INVESTIGATION OF THE THYMUS AS A SOURCE OF A HUMORAL FACTOR STIMULATING ANTIBODY FORMATION

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By fractionation of calf thymus extract No. 1A, prepared by the method of Goldstein et al., on a column with Sephadex G-25 a fraction stimulating antibody formation in newborn animals was obtained. Tests of the activity of analogous fractions of extracts of calf tonsils, spleen, lymph glands, and liver suggested that the thymus is the sole source of a specific humoral factor stimulating antibody formation which may accumulate in peripheral lymphoid structures. The low activity of tonsillar extracts does not confirm the hypothetical role of the tonsil as the central organ of immunity, corresponding to the bursa of Fabricius.

KEY WORDS: thymus; humoral factors; antibody formation.

The thymus has been shown to produce humoral factors that induce immunocompetence [3, 7, 12, 17-19]. Data on the effect of thymus extracts on antibody synthesis are few in number and contradictory in nature [12, 14, 20].

By fractionating calf thymus extract No. 1A, obtained by the technique of Goldstein et al. [11], on a column with Sephadex G-25 the writers isolated a fraction which stimulates antibody formation in newborn BALB/C mice and in puppies [1, 2, 5]. However, the effect of fractions of extracts isolated by the same method from other lymphoid structures was not investigated in these experiments. Such investigations are evidently essential if the thymus is to be regarded as the source of the active factor, more especially because of reports in the literature to the effect that some biologically active substances isolated from the thymus are also present in other lymphoid formations and do not disappear from them after thymectomy [4].

The object of the present investigation was to study whether the thymus is the sole source of the factor stimulating antibody formation discovered by the writers. Another interesting aspect was the performance of experiments with tonsillar extracts, for it has been postulated that the palatal tonsils are one of a series of lymphoid structures corresponding to the bursa of Fabricius in birds [7, 13].

EXPERIMENTAL METHOD

The original preparations and fractions of extracts from the calf thymus, tonsils, spleen, lymph glands, and liver were obtained as described previously [11]. Activity of the fractions was tested on noninbred newborn mice and rats. The preparations were injected subcutaneously into the animals in a dose of 0.05 ml within 12 h after birth: the mice received 20 μg and the rats 100 μg as protein [16]. In some experiments the first fraction of extracts of thymus and spleen was injected in a dose of 5 and 10 μg.

After 10 days the animals were immunized intraperitoneally with sheep's red cells: the mice received 2.5×10^8 and the rats 5×10^8 red cells. On the 4th day the number of antibody-forming cells (AFCs) in the animals' spleens was determined by the local hemolysis in gel test [15].

The results were subjected to statistical analysis [6].

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TABLE 1. Number of AFCs (per 10^6 nucleated cells) in Spleen of Mice Receiving Fractions of Extracts of Various Calf Organs in the Neonatal Period

Statistical index	No. of antibody-forming cells per 10^6 nucleated cells							
	0.15 M NaCl	bovine serum albumin	bovine serum	original prepn. of liver	fraction I of liver	original prepn. of lymph gland	fraction I of lymph gland	original prepn. of tonsil
M	2,7	3,0	2,0	2,4	1,8	3,8	3,2	2,7
±*m*	0,28	—	—	—	—	1,16	0,72	—
n	38	8	12	9	12	9	16	12
P*	—	—	—	—	—	>0,05	>0,05	—

Statistical index	No. of antibody-forming cells per 10^6 nucleated cells								
	fraction I of tonsil	original prepn. of spleen	fraction I of spleen (μg)			original prepn. of thymus	fraction I of thymus (μg)		
			5	10	20		5	10	20
M	5,3	3,3	2,4	3,4	5,7	2,8	10,9	18,0	17,0
±*m*	0,66	—	—	—	1,37	—	3,3	4,3	1,9
n	28	13	14	10	30	17	10	8	46
P*	<0,001	—	—	—	<0,05	—	<0,01	<0,001	<0,001

*Here and in Table 2, P calculated relative to number of AFCs in group of animals receiving 0.15 M NaCl.

