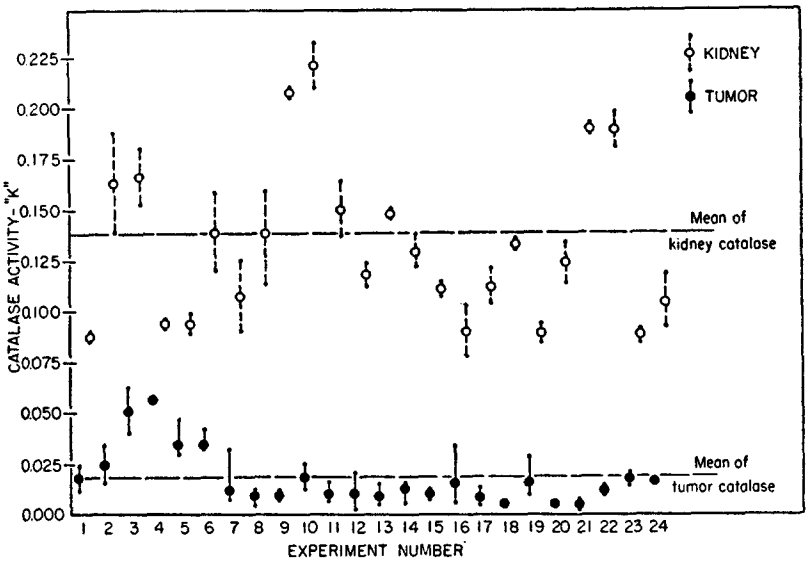

FIG. 1. Comparison between catalase activity of normal kidneys and of kidney tumors. The mean values for 24 experiments are given by the 2 horizontal lines. The vertical lines represent the spread obtained in individual experiments, *i.e.*, in the case of normal kidneys the activity from several slices from the right and left kidney; for tumor, the values of from 3 to 5 slices from different portions of the tumor.

TABLE I

Representative Assays of Catalase Activity in Normal Kidneys and in Bilateral Kidney Tumors

Experiment	Normal kidney		Experiment	Kidney tumor	
	Left	Right		Left	Right
A	0.132	0.179	D	0.016	0.038
	0.149	0.196		0.012	0.034
	0.141	0.191		0.014	0.035
	0.134	0.190		0.016	0.032
	Mean = 0.139	0.189		Mean = 0.015	0.035
B	0.089	0.084	E	0.042	0.056
	0.104	0.089		0.034	0.067
	0.104	0.091		0.037	0.067
	0.100	0.091		0.031	0.064
	Mean = 0.099	0.089		Mean = 0.036	0.064
C	0.097	0.125	F	0.045	0.031
	0.087	0.123		0.053	0.029
	0.091	0.128		0.050	0.032
	0.090	0.128		0.041	0.031
	Mean = 0.091	0.126		Mean = 0.047	0.031

obtained from 4 frogs in which only one kidney was neoplastic, and 8 other frogs having bilateral tumors with, however, considerable parts of one or both kidneys uninvolved. (In these cases care was taken to verify by microscopic examination the absence of cancerous infiltration.) The activity values are summarized in Fig. 2. Comparison with Fig. 1 shows that catalase activity of the "normal" renal tissue is now markedly diminished. The mean for normal portions of the tumor-bearing kidney is 0.047, a reduction to 34 per cent of values

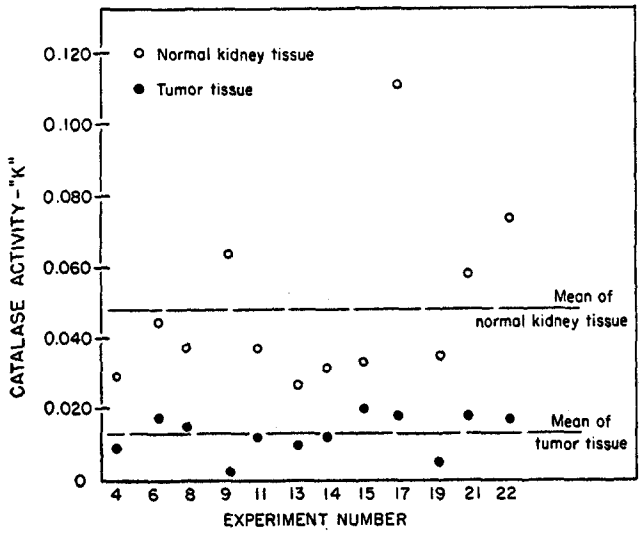

FIG. 2. Comparison between catalase activity of normal parts of tumor-bearing kidneys and of kidney tumors. Reference to Fig. 1 shows that activity values of tumor-bearing kidneys are diminished to about one-third the value obtained in normal frogs. (The numbers given to the experiments are the same as those in Fig. 1. Slight differences in activity values of tumor catalase from those in Fig. 1 are the result of examining different portions of the cancers.)

