

# AUTORADIOGRAPHIC STUDY OF THE DISTRIBUTION OF PERTECHNETATE- $^{99m}\text{Tc}$ IN RAT TESTES

B. M. Mart'yanov and A. E. Marinbakh

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Information on the accumulation of pertechnetate- $^{99m}\text{Tc}$  in the gonads in sufficiently high concentrations [3-5] has formed the basis for development of a technique of scintigraphy of the testes [2]. No special experimental studies of the behavior of pertechnetate in the gonads, which would provide a basis not only for visualization of the organ, but also for elucidation of the principles of assessment of its functional state, could be found in the literature.

The aim of this investigation was accordingly to study the character of distribution and excretion of pertechnetate- $^{99m}\text{Tc}$  on the tissues of the testis.

## EXPERIMENTAL METHOD

Pertechnetate- $^{99m}\text{Tc}$  of Soviet manufacture was obtained from a  $^{99}\text{Mo}$  generator immediately before the investigation. The  $^{99m}\text{Tc}$  isotope has a short half-decay period (6 h) and it emits  $\gamma$  rays with an energy of 140 keV, which are easily detected and collimated.

Noninbred male albino rats weighing 140-160 g were used. Pertechnetate- $^{99m}\text{Tc}$  with a specific activity of 4 mCi/ml was injected intravenously in a dose of 0.5 ml into each animal.

The distribution of the preparation in the blood and testes was studied by direct radiometry, for which purpose two equal standards containing the preparation in a dose of 1% of the injected dose were prepared. The content of the radionuclide was determined by counting specimens of the testes and blood by means of a Gamma (Hungary) well-type scintillation counter under identical geometric conditions. The results were expressed as percentages of the injected dose of pertechnetate- $^{99m}\text{Tc}$ . The animals were killed 10 and 40 min and 1, 2, 3, and 5 h after injection of the preparation (three rats at each time).

An autoradiographic investigation was carried out at the same times, for which purpose histological sections 20  $\mu$  thick were cut from testes of the experimental animals, frozen in dry ice, mounted on a slide, placed in contact with ORWO-300 x-ray film (East Germany), and exposed at between -10 and -12°C for 24 h. The sections were then stained with hematoxylin-eosin and the x-ray film was developed. The autoradiographic and histological preparations of the testes were photographed and enlarged and the histological and autoradiographic pictures were compared.

The optical density of the autoradiographs was measured by means of an Mk-III-C microdensitometer (Joyce, England). By taking into account the linear relationship between exposure and density of the autoradiographic image, and also between the injected activity and the density of the autoradiograph [1, 6], identical experimental conditions (thickness of the sections, length of exposure) were strictly observed. The density of the background, the density above the seminiferous tubules of the testes, and the density above their large blood vessels were measured on the densitograms and expressed in conventional units.

## EXPERIMENTAL RESULTS

A graph of elimination of pertechnetate- $^{99m}\text{Tc}$  from the blood (Fig. 1, I) shows that clearance follows an exponential rule. The top part of the curve (before 3 h, half-elimination period 30 min) reflects discovery of the preparation in the blood and a rapid fall in its concentration as a result of elimination from the blood stream and its transfer into the extracellular space. Starting with 3 h, when the curve becomes almost hori-

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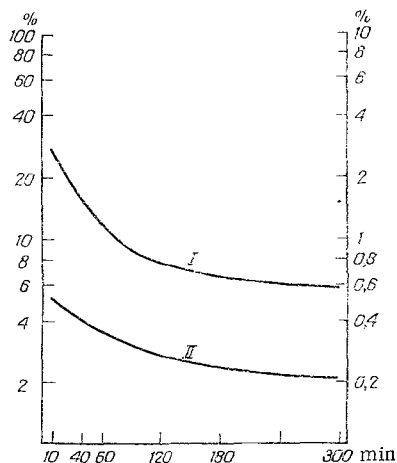


Fig. 1

Fig. 2

Fig. 1. Distribution of pertechnetate- $^{99m}\text{Tc}$  in peripheral blood (I) and in testis (II) of rat studied by direct radiometry. Abscissa, time after injection of pertechnetate (in min); ordinate: on left, % accumulation of pertechnetate in blood, on right, % accumulation of pertechnetate in rat testis.