TABLE 2. Number of AFCs in Spleen of Rats Receiving Fraction I of Calf Thymus and Spleen Extracts in the Neonatal Period

Statistical index	No. of antibody-forming cells per 10^6 nucleated cells		
	0.15 M NaCl	fraction I of spleen	fraction I of thymus
M	3,5	3,4	11,7
±*m*	1,2	1,1	2,36
n	15	11	15
P	—	—	<0,01

EXPERIMENTAL RESULTS

The results of the experiments on mice are given in Table 1.

As Table 1 shows, the first fraction of the thymus extract, when injected in a dose of 20 μg, caused an approximately sixfold increase in the number of AFCs compared with the control animals receiving physiological saline. The first fractions of extracts of the tonsils and spleen also possessed some activity. However, injection of 10 μg of the spleen extract did not lead to an increase in the number of AFCs, whereas fraction I of the thymus extract was highly active even in the dose of 5 μg.

The results of the experiments on newborn rats are given in Table 2.

They show that as a result of injection of fraction I of thymus extract the number of AFCs in the spleens of the experimental rats was increased threefold compared with the control animals receiving 0.15 M NaCl. Meanwhile fraction I of spleen extract did not affect AFC formation.

The results suggest that the thymus produces humoral factors which stimulate antibody synthesis.

The activity exhibited by extracts of the tonsils and spleen in the experiments on mice can be regarded as the result of circulation of the factor and its delivery from the bloodstream into the peripheral lymphoid structures, as has been demonstrated for extracts of a different type [8-10].

The low activity of the tonsillar extracts does not support the view that the palatal tonsils are analogues of the bursa of Fabricius. However, the possibility cannot be ruled out that other methods would be required in order to detect the tonsillar bursa-like factors.

LITERATURE CITED

1. É. V. Gyulling, V. M. Kavsan, O. F. Mel'nikov, et al., Zh. Ush. Nos. Gorl. Bol., No. 6, 25 (1971).
2. V. M. Kavsan and I. S. Nikol'skii, in: Immunology [in Russian], No. 6, Kiev (1973), p. 16.
3. I. N. Kendysh, Uspekhi Sovr. Biol., 73, 342 (1972).

4. D. A. Kostadinov and B. B. Fuks, Vestn. Akad. Med. Nauk SSSR, No. 5, 88 (1973).
5. I. S. Nikol'skii, Zh. Ush. Nos. Gorl. Bol., No. 6, 3 (1973).
6. I. A. Oivin, Pat. Fiziol., No. 1, 76 (1960).
7. A. Ya. Fridenshtein and I. L. Cherkov, The Cellular Basis of Immunity [in Russian], Moscow (1969).
8. J. F. Bach, Rev. Europ. Etud. Clin. Biol., 17, 545 (1972).
9. N. A. Bezssonoff and J. Comsa, Ann. Endocrinol. (Paris), 19, 222 (1958).
10. P. Falk, Arch. Ohr.-Nas.-Kehlk.-Heilk., 176, 713 (1960).
11. A. L. Goldstein, F. D. Slater, and A. White, Proc. Nat. Acad. Sci. U.S.A., 56, 1010 (1966).
12. A. L. Goldstein, G. Asanuma, J. R. Battisto, et al., J. Immunol., 104, 359 (1970).
13. R. A. Good and A. A. Gabrialsen, in: Transplantation of Organs and Tissues in Man [in Russian], Moscow (1973), p. 422.
14. T. J. Hand, W. S. Ceglowski, H. Friedman, et al., J. Immunol., 105, 442 (1970).
15. N. Jerne and A. Nordin, Science, 140, 405 (1963).
16. O. Lowry, N. Rosebrough, A. Farr, et al., J. Biol. Chem., 193, 265 (1951).
17. J. Miller, Austral. J. Sci., 32, 87 (1969).
18. J. Miller and D. Osoba, Physiol. Rev., 47, 437 (1967).
19. D. Osoba, Proc. Soc. Exp. Biol. (New York), 127, 418 (1968).
20. M. Small and N. Trainin, Nature, 216, 377 (1967).