# UTILIZATION OF PARENTERALLY INJECTED $C^{14}$-HYALURONIDASE BY ORGANS AND TISSUES

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After injection of hyaluronidase labeled with tyrosine-$C^{14}$ into sexually immature albino rats, radioactivity was found in various organs and tissues, and was particularly high in the thymus, pancreas, kidneys, and ovaries. In animals with a regenerating liver the level of radioactivity in the thymus under corresponding conditions was much lower. Differences were found in the distribution of $C^{14}$-hyaluronidase and tyrosine-$C^{14}$.

The writers have previously shown that $C^{14}$-hyaluronidase is utilized in sexually mature animals (albino rats) by organs and tissues mainly as larger fragments than amino acids [3], and that the distribution of this enzyme or of products of its incomplete degradation among organs and tissues was distinctive in character and differed significantly from that following administration of other labeled proteins ($C^{14}$-ribonuclease, $C^{14}$-albumin) [1].

The object of the present investigation was to study the ways and character of utilization of parenterally injected $C^{14}$-hyaluronidase in sexually immature animals and also to determine any special features of the utilization of this enzyme during tissue regeneration. This latter subject is particularly important in the light of data in the literature indicating utilization of hyaluronidase to stimulate regeneration of injured tissue, for this enzyme inhibits the development of coarse fibrous tissue at the site of injury.

## EXPERIMENTAL METHOD

Crystalline lyophilized bovine testicular hyaluronidase (Reanal, Hungary), with an enzymic activity of the order of 300 f.u./mg, not possessing proteolytic activity, was used in the experiments. Radioactive preparations of hyaluronidase were obtained by labeling with tyrosine-$C^{14}$ (specific activity 53 $\mu$Ci/mg) in a protein–amino acid system [1-3]. The specific activity of the labeled enzyme averaged 0.1 $\mu$Ci/mg. Enzyme activity of the labeled hyaluronidase [4] was the same as that of the original unlabeled preparation.

Male and female albino rats with a mean weight of 100 g were used in the experiments. The regenerating liver, after removal of 50-60% of liver tissue from the animals under ether anesthesia (from the anterior lobe), was used as the experimental model of tissue regeneration. The rats took part in the experiment 48 h after the operation, when high mitotic activity of the regenerating cells was observed.

The labeled enzyme was injected into the intact animals and into animals with a regenerating liver in a dose of 2000 pulses/min/g body weight. On the average the animals received 8-10 mg labeled hyaluronidase. The animals were decapitated after 3 h. Proteins were isolated from the organs and tissues taken for investigation, and the radioactivity of the proteins was determined with an end-window counter. In addition, tyrosine-$C^{14}$ together with 8-10 mg unlabeled hyaluronidase, also in a dose of 2000 pulses/min/g body weight, was injected intraperitoneally into a group of intact animals. The distribution of radioactivity was compared in the organs and tissues of animals receiving $C^{14}$-hyaluronidase and tyrosine-$C^{14}$ in the presence of unlabeled hyaluronidase.

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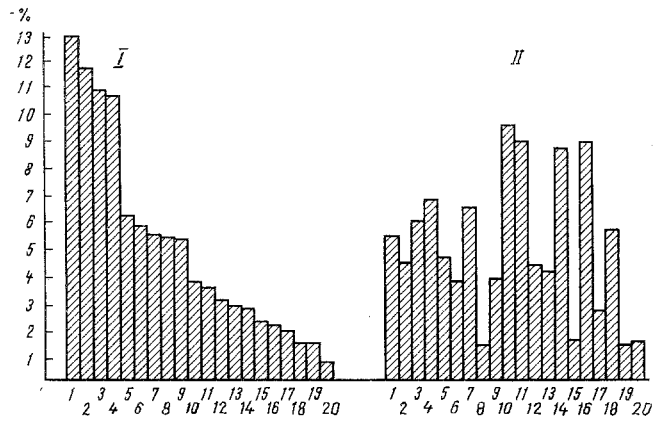


Fig. 1. Distribution of radioactivity in organs and tissues of rats receiving parenteral injections of hyaluronidase labeled with tyrosine-$C^{14}$ (I), and tyrosine-$C^{14}$ alone (II). 1) Thymus 2) ovaries; 3) kidneys; 4) pancreas; 5) spleen; 6) uterus; 7) nuclei of liver cells; 8) aorta; 9) adrenals; 10) serum globulin; 11) blood serum; 12) liver; 13) cytoplasm of liver cells; 14) serum albumin; 15) skeletal muscles; 16) small intestine; 17) lungs; 18) thyroid; 19) heart; 20) brain.

