Variations of the Content of Lysozyme in Normal Rats and in Rats Bearing Jensen Sarcoma Following Surgery *

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SUMMARY

Lysozyme activity increased in rats bearing the Jensen sarcoma transplantable tumor. The increase was maximal in the kidneys. Lysozyme activity in the lung and intestine was little affected by tumor growth. Removal of the tumor mass by surgery was followed by a rapid return to normal levels of renal lysozyme activity. A decrease also occurred if the tumor spontaneously regressed. Splenectomy produced a significant decrease of lysozyme activity in the kidneys of normal rats. The same operation in tumor-bearing rats lowered the lysozyme activity in the kidneys but to a lesser extent than in normal rats.

Many physico-chemical properties of the lysozyme of egg white1 are known. Its amino acid composition has been studied in many laboratories (9), and its mechanism of action as an enzyme has been partially elucidated (28); but little is known about the function of lysozymes in mammalian tissues. Other lysozymes have been described in the literature. Enzymes with the same substrate as egg white lysozyme have been described in plants (15), bacteria (24, 29), viruses (12), invertebrates (11), and vertebrates (8). In man, lysozyme has been found in many organs (6, 14, 25, 27) and in secretions from glands (10). Macrophages and polymorphonuclear cells are known to be rich in lysozyme (2, 4). In pathological conditions, lysozyme levels increase (7, 21, 22). Recently, Cappuccino (5) and co-workers found a constant, marked increase in activity of the lysozyme of the kidney and spleen in animals bearing transplantable tumors. Perri and Anigstein (19) have shown that there is a rapid increase of a basic protein rich in tryptophan in Sprague-Dawley rat kidneys after implantation of Jensen sarcoma tumor. This protein has been purified, obtained in crystalline form, and identified as a lysozyme (20).

To clarify the relationship between transplantable tumor growth and the elevation of lysozyme activity in the kidneys, various surgical treatments were performed on normal rats and those bearing the Jensen sarcoma tumor.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing 75–100 gm. were used. The rats were maintained on a Purina chow diet. Those animals which had been given implants by trocar of Jensen sarcoma in the axillary region were selected at random from the cages and were decapitated under ether at varying stages of tumor growth. Others, upon which surgery was performed after tumor implantation, were sacrificed 10 days postoperative. All the body weights were recorded; then the organs were rapidly excised, weighed, and homogenized as previously described (19). These homogenates were...
rehomogenized with equal volumes of 1 per cent acetic acid in 95 per cent ethanol to precipitate the proteins. After having been allowed to stand overnight at 4° C., the suspension was spun down, and the clear yellow supernatant fluid was used for the various chemical tests.

In these studies tryptophan was determined by the method of Udenfriend and Peterson (28), which gives a relatively good indication of lysozyme content (the higher the tryptophan values in the sample, the higher the lysozyme). Bound, inactive lysozyme (such as complexes with heparin, RNA, and DNA) still gives a tryptophan reaction even when no enzymatic activity can be detected. Therefore, the tryptophan determination, giving only a rough estimate of the actual active lysozyme present in the extract, facilitates the choice of the proper dilution of the sample for the enzymatic test, which is about 50 times more sensitive than the chemical method.

Lysozyme activity was determined enzymatically on acetone-dried cell suspensions of Micrococcus lysodeikticus with a modification of Litwack's method (13). The activity of the unknown was determined from the change in optical density per minute against a standard curve obtained with egg white lysozyme; the values are expressed in mg., equivalent to egg white lysozyme/gm of wet tissue.

RESULTS

A comparison of the distribution of lysozyme activity in the organs of normal and tumor-bearing rats is shown in Table 1. In normal rats, the lung had the highest lysozyme activity (mg/gm of wet tissue), and the kidney contained slightly less, followed in decreasing order by the spleen, intestine, and liver. On the other hand, the growth of Jensen sarcoma in the rat was accompanied by an average of a 67-fold increase of lysozyme activity in the kidney (Table 1); lysozyme in the spleen and liver increased much less. The increase of lysozyme activity in the spleen was accompanied by a fourfold enlargement of this organ in the tumor-bearing animals. Intestine and lung lysozyme activity did not seem to be affected by the growth of neoplastic tissue in the host. The lysozyme activity in the kidneys started to increase a few days after implantation of the tumor, reached a maximum after about 18 days, and declined steadily thereafter (Chart 1).

