# CCXLIX. CATHEPSIN IN RATS WITH TRANSPLANTABLE CANCER.

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In a recent paper Waldschmidt-Leitz, McDonald and co-workers [1933] reported on the general distribution and state of activity of cathepsin in transplantable rat cancers of various stages of development. It was found that with the ageing of the tumour (increase of necrosis) the cathepsin content decreases, indicating that the cathepsin occurs chiefly in the parenchymal tissue rather than in the necrotic portions of the tumour. At the same time a number of cases were met in which the cathepsin contents of livers of cancerous animals were considerably altered from that of normal rat liver.

These findings suggested further studies of the general distribution of cathepsin in the organs of healthy, cancerous and cancer-resistant rats. Such studies disclose that in normal rats the cathepsin content of the liver (measured as full activity) is almost constant from animal to animal, while the muscular system of the same rats is practically free from cathepsin (Table II). In rats with transplantable cancer, however, there is a significant increase of the cathepsin concentration (as judged by the activity) in the liver, while coincidentally a considerable amount of this enzyme appears in the muscle tissue. Edlbacher and Merz [1927] have found that arginase shows a similar behaviour, occurring in large amounts in the muscle of animals with transplantable cancer but not in normal muscle. In this increased arginase activity these authors see a manifestation of a specific growth factor which, like the disturbed glycolysis (known through the investigations of Warburg [1926]), is believed to be characteristic of malignancy. In view of our experimental findings, the increased catheptic activity of liver and muscle must likewise be considered as a manifestation of the growth factor.

In this connection, it was of interest to study the cathepsin distribution in the organs of cancer-resistant rats as well (rats in which transplants failed to grow). It was found that these animals have a particularly high cathepsin content in liver and muscle, as compared with the normals (usual laboratory albino rats). Koehler [1934] has likewise reported that the kidney-phosphatase of cancer-resistant rats is considerably higher than that of the normal kidney. It seems to be characteristic of cancer-resistant organisms that they have a high and uniform enzymic metabolism.

A number of experiments were carried out to test further the original conclusion of Waldschmidt-Leitz, McDonald and co-workers [1933] that tumour-cathepsin occurs chiefly in the rapidly growing parenchymal tissue. As shown in Table III, analysis of histologically homogeneous portions of tumour material confirmed the previous findings that cathepsin occurs chiefly in the rapidly growing parenchymal tissue, to a less extent in the fibrous tissue and is practically

absent from the necrotic portions of the tumour. To a certain extent, arginase shows the opposite picture.

The physiological interpretation of these findings is opposed to the hypothesis set up several years ago by Waldschmidt-Leitz and Schaeffner [1930] on the activity of proteolysis in tumours. According to this hypothesis, the increased proteolytic activity of the tumour cell (which Waldschmidt-Leitz considered as proved) is due to an activation of the intracellular proteinase (cathepsin) by increasing amounts of reduced glutathione. The increased formation of reduced glutathione is ascribed to a preponderance of reduction processes in the tumour, due to a fall in oxidation-reduction potential and decrease of respiration. This relationship between proteolysis and respiration in the cell he believed to be responsible for the necrosis occurring in the tumour tissue.

The hypothesis of Waldschmidt-Leitz is certainly not correct in its present form; in fact, it has been questioned by other investigators [Krebs, 1931; Kleinmann and Werr, 1931] and recently also by Waldschmidt-Leitz, McDonald and co-workers [1933]. The results reported in the present paper not only fail to support this hypothesis, but also indicate that the action of cathepsin during the growth of the tumour may become exerted in the direction of synthesis. Nothing has been found which indicates that the autolytic cell disintegration in cancer tissue can be directly connected with the enzymic action of cathepsin. It is much more likely that arginase is connected with this autolysis.

## Experimental.

*Catheptic activity of liver, muscle and tumour.* Immediately after extirpation, the organs to be examined for catheptic activity were cut up with scissors, frozen in liquid nitrogen and then crushed with a hammer. This pulverised material was suspended (1 : 10) in 90 % glycerol. For activation, 2·0 ml. of neutral cysteine HCl solution (containing 20 mg. of cysteine, HCl) were added to 7·0 ml. of the well mixed enzyme tissue suspension and allowed to stand for 30 minutes at 30°, at $p_H$ 7. 5·0 ml. of 8 % gelatin (Kahlbaum "gold label") and 2·0 ml. of 1 *M* acetate buffer ($p_H$ 4) were then added, and the volume was made up to 25 ml. After incubation at 30° for 24 hours, the increase in amino-nitrogen was determined by the Van Slyke method.

Table I shows the changes in cathepsin concentration, during the ageing of the tumour, in muscle, liver and tumour tissue of rats into which cancer had