obtained in control frogs. Tumor catalase is about the same, 0.013, as in the preceding series.

These results demonstrate a pronounced effect of the tumor upon kidney catalase activity. The diminution of kidney catalase activity associated with the renal tumors of frogs is paralleled by assays made on a number of comparable renal cancers and non-involved portions of human kidneys obtained fresh at operation (7).

Catalase Activity of Normal Liver and of Liver from Frogs with Renal Tumors

As stated above, one of the most definite of the systemic effects of cancer upon enzyme systems of the mammalian host is diminution of the level of liver

catalase activity (4, 8). The following experiments demonstrate that a similar change takes place in renal carcinoma of frogs. In Figs. 3 are given the liver catalase values for 24 normal and for an equal number of tumor-bearing frogs. The mean for the livers from the controls is 1.096 (standard error of the mean = 0.083). For the livers of frogs with kidney tumors the mean is 0.549 (standard error of the mean = 0.044). That is, the activity level has been diminished to almost exactly one-half the normal.

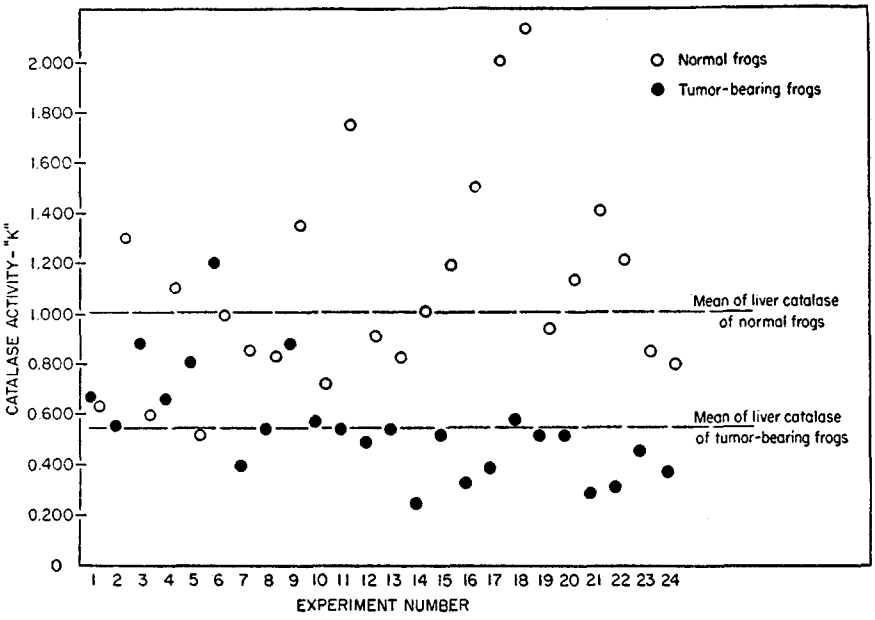

FIG. 3. Comparison between liver catalase activity of normal and of tumor-bearing frogs.

Correlation between Catalase Activity of Tumor and of Liver Catalase of the Tumor-Bearing Animals

As is shown in Fig. 1, catalase values of renal tumors are in most cases grouped closely around the mean, although there are some exceptions. Thus, in Experiment 20 the activity level is 0.005, whereas in Experiment 4 the level is 0.057—a 10-fold difference. Similar fluctuations are noted in the activity values of liver catalase from tumor-bearing frogs; in Fig. 3 the lowest level recorded is 0.241 (Experiment 14) and the highest 1.200 (Experiment 6). In tumors the variations are perhaps accounted for by size and degree of malignancy. The effect of a distant cancer on liver catalase is probably produced by one or more unknown substances elaborated and released by the cancer cells and carried by the blood stream to the liver (4, 8). It is of interest therefore to find out

whether the level of catalase activity of the cancer is correlated with the extent of diminution of liver catalase. That correlation between these two variables is positive is evident from Fig. 4, in which activity values of tumor catalase are plotted against liver catalase from the same frogs. When the activity of catalase is low in the cancer, diminution of liver catalase is marked, and the reverse also holds true. This correlation supports inferences drawn from experiments in warm blooded animals.

