A FACTOR STIMULATING DNA SYNTHESIS IN THYMOCYTES IN VITRO: ITS DISTRIBUTION IN THE TISSUES AND SOME PROPERTIES

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Extracts of some organs were analyzed for their content of a factor stimulating DNA synthesis in thymocytes by the method of cultivation of mouse thymocytes in the presence of labeled DNA precursor (thymidine-C^{14}). This factor is found in the blood serum, thymus, spleen, lymph glands, liver, and kidney. The factor was found to be relatively thermostable at 100°C and it dialyses readily.

TABLE 1. Action of Native Dialyzates of Unpurified Extracts of Thymus, Lymph Glands, and Spleen, and of Dialyzates Heated to 100°C, on DNA Synthesis in Thymocytes in vitro

Extract	Native dialyzate		Dialyzate heated to 100°C for 10 min	
	protein conc. (μg/ml)	IS	protein conc. (μg/ml)	IS
Mouse thymus	95	2,41±0,05	95	1,94±0,04
	40	2,33±0,09	47	2,65±0,05
	20	2,12±0,05	16	1,88±0,03
	10	1,72±0,06	10	1,48±0,11
	—	—	5	1,28±0,03
	—	—	1	1,07±0,04
Mouse lymph glands	49	2,26±0,04	41	2,48±0,05
	20	2,00±0,08	—	—
	10	1,75±0,05	10	1,74±0,02
	—	—	5	1,32±0,02
	—	—	1	1,08±0,04
Mouse spleen	40	2,25±0,02	44	2,53±0,08
	20	2,18±0,02	—	—
	10	1,79±0,01	—	—
Calf thymus	40	1,80±0,09	37	2,21±0,04
	20	1,79±0,08	—	—
	10	1,70±0,07	—	—

Note: IS) Index of stimulation – ratio between incorporation of thymidine-C^{14} into experimental cultures of thymocytes and its incorporation into control cultures, calculated from results of 3-5 separate samples (expt.). For IS = 1.28 or above, $0.05 < P < 0.001$.

The study of the mechanism of action of hormonal factors on the development of lymphoid tissue (proliferation, differentiation, and immunocompetence) in animals and man is an urgent problem in immunology. Corticosteroids are known to produce involution of lymphatic organs and the thymus [2, 11]. Atrophy of the thymus, for instance, has been induced in mice by injection of rabbit antiserum against mouse pituitary [9, 10]. Involution of the thymus in these mice was accompanied by the absence of small lymphocytes from the cortex. One or more hypothetical thymus hormones may perhaps play a role in the development of immunocompetence [8, 15]. One of the points at which hormones may act is the genesis of the cells reacting to antigen, which is dependent on activity of the thymus and Fabricius' pouch [14]. Regulation of the functions of lymphocytes by hormones evidently takes place at the level of protein and nucleic acid synthesis [7, 12].

The authors have previously shown [6] that mouse thymus extract contains a factor exhilarating DNA synthesis in thymocytes in vitro. Extracts of other organs have not previously been investigated. For simultaneous testing of the action of different extracts on the same cell suspension, the method used previously had to be simplified. Sufficiently good reproducibility of the results was obtained.

The object of this investigation was to compare the action of extracts from the thymus and other organs on DNA synthesis in thymocytes in vitro and also to analyze certain properties of the factor discovered.

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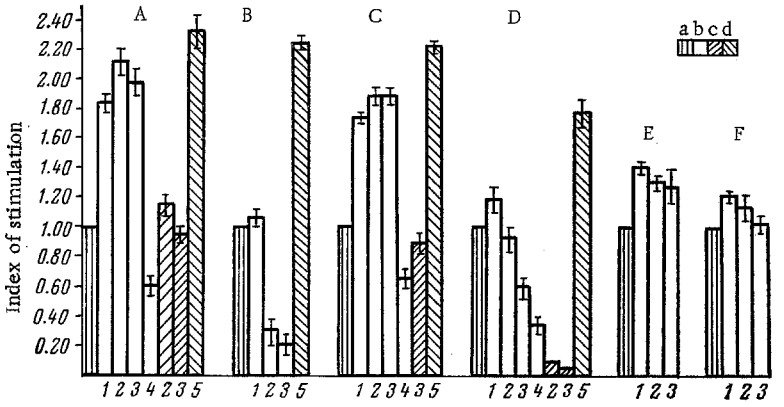

Fig. 1. Comparative action of extracts of different organs on DNA synthesis in thymocytes in vitro: A) extract of mouse thymus; B) of mouse lymph glands; C) of mouse spleen; D) of calf thymus; E) of mouse kidney; F) of mouse muscle. a) Control; b) undialyzed extract; c) dialyzed extract; d) dialyzate. Protein concentration (in μg/ml): 1) 250, 2) 500; 3) 1000; 4) 2000; 5) 40.