After surgical removal or spontaneous regression of tumor, the lysozyme activity of the kidney fell rapidly. At the time of surgical removal of the tumor, if the lysozyme content of the kidney was of the order of 28.4 mg/gm of wet tissue and 10 days later the other kidney at autopsy was found to contain 0.89 mg lysozyme/gm of wet tissue, it can be calculated that the average disappearance of lysozyme activity from the kidney after the removal of the tumor should be of the order of .115 mg/hour. Similar calculations can be made for the accumulation rate of the lysozyme in the kidney from the blood stream. Normal levels of lysozyme are of the order of 0.59 mg/gm of wet tissue. After

\[
\begin{array}{c|c|c|c}
\text{Organ} & \text{LYSOZYME (mg/gm of wet tissue)} \\
& \text{No. rats/} & \text{Normal} & \text{Tumor-bearing} \\
& \text{group} & \text{group} & \\
\hline
\text{Kidney} & 22 & 0.366 ± 0.0.886 & 28 & 24.8 ± 15.6 \\
\text{Spleen} & 6 & 0.165 ± 0.0.054 & 9 & 0.277 ± 0.145 \\
\text{Liver} & 6 & 0.019 ± 0.0.007 & 10 & 0.072 ± 0.0.006 \\
\text{Lung} & 6 & 0.689 ± 0.158 & 9 & 0.706 ± 0.138 \\
\text{Intestine} & 5 & 0.106 ± 0.0.017 & 4 & 0.106 ± 0.0.009 \\
\end{array}
\]
18 days of tumor growth, the average levels are 20 mg. This gives an average rate of accumulation of lysozyme of .046 mg/hour.

Various surgical treatments performed on several groups of animals are summarized in Table 2. Monolateral nephrectomy on normal rats was performed, and the lysozyme activity of the surgically removed kidney was compared with that of the remaining kidney removed at autopsy 10 days later. Although the weight of the remaining kidney increased 50 per cent, its lysozyme activity approximately doubled (Line A). The removal of one kidney in tumor-bearing animals was often accompanied by regression of the tumor. When the tumor did not regress, the remaining kidney contained a high concentration of lysozyme; but with regression (Line F), the lysozyme content diminished markedly. In the tumor-bearing animals when monolateral nephrectomy was performed, together with surgical removal of the tumor, lysozyme activity dropped to normal levels (Line E) 10 days after operation. A similarly striking drop in lysozyme activity in the kidneys was produced by surgical removal of the tumor only (Line D).

In normal animals splenectomy produced a significant decrease of lysozyme activity in the kidneys 10 days postoperative (Line C). In tumor-bearing animals, the same surgical treatment produced a slight decrease of the lysozyme activity, but the per cent of decrease is not comparable with that produced in normal animals. It should be noted that splenectomy, to affect the level of lysozyme in the kidneys, must be performed after the tumor is implanted. Animals splenectomized 10 days before tumor implantation did not differ from untreated tumor-bearing animals. Sham operation did not affect the content of renal lysozyme in either normal or tumor-bearing rats.

**DISCUSSION**

Our data confirmed the findings of Cappuccino (5) that the growth of transplanted tumors is accompanied by an elevation in content of lysozyme in the organs of the host, especially in the kidneys. An increase of lysozyme activity has been found in all tumor-host systems studied; the species and tumor-type determined the quantitative response. For instance, rats generally responded to tumor implantation with higher increases of lysozyme than did mice and hamsters. In Sprague-Dawley strain rats bearing Jensen sarcoma, lysozyme in the kidney increased gradually to reach peak values of 70–100 times the normal level, 18 days after tumor implantation.

