THE IMPORTANCE OF SPECIFIC SENSITIZATION OF THE BODY TO THE RESORPTION OF PROTEIN FROM THE TISSUES

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The subject of tissue resorption of substances of different molecular weight by different tissues is of considerable interest, both in connection with the question of tissue permeability and in relation to the effect of the physical properties of different substances on their ability to undergo resorption by particular tissues. This problem has been discussed in the literature, but some of its aspects, of definite pathophysiological importance, have not been studied in any great detail. In particular, the study of the effect of specific sensitization of the body on the resorption of protein from different tissues is very important for an understanding of the pathophysiology of the processes of tissue resorption.

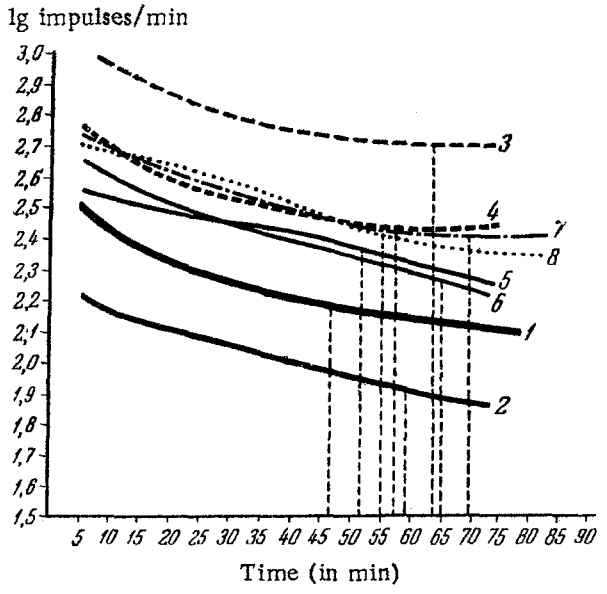

Fig. 1. Resorption of labeled protein from the tonsils and peritonsillar region in normal dogs. The curves are drawn on a semilogarithmic system of coordinates.——— Resorption from the tonsils; – – – – resorption from the peritonsillar region. 1, 2, 3, 4) In the dog Chernushka; 5, 6, 7, 8) in the dog Yellow Paws. The vertical broken lines denote the time for the initial activity to be halved.

According to information in the literature, increased resorption of colloidal particles is observed during sensitization. This conclusion was reached, for example, by S. K. Yurchenko [3] in respect of the mucous membrane

of the upper respiratory tract. In the experiments of Strömme [7, 8] and Naumann [6], the permeability of the mucous membrane of the nose and the antrum of Highmore to a suspension of pollen was considerably increased after preliminary sensitization of the animals to the same pollen. In work by V. I. Oivin [2], on the other hand, the resorption of fluorescein from the subcutaneous areolar tissue in rabbits sensitized to horse serum remained the same as in intact (nonsensitized) animals.

These differences in the results may be due to several circumstances. In the first place they may be related to the specificity of sensitization. Secondly, the importance of difference in the technique of investigation cannot be excluded. The physiological properties of the tissues studied by different workers may also have some bearing on the problem.

We were interested in the effect of the specific sensitization of the organism on the resorption of substances of such a molecular size that resorption was possible only through the lympathic spaces. We therefore studied the resorption of serum proteins from the tissues of the tonsils and the peritonsillar region, taking into consideration the great importance of the role of resorption from these tissues in the development of tonsillar diseases and their complications.

EXPERIMENTAL METHOD

Experiments were conducted on rabbits and dogs. Thirty animals were used—20 rabbits and 10 dogs. In dogs we studied the resorption of labeled protein from the tonsils and peritonsillar region to compare its rate from these tissues. In 6 rabbits a comparative study was made of the character of resorption of labeled protein from the retrotonsillar region on both sides of the body. In the remaining rabbits we studied the role of sensitization in the resorption of labeled proteins from the retrotonsillar region.