TABLE 2. Action of Unpurified Extracts after Heating to 100°C on Incorporation of Thymidine-C^{14} into Thymocytes in vitro

Extract	Quantity of IS added (in ml/sample)						
	0,4	0,2	0,1	0,05	0,025	0,01	0,001
Mouse thymus	0,91	2,22	2,48	2,44	2,31	1,78	1,19
Lymph glands	—	2,48	2,48	1,93	1,70	1,25	1,00
Spleen	—	2,56	2,73	2,64	2,17	1,68	1,02
Kidneys	—	2,49	2,00	1,75	1,75	1,19	1,00
Liver	—	—	2,45	2,31	2,06	—	—
Serum	—	—	2,67	2,43	—	—	—
Native serum	—	—	1,27	1,61	—	—	—

EXPERIMENTAL

The donors of the various tissues used to obtain the extracts were 400 C57B1/6 mice (males and females), aged 2-6 months. Calf thymus, freshly frozen to −70°C, was kept at −20°C until the experiment.

The freshly frozen tissues were minced with scissors and homogenized in a glass homogenizer in cold (4°C) 0.15 M NaCl at pH 7.0-7.2 in the ratio of 1 : 5 for 5-10 min. The homogenate was centrifuged after 30 min at 1800 rpm for 30 min at 4°C. The supernatant was filtered through filter paper. The extracts were kept at −20°C. In other cases the extracts were dialyzed against 0.15 M NaCl for 36 h at 2-4°C. The protein concentration in the extracts was determined before the experiment by Lowry's method.

The thymus of C57B1/6 mice (300 animals) aged 4-8 weeks was squeezed through a nylon mesh into cold Eagle's medium or medium No. 199 containing penicillin and streptomycin (100 units/ml of each). The cell suspension was then washed twice with fresh medium. The residue was resuspended in a small volume of medium, dissociated with a Pasteur pipet, and filtered through Kapron gauze, after which the number of cells was counted.

The cells (20×10^6) were cultivated in 1 ml medium in revolving tubes (15 × 150 mm) in an atmosphere containing 10.2% CO_2, 5.5% O_2, and 84.3% N_2 for 2 h at 37°C. Before incubation began, the extracts to be tested (in volume of 0.1-0.2 ml) and thymidine-C^{14} (0.125 μCi in a volume of 0.05 ml) were added to the tubes. At the end of incubation the samples were quickly cooled in an ice bath. To each tube 5 ml of cold 5% TCA was added. The precipitate was transferred to Millipore filters (RUFS 0.8-1.2 M) and washed with cold 5% TCA and 96% alcohol. Radioactivity was determined by means of a type USS-1 scintillation counter.

EXPERIMENTAL RESULTS

The results of experiments to study the action of extracts of the thymus and other organs on DNA synthesis in thymocytes in vitro are shown in Fig. 1. With an increase in the protein concentration up to a certain optimal value the incorporation of labeled thymidine into thymocytes increased. With a further increase in the protein concentration the effect decreased. Differences were found in the action of the whole thymus extracts from mice and calves. The concentration of inhibitory factors found by some investigators in extracts of calf thymus in experiments with peripheral lymphocytes [3, 4] was evidently higher than in extracts of mouse thymus. In the present experiments the inhibitory action of an extract of mouse lymph glands was particularly marked, whereas extracts of the kidney and skeletal muscle gave a much smaller effect. The differences between the action of the whole extracts obtained from the mouse organs can evidently be attributed to the characteristic assortment of proteins found in each organ, including proteins more or less specifically or nonspecifically inhibiting DNA synthesis in the thymocytes. These proteins probably remain behind in the cellophane bag during dialysis or are precipitated during strong heating of the whole extract (Table 1). The factor stimulating DNA synthesis in thymocytes in vitro was evidently present in all the dialyzates tested. Meanwhile the dialyzed extracts did not possess the activity of the whole extracts.

The behavior of the native dialyzates after heat treatment was investigated. The activity of the factor remained as high as before when heated to 100°C for 10 min (Tables 1 and 2).

In view of the relative thermostability of the factor, full extracts were heated in one of the experiments. Tissue homogenates were centrifuged at 18,000 rpm. The supernatant was kept at 100°C for 5 min, after which it was again centrifuged and the new supernatant was kept at between 2 and 4°C until the experiment. The results of this experiment are given in Table 2.

It will be clear from Table 2 (see also Table 1) that with an increase in the concentration of the factor its effect became stronger and ultimately the curve became a plateau. The factor stimulating DNA synthesis in the thymocytes in vitro is present in normal mouse serum. It can be assumed that the factor under investigation is formed by one organ, is liberated into the blood stream in sufficiently high concentrations, and thus reaches other organs.

However, the data are not yet sufficient to allow an unequivocal answer to be obtained to this question. It is also difficult to say to what extent this factor resembles the substances found by other workers in extracts of calf thymus [1, 3, 4, 13], for there were differences both in the technique used and in the physicochemical properties of the factors compared. A factor similar to that now described has been found in an extract of mouse and sheep bone marrow cells [5] in a similar test system. However, the authors cited were unable to detect its activity in extracts of mouse spleen and kidney cells, evidently because of the method used to prepare the extracts and the conditions of analysis.

According to the results of these experiments the factor stimulating DNA synthesis in thymocytes in vitro is thus present in the serum of normal mice and also in some of the organs tested. This factor is relatively thermostable and has low molecular weight.

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