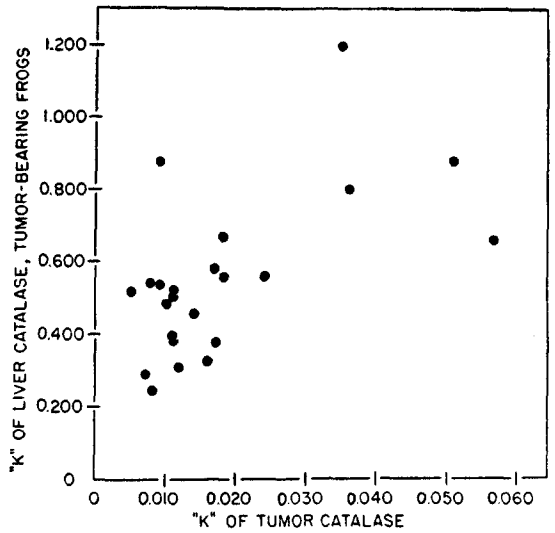

FIG. 4. Relation between catalase activity of tumors and of livers from tumor-bearing frogs. Inspection shows that the correlation is positive.

Effect of Temperature upon Catalase Activity of Tumors and of Livers of Normal and of Tumor-Bearing Frogs

The mechanism by which neoplasia leads to the great diminution of catalase activity in the cancerous tissue itself, and to the less marked depression of liver catalase of the tumor-bearing host, remains unknown. Because of the influence that temperature exerts upon chemical (or biological) processes, an attempt was made to alter catalase activity by changing the temperature of the environment and hence of the frog. In previous experiments with the frog renal carcinoma we had found that processes such as growth rate, in which many enzymatic reactions are involved, are profoundly affected by temperature (9, 10). On the contrary, in the present experiments, we were unable to observe any obvious effects of temperature upon catalase activity, either in the tumors or in the livers from tumor-bearing and normal frogs. Three groups of frogs with palpable, that is to say large, kidney tumors were maintained at constant temperatures

of 8, 18, and 26.5°C., respectively. The exposure in the majority ranged from 25 to 29 days; a few were kept for from 11 to 19 days. The results of catalase assays are summarized in Fig. 5. It will be seen that the activity values for the tumors are about the same in the 3 groups. But the levels of liver catalase of the controls and of the tumor-bearing frogs kept at 8 and at 26.5°C. are somewhat below that of the group maintained at 18°C., which may be regarded as the average "normal" temperature for leopard frogs. When these results are analyzed statistically (11) the differences are not significant, except perhaps between liver catalase of the 8 and 18° control groups.

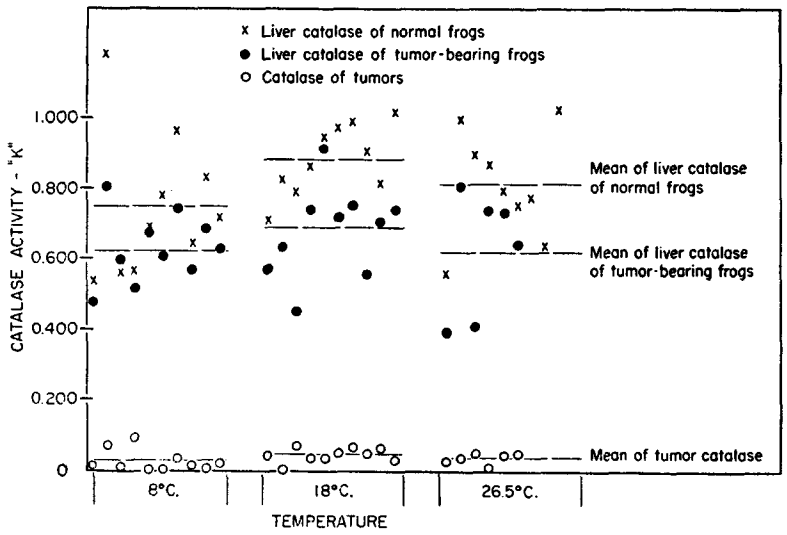

FIG. 5. Negative effect of temperature upon liver catalase activity of normal and of tumor-bearing frogs and upon catalase activity of renal tumors.