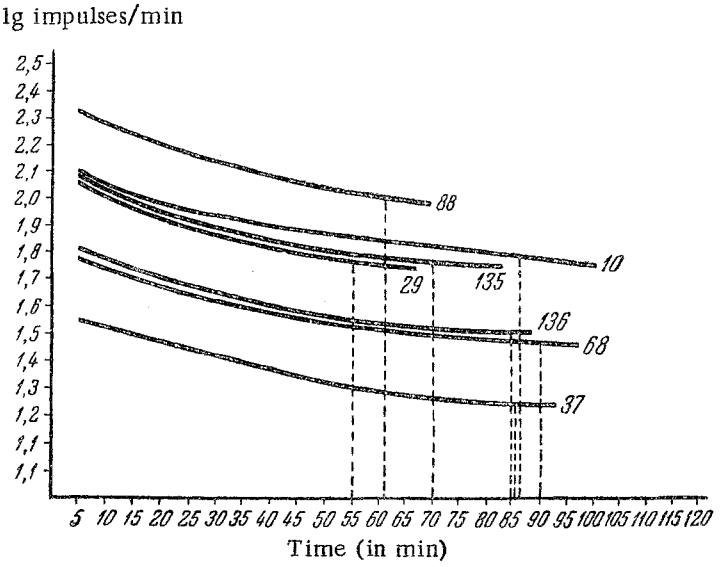

Fig. 2. Resorption of labeled protein from the peritonsillar region in normal rabbits. Semilogarithmic system of coordinates. The figures on the curves denote the number of experimental rabbits. Remaining legend as in Fig. 1.

Serum proteins were labeled with radioactive iodine (I^{131}). The iodine was incorporated in horse serum by the method of Liebster and co-workers [5]. We were able to obtain a very high percentage of incorporation of radioactive iodine in the proteins (up to 50%), which ensured that the experiments could be successfully performed.

Briefly, the method was as follows. Horse serum was purified by dialysis against 2 liters of physiological saline at 4-5° for 24 hours. The following mixture was then prepared in a flask (the amounts are computed for 10 ml of 7-10% protein solution: 3 ml 0.02 M NaI solution, 1 mC I^{131}, 3 ml 0.02 M $NaNO_2$ solution and 5 ml 0. 1 M HCl solution. The mixture was agitated periodically for 1 hour, after which 3 ml of 0.1 N NaOH solution, the protein solution, and 10 ml of a 0.02 M solution of phosphate buffer (pH 7.5) were added.

After a few minutes of energetic agitation, 0.2 ml of 30% H_2O_2 solution was added. The mixture was kept for 24 hours at room temperature, after which it was dialyzed against 2 liters of physiological saline at 4-5° for 24 hours, the physiological saline being changed 3 or 4 times during this period, until all the iodine which was not bound to protein had been removed.

After activity tests the solution was used for the experiments. The mean activity of 0.1 ml of solution on the target was 1500-3000 impulses per minute.

Labled serum was injected into the tonsils or retrotonsillar region of the animals in a dose of 0.1-0.15 ml. In dogs, this volume of serum could be injected directly into the tonsils. In rabbits, on the other hand, in which the tonsils are poorly developed, the serum was injected into the peritonsillar region.

The activity of the injected iodine was determined by means of two B-2 apparatuses and two T-25 counters for use in vivo, placed by the outer surface of the neck of the immobilized animals on both sides simultaneously in the projection area of the tonsils.

The impulses were recorded continuously for a period of several hours. The time taken for the initial iodine activity to be halved was determined, and the appropriate semilogarithmic curve was drawn up. The results of the rate of resorption were treated by Kety's method [4] as modified by I. I. Islamov [1], and from them the resorption constant was deduced in accordance with the formula: $\lambda = \frac{0.693}{t^{1}/_{2}}$, where λ is the resorption constant and $t^{1}/_{2}$ the time taken for the initial activity to be halved.

EXPERIMENTAL RESULTS

These experiments showed that the resorption of labeled protein from the tonsils and peritonsillar region is characterized by a similar curve on both sides of a given animal, and the differences between individual animals are purely quantitative in character.

In all cases, soon after the injection of labeled protein resorption took place comparatively quickly, after which the rate gradually slowed until the resorption curve became almost parallel to the axis of abscissas. It is difficult to explain this slowing of resorption. It has been suggested that it may be due to the combination of labeled protein with the tissues. The rate of resorption was independent of the initial activity of the serum injected into the animal, provided, of course, that this activity was not too low (not less than 50 impulses per minutes), when the experiment could not be performed, and not too high (not more than 1000-1500 impulses per minute).