Fig. 2. Autoradiograph of rat testis 1 h after injection of pertechnetate- $^{99m}\text{Tc}$ . A) Histological section through testis. Hematoxylin-eosin, 7 $\times$ ; B) autoradiograph from this section.

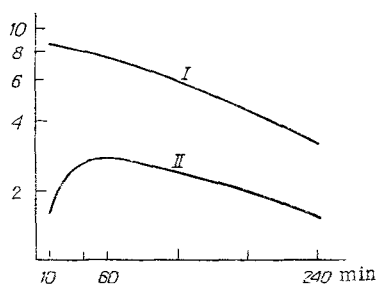


Fig. 3. Changes in density of autoradiographs above various structures of rat testes, studied by microdensitometry. Abscissa, time after injection of pertechnetate (in min), ordinate, density of autoradiographs (in conventional units). I) Density of grains of silver above parenchyma of testis. II) Density of the autoradiograph on the parenchyma egg.

zontal in direction, dynamic equilibrium takes place between the concentrations of the preparation in the blood and tissues. Curve II in Fig. 1, reflecting the concentration of isotope in the testes, is also exponential in shape, almost repeating the one described above. The reason was that most of the pertechnetate- $^{99m}\text{Tc}$  which entered the testes was located in their vascular system. The more sloping character of curve II during the first 3 h (half-elimination period about 1 h) is evidence of slower elimination of the compound from the organ, evidently because of its retention in the parenchyma. Accumulation of pertechnetate- $^{99m}\text{Tc}$  in the testis reached a maximum during the first 10 min after injection, at the level of 0.5% of the injected dose per gram of tissue.

Autoradiographs of the testes (Fig. 2) show that against the background of a more or less uniform increase in density over the whole surface of the section through the testis, a considerable and fairly sharply outlined increase in density was observed in the form of dots and small stains at the site of projection of the

blood vessels, and in the form of lines above the capsule of the organ, repeating their dimensions and arrangement. Whereas 10 min after injection of the preparation the picture above the large vessels had the appearance of confluent stains, after 1 and 3 h small translucencies appeared in the center of these stains, the density of which weakened at each successive time of investigation, and the stains themselves assumed the appearance of steering wheels.

Measurement of the optical density of the autoradiographs by means of a microdensitometer showed that whereas density above blood vessels of the testes became maximal 10 min after injection of the pertechnetate- $^{99m}\text{Tc}$ , and subsequently fell gradually, optical density above the parenchyma of the organ reached a maximum after 1 h (Fig. 3).

Comparison of curve II in Fig. 1, reflecting the concentration of the preparation in the isolated testis as a whole, and curves I and II in Fig. 3, showing indirectly the concentration of radionuclide separately in the vascular system of the testis and in its parenchyma, shows that curve II in Fig. 1 and curve I in Fig. 3 repeat one another to a large extent, which is not the case with curve II in Fig. 1. Analysis of these results confirms once again that the integral index of radioactivity in the testis obtained by scintigraphy is determined primarily by the concentration of pertechnetate- $^{99m}\text{Tc}$  in the blood vessels of the organ studied.

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#### INHIBITORY EFFECT OF LEU-ENKEPHALIN ON GASTRIC SECRETION IN DOGS

V. G. Smagin, V. A. Vinogradov,  
V. N. Shatalov, V. P. Polonskii,  
S. A. Bulgakov, V. V. Anokhina,  
and Zh. D. Beshpalova

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The attention of research workers in recent years has been drawn to the regulatory role of a new class of biologically active substances; the neuropeptides. The importance of leu- and met-enkephalins, morphine-like pentapeptides of endogenous origin, has been particularly widely discussed [4, 5, 8].

The object of this investigation was to study the action of leu-enkephalin (EK) on gastric secretion and to elucidate some of its mechanisms. The EK used was synthesized in the laboratory of peptide synthesis (Head, M. I. Titov), All-Union Clinical Scientific Center, Academy of Medical Sciences of the USSR.

#### EXPERIMENTAL METHOD

Several experimental models were used, namely: two groups of mongrel dogs (four dogs in each group) with different types of fistulas; the isolated gastric mucosa of the frog *Rana temporaria*; the effect of EK on the blood gastrin concentration in dogs also was studied.

During a preliminary operation on the dogs of group 1 a large Basov's fistula was formed; denervated gastric pouches were created by Heidenhain's method in the animals of group 2. Before the experiments, the

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