INVESTIGATION OF EJECTION OF DNA-CONTAINING MACROTHREADS FROM LYMPHOCYTE NUCLEI

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Ejection of DNA-containing macrothreads was demonstrated by cells of the lymph glands and spleen in mice and hamsters and also by circulating human lymphocytes in tissue culture at slightly acid, neutral, and alkaline pH values in isotonic phosphate buffer solution, and also at neutral and alkaline pH values in medium No. 199 and in Hanks's solution. No ejection of DNA-containing macrothreads was observed in the tested media at acid pH values, in 5% crystalline egg albumin solution, or in autologous or heterologous bovine inactivated serum at alkaline pH values.

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DNA-containing bridges between lymphocytes and target cells were described previously during cytochemical studies of interaction between these cells in tissue culture [1]. DNA-containing bridges between two or three lymphocytes were observed in the same system [2], having the appearance of dense, twisted bands, of discretely stained isthmuses between pairs of lymphocytes, or a thin, twisted thread. A further study showed that the ejection of DNA threads from the nuclei of lymphocytes is evidently a stereotype reaction of the lymphocyte to injury.

Ejection of DNA-containing macrothreads from the nuclei of lymphocytes in various media and under different conditions was investigated in the present study.

EXPERIMENTAL METHOD

Lymphocytes from lymph glands and spleen of golden hamsters, BALB/c mice, and human peripheral blood were used in the investigation. The following media were used to incubate the lymphocytes: 0.15M, 0.3M, and 0.06M phosphate buffer, pH 5.0, 5.6, 6.0, 6.5, 7.0, 7.6, 8.0, and 8.4; 0.15M phosphate buffer with the addition of crystalline egg albumin in concentrations of between 0.001 and 5%, with pH adjusted to 7.0 and 8.0; Hanks's solution, pH 5.6, 6.0, 6.5, 7.0, 7.6, and 8.4 without albumin and with the addition of 5% albumin; medium No. 199, pH 6.0, 6.5, 7.0, 7.2, 7.4, 7.6, 7.8, and 8.4; 6% and 3% polyvinol solution (the 3% polyvinol solution was obtained by dilution of the 6% solution with 0.15M phosphate buffer, pH 7.0, and 8.0);

TABLE 1. Substances Protecting Lymphocyte Nuclei against Ejection of DNA Macrothreads

pH	Serum		Polyvinol		Egg albumin						
			%								
	autologous	heterologous	3	6	0,01	0,1	0,5	1	2	3	5
7,0	—	±	—	—	++	++	±	±	±	±	—
8,0	—	±	±	+	+++	+++	++	±	±	±	±—

Note: —) Threads absent; ±) a few threads in several fields of vision; +) approximately 25% of nuclei eject a DNA macrothread; ++) about 50% of nuclei eject a DNA macrothread; +++) network of thin interwoven threads with adherent whole lymphocyte nuclei.

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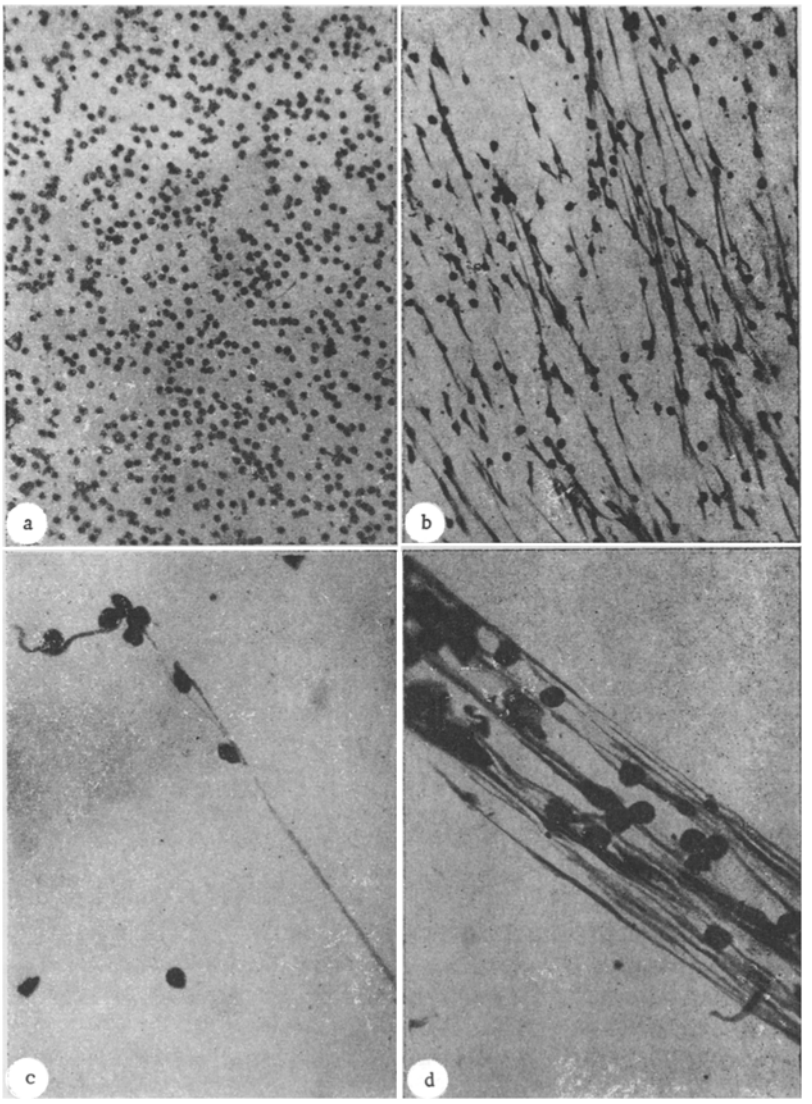