Table I. *Catheptic activity of liver, muscle and tumour of cancerous animals.*

| | Sarcoma | | | | | Liver | | Muscle | |
|---|---|---|---|---|---|---|---|---|---|
| Rat | P. | F. | N. | Initial activity | Full activity | Initial activity | Full activity | Initial activity | Full activity |
| 1 | 85 | 15 | — | 1·0 | 2·90 | — | — | — | — |
| 2 | 65 | 35 | — | 1·05 | 2·80 | 0·84 | 1·80 | 0·12 | 0·35 |
| 3 | 65 | 30 | 5 | 0·70 | 2·40 | — | — | — | — |
| 4 | 50 | 45 | 5 | 0·64 | 2·0 | 0·32 | 1·20 | 0·10 | 0·25 |
| 5 | 40 | 45 | 15 | 0·40 | 1·70 | 1·0 | 1·50 | 0·08 | 0·33 |
| 6 | 40 | 40 | 20 | 0·56 | 1·90 | 0·50 | 1·70 | — | — |
| 7 | 35 | 55 | 15 | 0·52 | 1·80 | 1·31 | 2·80 | 0·10 | 0·30 |
| 8 | 35 | 65 | — | 0·80 | 2·50 | 0·70 | 1·65 | 0·12 | 0·20 |
| 9 | 30 | 70 | — | 0·16 | 1·00 | 0·30 | 1·31 | — | — |
| 10 | 30 | 50 | 20 | 0·90 | 1·56 | 0·42 | 1·31 | 0·09 | 0·25 |
| 11 | 30 | 50 | 20 | 0·56 | 1·44 | 1·20 | 2·32 | 0·10 | 0·28 |
| 12 | 25 | 50 | 25 | 0·56 | 1·00 | 0·84 | 2·10 | — | — |
| 13 | 25 | 25 | 50 | 0·65 | 1·65 | 1·68 | 2·52 | 0·10 | 0·25 |
| 14 | 25 | 20 | 55 | 0·72 | 1·00 | 2·80 | 3·36 | 0·15 | 0·33 |
| 15 | 20 | 60 | 20 | 0·24 | 0·75 | 1·68 | 2·10 | 0·09 | 0·28 |

successfully been transplanted. The tumour used was a rapidly growing sarcoma, Philadelphia #1, the histological description of which may be found in the paper by Waldschmidt-Leitz, McDonald and co-workers [1933]. The results are given for 10 ml. of the reaction mixture (corresponding with 2·8 ml. of enzyme suspension) and indicate the increase of $NH_2$ expressed as ml. of 0·05 *N* KOH. P. indicates parenchymal tissue; F. fibrous tissue; N. necrotic portions of the tumours, stated in percentages.

Table II shows the catheptic activity of liver and muscle of healthy normal rats and of cancer-resistant rats (in which transplants failed to grow). The experimental details are identical with those described for Table I.

Table II. *Catheptic activity of liver and muscle of healthy and cancer-resistant rats.*

| Healthy rats (average body weight—90 g.) | | | | Cancer-resistant rats (average body weight—85 g.) | | | |
|---|---|---|---|---|---|---|---|
| Liver | | Muscle | | Liver | | Muscle | |
| Initial activity | Full activity | Initial activity | Full activity | Initial activity | Full activity | Initial activity | Full activity |
| 1·05 | 1·75 | 0·05 | 0·09 | 1·75 | 2·62 | 0·10 | 0·30 |
| 0·56 | 1·68 | 0·02 | 0·05 | 1·12 | 2·27 | 0·09 | 0·15 |
| 0·25 | 1·45 | 0·00 | 0·00 | 1·20 | 2·30 | 0·08 | 0·20 |
| 0·56 | 1·38 | 0·00 | 0·00 | 1·80 | 2·90 | 0·10 | 0·25 |
| 0·56 | 1·12 | 0·00 | 0·00 | 1·50 | 3·10 | 0·15 | 0·30 |
| 0·32 | 1·30 | 0·00 | 0·05 | 1·00 | 2·50 | 0·08 | 0·12 |
| 0·65 | 1·50 | 0·00 | 0·00 | 1·20 | 2·50 | 0·05 | 0·15 |

*Catheptic activity of histologically homogeneous parts of cancer tissue.* The experimental material in this series of experiments was obtained from a transplantable rat sarcoma, Philadelphia #1, and a transplantable rat carcinoma, Walker #256. The latter is a carcinoma tending to be necrotic in the early stages. The histological description of both tumours is given by Waldschmidt-Leitz, McDonald and co-workers [1933].

The experimental material of the desired histological structure was first prepared macroscopically and then microscopically by staining a section with haematoxylin-phloxin. The further procedure of the experiment was the same as that described under Table I.

Table III. *Catheptic activity of histologically homogeneous parts of cancer tissue.*

| Cancer | | Parenchymal tissue | | | Fibrous tissue | | | Necrotic tissue | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Strain | Age of tumour (weeks) | $p_H$ of tissue | Initial activity | Full activity | $p_H$ of tissue | Initial activity | Full activity | $p_H$ of tissue | Initial activity | Full activity |
| Phila. #1 | 6 | 6·3 | 0·5 | 1·4 | 6·4 | 0·28 | 0·60 | — | — | — |
| ,, | 4 | 6·0 | 0·7 | 1·8 | 6·5 | 0·20 | 0·90 | — | — | — |
| ,, | 3 | 6·0 | 1·0 | 2·5 | — | — | — | — | — | — |
| Walker #256 | 4 | 6·3 | 0·40 | 1·10 | — | — | — | 6·9 | 0·00 | 0·20 |
| ,, | 3 | 6·0 | 0·50 | 1·50 | — | — | — | 7·0 | 0·00 | 0·10 |
| ,, | 3 | 6·2 | 0·70 | 1·90 | — | — | — | 7·0 | 0·00 | 0·09 |
| Arginase activity* | | | | | | | | | | |
| Fe″ Cysteine | | | | 4·0 | | | | | | 17·6 |

* See Waldschmidt-Leitz, McDonald and co-workers [1933].

SUMMARY.

1. Untreated laboratory albino rats show a significant and uniform cathepsin content in liver, while the muscular system is free from this enzyme or has it in insignificant amount.

2. In rats into which cancers have successfully become transplanted there is a significant increase of the cathepsin concentration in the liver and coincidentally a large amount of cathepsin now appears in the muscle. The increase of cathepsin in liver and its appearance in muscle do not run parallel with the decline of the cathepsin concentration in the ageing cancer tissue (increase of necrosis), but are irregular and indicate an unsettled enzymic metabolism.

3. Rats into which efforts to transplant cancer have failed prove to have a particularly high concentration of cathepsin in liver and a significant concentration of cathepsin in muscle. This contrasts with the findings in normal rats in which no effort to transplant has been made.

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