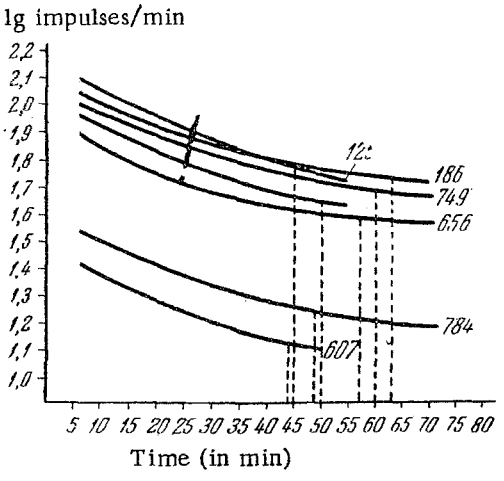

Fig. 3. Resorption of labeled protein from the peritonsillar region in specifically sensitized rabbits. Legend as in Figs. 1 and 2.

Typical curves of resorption of labeled protein in dogs and rabbits are shown in Figs. 1 and 2. It will be clear from Fig. 1 that the character and time of resorption in dogs from the tonsils and the peritonsillar region do not differ significantly. On the other hand, the character of resorption of protein from the peritonsillar region in dogs and rabbits was also found to be identical. The results of the investigation of resorption from the peritonsillar region could thus be regarded as analogous to the results of resorption from the tonsils. In order to compare resorption during sensitization and in normal conditions, some rabbits were sensitized with horse serum by repeated intraperitoneal injections of serum at weekly intervals. Three weeks after the last sensitizing injection, the character of protein resorption from the serum used to produce sensitization was determined in these animals. The resorption of labeled protein from the peritonsillar region was estimated in the same number of control animals without previous sensitization. All the numerical results were treated by statistical methods.

We found that sensitization considerably increased the rate of resorption of labeled horse serum protein. Whereas in intact animals the period $t^{1}/_{2}$ was 71.7 ± 5.2 minutes and the resorption constant $\lambda - 0.0096$, in the sensitized ani-

mals the time taken for resorption of half the labeled protein was shortened on the average by 24 minutes: $t^1/_2$ amounted to 47.7 ± 2.7 minutes and $\lambda - 0.0147$ respectively. These results were reflected in the curves of half-resorption on a semilogarithmic system of coordinates by a steeper fall and a shortening of their course (Fig. 3). The difference between the resorption values was significant ($P < 0.01$ and > 0.001).

Our results thus showed clearly that sensitization speeds the resorption of the specific sensitizing agent—horse serum protein—from the peritonsillar tissue.

SUMMARY

Resorption of proteins from the peritonsillar area was studied in experiments on rabbits both in normal conditions and in the state of specific sensitization. The process of resorption was studied by labeling the horse serum proteins with radioiodine and estimating its activity directly in the living organism. As shown experimentally, specific sensitization of rabbits considerably accelerates the resorption of horse serum proteins from the tonsillar area.

LITERATURE CITED

1. I. I. Islamov, The Study of the Processes of Resorption from Normal and Inflamed Skin by Means of Labeled Atoms. Candidate dissertation [in Russian], Dushanbe (1954).
2. V. I. Oivin, Byull. Éksper. Biol. (1950), No. 10, p. 255.
3. S. K. Yurchenko, In: Collected Papers of the Leningrad Research Institute of Diseases of the Ear, Nose, and Throat and Speed [in Russian] (1948), Vol. 9, p. 137.
4. S. Kety, Am. Heart J. (1949), Vol. 38, p. 321.
5. J. Liebster, A. Babický, J. Kozel, et al., Folia biol. (Praha) (1957), Vol. 3, p. 183.
6. H. H. Naumann, Arch. Ohr.-Nas u. Kehik.-Heilk. (1958), Bd. 173, S. 127.
7. O. Strömme, Studies on the Histopathology of Polyposis, Especially Concerning the Absorption of Pollen Through the Nasal Mucosa, Oslo (1952).
8. Idem, Acta allerg. (Kbh.) (1955), Vol. 8, p. 251.