Fig. 1. External appearance of cells after incubation for 3 h in 0.15M phosphate buffer. a) pH 5.6 (200×); b) pH 7.0 (200×); c) pH 7.4 (600×); d) pH 8.4 (600×). Azure-eosin or Feulgen's method.

bovine inactivated serum, whole (pH 7.8) and diluted with 0.15M phosphate buffer, pH 8.0, to concentrations of 5, 10, 20, and 50%. Human blood plasma was added to the human lymphocytes, and inactivated hamster serum to the hamster lymphocytes. The lymphocytes (4-5 million/ml) were incubated in flasks with cover slips for 3 h. In two experiments the lymphocytes were incubated in 0.15M phosphate buffer, pH 8.0, with the addition of 2% albumin and in 6% polyvinol solution in flasks without cover slips. The flasks were centrifuged for 5 min at 1000 rpm before incubation or after incubation for 3 h. After fixation, the bottoms of the flasks were sawn off and stained for subsequent microscopic investigation. Specimens were fixed by Carnoy's method and stained with azure-eosin by Feulgen's method and with acridine orange after preliminary hydrolysis [3]. Control specimens were treated with desoxyribonuclease in a concentration of 100 μg/ml in 0.005M $MgSO_4$ solution for 2 h at 37°. In two experiments the DNA of the lymphocytes was labeled with thymidine-H^3 in vivo. For this purpose, 100 μg thymidine-H^3 with specific activity of 1 Ci/mg was injected intramuscularly into BALB/c mice. The mice were sacrificed 1 h later and lymphocytes taken from the spleen. Some labeled lymphocytes were used for making films. Specimens fixed by Carnoy's method were stuck to slides, treated with 5% TCA solution at 4° to remove precursors, and covered with type M NIKFI emulsion. The period of exposure was 2 weeks. The developed specimens were stained with methyl green–pyronine.

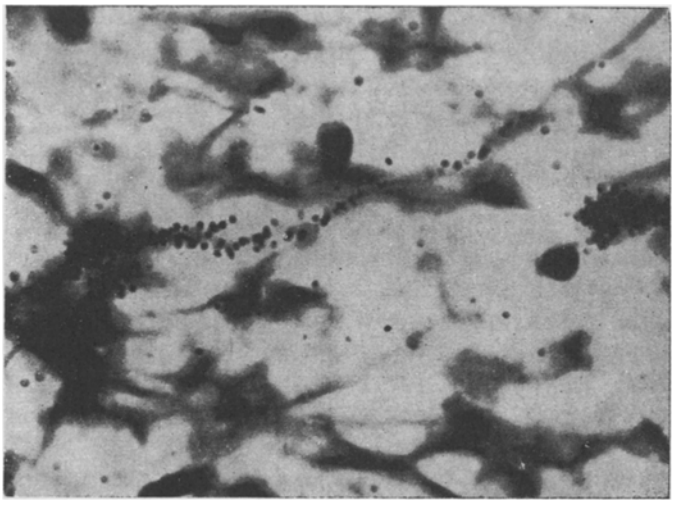

Fig. 2. DNA macrothread labeled with thymidine-H^3. Methyl green–pyronine, 900×.