The fact that surgical removal of the tumor or its spontaneous regression was followed by a decrease in lysozyme activity toward normal values seems to point to some direct relationship between the presence of a growing neoplastic mass and the lysozyme activity in the kidney. On the other hand, several considerations tend to exclude such a direct cause-effect relationship and support the opinion that the increase of lysozyme activity in the kidney is not a specific response to, but an indirect effect accompanying transplantable tumor growth. In our investigations the spontaneous or methylcholanthrene-induced tumors have not produced a significant increase of lysozyme activity. Experiments in progress indicate that specific (nontumor) challenges to the defense mechanism of the host by Calmette-Guerin bacillus (BCG) injection, bacterial antigens, etc., produce an in-

**TABLE 2**

<table>
<thead>
<tr>
<th>Rats</th>
<th>Treatment</th>
<th>Line no.</th>
<th>Lysozyme (mg/cm of wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Remove one kidney (5)</td>
<td>A</td>
<td>0.462 ± 0.157</td>
</tr>
<tr>
<td></td>
<td>Untreated (5)</td>
<td>B</td>
<td>0.866 ± 0.346</td>
</tr>
<tr>
<td></td>
<td>Untreated splenectomy (6)</td>
<td>C</td>
<td>0.598 ± 0.172</td>
</tr>
<tr>
<td>Tumor-bearing*</td>
<td>Remove tumor (5)</td>
<td>D</td>
<td>0.154 ± 0.062</td>
</tr>
<tr>
<td></td>
<td>Remove kidney + tumor (5)</td>
<td>E</td>
<td>0.551 ± 0.145</td>
</tr>
<tr>
<td></td>
<td>Regression of tumor after removal of one kidney (7)</td>
<td>F</td>
<td>0.890 ± 0.330</td>
</tr>
<tr>
<td></td>
<td>Untreated JRS at 18 days (16)</td>
<td>G</td>
<td>1.17 ± 0.341</td>
</tr>
<tr>
<td></td>
<td>Splenectomy + JRS at 18 days (11)</td>
<td>H</td>
<td>12.6 ± 12.0</td>
</tr>
</tbody>
</table>

* Jensen rat sarcoma.
Nos. in parentheses = no. of animals treated.
crease of lysozyme activity in the kidney, comparable to that produced by tumor implant. Furthermore, it is well known that lysozyme activity increases in pathological conditions other than tumor growth. An indirect response of the host to transplantable tumor growth appears to be the most plausible explanation of the increase of lysozyme activity, although it is difficult to pinpoint the biochemical mechanisms involved.

To date, the substrate upon which lysozymes act has been found only in the cell walls of microorganisms, so that, on the basis of data at hand, we must concede that the action of lysozyme is confined to bacterial cell walls. Lysozyme participates in synergistic association with other factors such as antibodies (1) and enzymes (3) in the elimination of bacteria from the organism.

Available experimental evidence indicates that lysozyme participates in the reaction mechanisms of the host in the activity of the reticuloendothelial systems (RES). For instance, it is well established that lysozyme increases in chronic infections (7) and that granulomatous tissue (22), macrophages (4), and polymorphonuclear cells (2) are rich in lysozyme. Moreover, the amount of lysozyme present in lung macrophages increases with BCG infection (18). We can therefore assume that, when RES is stimulated by a challenge, it will react with the proliferation of the types of cells involved in the defense mechanism. Among these cells macrophages and polymorphonuclear cells will increase in number, and their functional activity will be enhanced (16). It has been shown that transplantable tumors stimulate the RES system because of accompanying bacteria or viral-like agents (17). Stimulation by the tumor tissue itself cannot be excluded. Under conditions of RES stimulation, the production of lysozyme will increase with the activity of the RES, as indirect response to a challenge to the defense mechanisms of the host.

Lysozyme is produced in larger amounts by RES cells which have been challenged by tumor growth and will be released into the blood stream. Through its tubular activity the kidney absorbs and accumulates the enzyme. Lysozyme has not been found in the urine of normal animals (6, 21).

The regulating mechanisms involved in the accumulation and release of lysozyme from the kidney are not known. However, through the use of the biological system described, which is an effective tool for the study of mammalian lysozymes, we hope to clarify this problem. Our data focus attention on the important role which lysozyme plays in the mammalian systems. As a reflection of enhanced RES activity, increase of lysozyme during transplantable tumor growth represents a new parameter for exploration of the complex mechanisms of the tumor-host relationship.

REFERENCES


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