Although the results of these experiments are mainly negative, they do raise questions that need further exploration. In all 3 groups mean activity values for liver catalase in the control frogs are lower than the mean recorded in Fig. 1, and, conversely, activity values of the tumors are higher—0.038 as against 0.018. Are these differences fortuitous or real? Our temperature experiments were performed during the winter months, when frogs under natural conditions lead a dormant existence. In captivity they are usually kept for several months at low temperatures before delivery to the laboratory. On the other hand, many of the assays recorded in Fig. 1 were made on recently caught animals during the spring or summer. The general metabolism of frogs is known to vary with the seasons. Are there seasonal differences in catalase activity of tumors and of normal tissues? If so, what are the factors responsible? It would seem possible to design experiments that could perhaps yield valuable information about the complex enzyme mechanism.

Effect of Intracoelomic Injection of Homogenates of Kidney Tumor and of Kidney upon Liver Catalase

Homogenates were prepared as for catalase assay. Each of a series of 106 normal frogs received a single intracoelomic injection of 33 mg. of kidney tumor, using 22 different tumors for these experiments. An equal number of frogs were injected with the same amount of normal kidney. A number of animals were sacrificed after 12 and 24 hours, and therefore at daily intervals for 6 days. The results of the experiments are summarized in Fig. 6. A prompt (and statistically significant) drop of liver catalase occurs within 24 hours, and continues for 5 days; then there is a slight return toward the normal. No significant change of

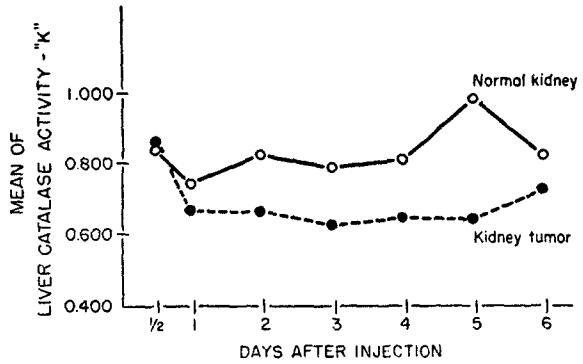

FIG. 6. Effect of intracoelomic injection of homogenates of kidney tumors and of normal kidneys upon liver catalase. During the first 4 days each point represents the mean of determinations on 22 frogs; after the 4th day on, from 8 to 10 frogs.

liver catalase follows injection of normal kidney. It may be inferred, therefore, that the cells of the frog tumor contain a liver catalase-depressing substance that is rapidly resorbed from the coelomic cavity. These results agree with experiments on warm blooded animals (12–14).

SUMMARY

The kidney carcinoma of the leopard frog has served for various studies on catalase activity, as a first step in gaining information on enzymatic properties of neoplasms in cold blooded animals.

It was found that the activity level of this tumor is reduced to approximately 13 per cent of that of the normal frog's kidney.

Systemic effects of the tumor on catalase activity of liver and kidney are evident. Liver catalase in tumor-bearing frogs is diminished to about 50 per cent of the normal, and kidney catalase to an even greater degree; *i.e.*, to about 34 per cent. A positive correlation exists between levels of catalase in tumor and in liver.

Frogs kept at 3 different temperatures, 8, 18, and 26.5°C., for upward of 29 days exhibited no significant change in activity levels either of tumor or of livers from normal or from tumor-bearing animals. It is suggested that a seasonal variation may occur in catalase activity of the frog. During the winter months catalase activity of the tumors was found to be higher than during the summer, whereas liver catalase was below the level of normal frogs examined during the summer.

Intracoelomic injections of homogenates of tumors promptly lead to diminution of liver catalase lasting for several days. Injection of normal kidney has no such effect.

These results with a spontaneous tumor of a cold blooded animal are in essential agreement with the many observations made with transplanted tumors of warm blooded animals. They lend support to the view that neoplasia is a ubiquitous biological process with similar characteristics in all species or classes of vertebrates.

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