EXPERIMENTAL RESULTS

The results of 10 experiments to determine the effect of pH on ejection of DNA threads from lymphocyte nuclei were as follows: at acid pH values (5.0-5.6) the cells were uniformly distributed over the glass, palely stained; the cytoplasm of most cells was invisible (Fig. 1a), and threads were absent. At pH 6.0-7.0, the lymphocytes settling on the glass ejected a thin thread (at pH 6.0-5.5 approximately 25% of nuclei ejected a thread, about 50% at pH 7.0), slightly widened at the base and narrowed at the end, and not exceeding 50-70 μ in length (Fig. 1b); frequently the threads ran in a definite direction. On these specimens the cells were distributed uniformly over the glass (Fig. 1c) or, as is usually observed with lymphocytes incubated at pH 7.0-7.4, numerous threads up to 200-400 μ in length appeared, with lymphocytes adherent to them, or apparently strung from them. In some places extensive interweaving of thin threads formed an azure network. Clusters of cells were grouped into discrete islets, mainly at the periphery of the cover slip. In some specimens, long, parallel threads with adherent lymphocytes formed massive bands. In an alkaline medium (pH 8.0-8.4), the same pattern was observed although it was quantitatively different: in this case there were fewer whole lymphocyte nuclei and more thin, long, interwoven or merging threads (Fig. 1d). In 0.3M and 0.06M buffer, pH 5.6-8.0, similar results were observed. The results obtained with the use of medium No. 199 buffered with 0.15M phosphate buffer were contradictory. The standard response was observed only at pH 5.0-5.6 and 8.0-8.4. At acid pH values no threads were formed, and at pH 8.0-8.4, the appearance of the cells was the same as in 0.15M phosphate buffer. Within the range pH 6.0-7.5, a uniform response could not be obtained, even during the same experiment. The same pattern was found when Hanks's solution was used. Lymphocytes placed in distilled water, pH 4.7 and 7.0, underwent lysis. No ejection of threads took place under these conditions. In 5% egg albumin solution in 0.15M phosphate buffer, pH 7.0 and 8.0, ejection of threads was almost completely absent. To determine the threshold concentration of albumin at which this phenomenon is inhibited, the albumin was titrated in the range from 0.0001 to 5% solution.

The results of these experiments are given in Table 1, showing that the phenomenon is inhibited at pH 7.0 in a 0.5% solution and at pH 8.0 in a 1% solution of albumin. Short, thick, twisted threads were still visible in the specimens, a few in several fields of vision. When albumin was used in the corresponding concentrations in medium No. 199 or in Hanks's solution, similar results were obtained. Bovine inactivated serum in concentrations of 5, 10, and 20% had hardly any effect on the appearance of the threads. However, whole homologous and heterologous serum almost completely abolished the phenomenon. Positive results were obtained by using the plasma substitute polyvinol, also in 6% solution.

In one series of experiments lymphocytes in 2% albumin solution and in 6% polyvinol solution, pH 8.0, were poured into flasks without cover slips and the cells were centrifuged 3 h later. The same pattern was found on the stained sawn-off flask bottoms on which the cells had settled as on cover slips in the parallel experiment without centrifugation.

After injection of the radioactive label thymidine-H^3 into mice, about 10% of the cells in films were labeled. The mean number of label granules per labeled lymphocyte was 30. This high intensity of thymidine-H^3 incorporation by the lymphocyte nuclei made it possible for label to be observed in the region of the threads (Fig. 2). Threads were seen in specimens stained by Feulgen's method and with acridine orange after preliminary hydrolysis. The staining of the nuclear threads disappeared after treatment of the specimens with desoxyribonuclease in control experiments.

The positive results obtained by means of special techniques for DNA detection (staining by Feulgen's method, with acridine orange after preliminary hydrolysis, incorporation of thymidine-H^3 using the appropriate controls) indicate that the phenomenon described above is one of ejection of macrothreads of DNA from lymphocyte nuclei.

In most experiments isotonic 0.15M buffer solution was used. Ejection of DNA microthreads took place in it at a weak acid pH (6.0), although the external appearance of the threads and the number of cells ejecting them gradually changed as the pH increased. At alkaline pH values only a few lymphocyte nuclei remained intact, and there was a delicate network of very thin threads. Relative hypotonicity (0.06M) or hypertonicity (0.3M) of the buffer solution did not affect the experimental results at different pH values. Colloidal solutions – a 2-5% solution of albumin and 3-6% solution of polyvinol – inhibited this process, although a few short, thick twisted threads were always visible in several fields of vision. The best medium for preservation of lymphocytes at alkaline pH values was autologous serum. The protein concentration in the bovine serum used was 4-6%, approximately equal to the concentration of egg albumin used. Human, hamster, and mouse lymphocytes behaved identically under the conditions studied.

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