TABLE 1. Utilization of Hyaluronidase (pulses/min/mg protein), Labeled with Tyrosine-$C^{14}$, by Organs and Tissues of Albino Rats (M ± m)

| Organ | Control animals | Animals with regenerating liver | P |
|---|---|---|---|
| Brain | 7±2,80 | 8±3,34 | >0,5 |
| Thyroid | 16±2,72 | 18±2,08 | >0,5 |
| Thymus | 146±40,00 | 33±5,62 | <0,05 |
| Heart | 16±4,30 | 18±4,36 | >0,5 |
| Aorta | 61±17,76 | 49±9,46 | >0,5 |
| Lungs | 21±2,55 | 23±5,10 | >0,5 |
| Liver | 34±4,70 | 32±4,02 | >0,5 |
| Liver nuclei | 62±10,30 | 52±3,58 | >0,5 |
| Liver cytoplasm | 32±2,78 | 26±4,38 | >0,5 |
| Pancreas | 121±23,50 | 115±5,95 | >0,5 |
| Spleen | 70±25,30 | 62±26,59 | >0,5 |
| Kidneys | 124±42,37 | 116±9,63 | >0,5 |
| Adrenals | 60±17,50 | 46±10,50 | >0,5 |
| Testicles | 9±0,88 | 5±3,00 | >0,2 |
| Uterus | 66±3,67 | 56±4,04 | >0,1 |
| Ovaries | 132±35,44 | 122±1,41 | >0,5 |
| Small intestine | 24±8,00 | 29±10,59 | >0,5 |
| Skeletal muscle | 25±6,36 | 25±9,37 | |
| Blood serum | 40±4,38 | 45±4,53 | >0,5 |
| Serum albumins | 31±5,40 | 28±5,67 | >0,5 |
| Serum globulins | 42±6,04 | 34±3,40 | >0,2 |

## EXPERIMENTAL RESULTS

Following injection of $C^{14}$-hyaluronidase into intact animals radioactivity was found in all investigated organs and tissues (Table 1), but its level varied depending on the structure and function of the organ. High radioactivity was found in proteins of the thymus, pancreas, ovaries, and kidneys. A relatively high level of radioactivity also was observed in proteins from the aorta, spleen, adrenals, and uterus. A low level of radioactivity was found in the total proteins of the liver, but the radioactivity of the nuclear proteins was almost twice that of the cytoplasmic proteins. The level of radioactivity was identical in proteins of the albumin and globulin fractions of the blood serum. The lowest radioactivity was observed in brain and testicular proteins.

Comparison of the distribution of radioactivity in the organs and tissues of two groups of rats, one of which received $C^{14}$-hyaluronidase and the other tyrosine-$C^{14}$ (Fig. 1), indicates the qualitatively different

pattern of distribution of radioactivity in the animals of these two groups, indicating that the body of sexually immature animals, just like sexually mature animals, can utilize hyaluronidase in the form of larger fragments than amino acids.

In animals with a regenerating liver (Table 1), virtually no difference was observed in the utilization of hyaluronidase by the organs and tissues compared with the animals of the control group. The level of radioactivity was significantly lower (more than 4 times) only in the thymus proteins.

These results thus show that $C^{14}$-hyaluronidase, injected parenterally into sexually immature animals, is utilized intensively by the organs and tissues, and in particular, by a number of actively secreting organs (the thymus and pancreas). Definite tissue selectivity is observed: the level of radioactivity in proteins from the aortic wall, relatively inert as regards protein metabolism but rich in connective-tissue elements, was higher than in the proteins of some parenchymatous organs, whereas when utilization of $C^{14}$-serum albumin (labeled with tyrosine-$C^{14}$) by the organs and tissues was investigated, this phenomenon was not observed [1].

The results show that parenterally injected hyaluronidase is utilized by organs and tissues of intensively growing animals, just as of sexually mature animals, without being broken down to amino acids. A distinguishing feature of sexually immature animals in this respect is the high level of utilization of hyaluronidase by the thymus, because in sexually mature animals this enzyme was utilized by the thymus to a much lesser degree than by the pancreas [3]. This peculiarity, and also the fact that in animals with a regenerating liver the thymus is the only organ in which the utilization of hyaluronidase was changed by a statistically significant degree compared with the control, indicate the important role of the thymus in hyaluronidase metabolism.

## LITERATURE CITED


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