

A COMPARATIVE STUDY OF THE FIXATION
OF ANTIBIOTICS OF THE TETRACYCLINE GROUP
BY HOMOGENATES OF ORGANS

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The least studied aspect of the distribution of antibiotics in the body is their fixation by the organs. The volume of experimental data in this field seems not in accordance with the importance of the subject. This is to some extent due to technical difficulties.

Our aim was to obtain comparative quantitative data on the fixation of chlortetracycline, tetracycline, and oxytetracycline by organ homogenates.

In the literature there are only isolated communications on this problem. In one of these it is stated that a homogenate of guinea-pig liver fixes tetracycline to a greater degree than homogenates of other organs [4]; there are similar reports concerning oxytetracycline, but in experiments with fragments of organs rather than with homogenates [3]. In both these researches, the workers confined themselves to a comparison of the degree of fixation of the antibiotic by various organs, without examining the quantitative aspect of the subject.

EXPERIMENTAL METHODS

Experiments were carried out on the organs of white rats. In order to secure exsanguination of the animals, the axillary artery and vein were divided under light ether anesthesia; the liver, kidneys, spleen, heart, lungs, and brain were then extracted from the animals and equal samples by weight of these organs were homogenized with a measured quantity of quartz sand. The homogenate was transferred into a centrifuge tube, where to it was added a solution of the antibiotic in phosphate buffer (pH = 7.4) in a dose calculated as 0.3 ml of a concentration of 20 γ /ml per 0.7 g of tissue. Thus 1 g of the mixture (antibiotic solution and organ homogenate) contained 6 γ of chlortetracycline, tetracycline, or oxytetracycline. The proportion of 0.3 ml to 0.7 g was adopted because in preliminary experiments it was found that the degree of fixation fell as the volume of antibiotic solution increased in relation to the weight of homogenate. A smaller volume of antibiotic solution was not used, for this made the determination of the activity of the mince much more difficult, on account of its viscosity. The contents of the centrifuge tube were agitated on a shaker (280 agitations per minute) for 30 minutes and were then centrifuged at 3000 rpm, also for 30 minutes. Subsequently the activity of the supernatant fluid was determined on plates seeded with the spore-bearing strain L_2 . The standard was also dissolved in phosphate buffer. Control experiments showed that none of the homogenates of the organs used in the experiment formed zones of inhibition of growth on the plates. From 5 to 14 experiments were performed with each organ and each antibiotic.

TABLE

Fall in the Activity of the Tetracyclines in the Presence of Homogenates of the Organs of Rats (Estimated Activity 6 γ /ml)*

Organs	Antibiotics								
	chlortetracycline			tetracycline			oxytetracycline		
	activity (in γ /ml)*	<i>m</i>	fixation (in %)	activity in γ /ml*	<i>m</i>	fixa- tion (in %)	activity in γ /ml*	<i>m</i>	fixation (in %)
Liver	2.3	0.18	61.6	2.9	0.19	51.6	3.8	0.28	38.3
Kidneys	2.5	0.12	58.3	3.1	0.42	48.3	4.5	0.44	25.0
Spleen	4.6	0.52	23.3	—	—	**	—	—	**
Lungs	3.9	0.37	35.0	4.4	0.55	26.6	—	—	**
Heart	5.4	0.64	10.0	5.1	0.83	15.0	—	—	**
Brain	2.3	0.19	61.6	3.8	0.21	38.3	5.6	0.56	6.6

* Results of the determination of activity were treated statistically; possible error in the value of $M \pm m$ does not exceed 0.1% (by Pomorski's method).

** No fixation discovered.

EXPERIMENTAL RESULTS

It was found that the organ homogenates fixed different quantities of the tetracyclines; here, as in the serum [2], chlortetracycline was fixed to the greatest degree, closely followed by tetracycline, and oxytetracycline was fixed to the smallest degree (see Table).

By the degree of fixation of the tetracyclines, the organ homogenates could be divided into 2 groups: those fixing considerable amounts of tetracyclines — the liver, kidneys, and brain, and those fixing appreciably smaller amounts — the lungs, heart, and spleen. In all cases the liver fixed the largest amount of tetracyclines (from 61.6% in the case of chlortetracycline to 38.3% in that of oxytetracycline).

The results obtained with homogenate of the brain were unexpected: It fixed chlortetracycline to the same degree as homogenate of liver and tetracycline to a less but still considerable degree (38.3%). Fixation of oxytetracycline by homogenate of brain was slight (6.6%), but it must be remembered that homogenates of the lungs, heart, and spleen did not in general fix this antibiotic in our experiments.

In earlier experiments in which rats were given tetracyclines by mouth, and their organs were subsequently homogenized and repeatedly rinsed, we found no antibiotics in the brain tissue [1]. The results of the present research suggest that this is explained by the low permeability of the blood-brain barrier to the tetracyclines, since when these antibiotics are brought into direct contact with brain tissue they undergo absorption by it in large quantities.

The problem of the reversibility of the bond between the tetracyclines and the brain homogenate remained unsolved. In previous work we were unable to explain this for, as has been stated, these antibiotics were not found in the brain tissue. In the present work, by repeated washing of chlortetracycline from the brain homogenate roughly the same amount of the antibiotic could be extracted from it as from liver homogenate, used as a control. The bond between brain, like the other organs, and the tetracyclines is thus reversible.

From these findings and the results of our previous work [1, 2], we can begin to understand some of the factors governing the distribution of the tetracyclines in the body. The low fixation of oxytetracycline by the serum and organs may account for its high concentration in the blood when injected intravenously. Chlortetracycline and tetracycline do not give such high blood levels, but because of the considerable deposition of the antibiotic in the organs and serum, it remains in the body far longer.

From the point of view of rational chemotherapy it may be supposed from our findings that chlortetracycline and tetracycline will be more effective in infectious diseases localized in the organs, and especially in the liver and kidneys. In generalized septicemic conditions oxytetracycline may be of essential importance. In these cases it may be rational to combine it with chlortetracycline or tetracycline.

SUMMARY

The authors established the quantitative fixation of chlortetracycline, tetracycline, and oxytetracycline by homogenates of the liver, kidneys, spleen, lungs, heart, and brain of white rats. Fixation by the organ homogenates was greatest by chlortetracycline, followed by tetracycline and oxytetracycline in that order. Homogenates of the liver, kidneys, and brain fixed the tetracyclines in considerable quantities, those of the lungs, heart and spleen — to a noticeably lesser degree.

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*Original Russian pagination. See C. B. translation.