EFFECT OF CHLORAMPHENICOL ON ANTIBODY FORMATION

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Experiments on CBA mice immunized with sheep's erythrocytes showed that chloramphenicol, if injected into animals 3 days after immunization, reduced the number of cells synthesizing 19S- and 7S-hemolysins. If chloramphenicol was injected together with antigen, the number of cells forming 19S-antibodies was increased.

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Chloramphenicol, as an inhibitor of protein synthesis, exerts an immunodepressive action [3, 7], but the manner in which it acts on the cell components of the immunologic response is unknown.

In this investigation the action of chloramphenicol was studied on the number of antibody-forming cells synthesizing 19S- and 7S- hemolysins.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice weighing 15 g. The animals were immunized intraperitoneally with sheep's erythrocytes ($5 \cdot 10^8$ erythrocytes). On the 4th day after immunization the mice were killed and cell suspensions prepared from their spleen ($5 \cdot 10^7$–$1 \cdot 10^8$ nucleated cells/ml). The number of antibody-forming cells was determined by the method of local hemolysis in agar. Direct hemolytic plaques (19S) were detected by the method of Jerne and Nordin [5]. Indirect plaques (7S) were identified after incubation with rabbit antiserum against mouse 7S-globulins [4]; 7S-globulins were obtained by fractionation of whole mouse serum on a column with Sephadex G-200 in 0.2 M tris-HCl buffer (pH 8.0) with 0.1 M NaCl solution. In Ouchterlony's agar precipitation test rabbit anti-7S-serum gave a positive result with 7S-globulins in dilutions of up to 1:32, in crossed reactions with 19S-globulins in a dilution of 1:4, but did not react with albumins.

Chloramphenicol was injected subcutaneously into the animals in a single dose of 0.2 mg/kg, which is nontoxic to mice. Altogether 5 series of experiments were carried out. In series I the compound was injected along with the antigen, and in series II 24 h, in III 48 h, and in IV 72 h after immunization. In the control series (V) the mice were injected with antigen only. In each series experiments were carried out on 5 animals.

EXPERIMENTAL RESULTS

The number of antibody-forming cells found in the spleen of the control mice receiving antigen only, on the 4th day after immunization, was $45{,}133 \pm 4330$. Of this number, $32{,}013 \pm 3018$ synthesized 19S-hemolysins and $13{,}120 \pm 1312$ synthesized 7S-hemolysins. Under the influence of chloramphenicol injected simultaneously with antigen, the number of 19S-antibody-forming cells was increased by 79% over the control, whereas the number of 7S- antibody-forming cells was unchanged (Table 1). If the antibiotic was injected 24 and 48 h after antigen, the changes in the number of antibody-forming cells were not significant. When chloramphenicol was injected 72 h after antigen, the number of antibody-forming cells was reduced, the most marked inhibition of antibody formation being observed in the population of cells

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TABLE 1. Effect of Chloramphenicol on Weight of Immunocompetent Organs and Number of Nucleated and Antibody-forming Cells in Spleen

Series of experiments	Weight of thymus (in mg)	Weight of spleen (in mg)	No. of nucleated cells in spleen ($\times 10^{-6}$)	No. of cells synthesizing 19S-antibodies in spleen	No. of cells synthesizing 7S-antibodies in spleen
I	34,0±5,0	79,5±0,9	157,4±27,3	57 608±9 224 ($P<0,05$)	15 740±4 722 ($P>0,05$)
II	38,0±5,0	77,0±8,0	184,5±9,0	55 830±11 254 ($P>0,05$)	18 450±1 845 ($P>0,05$)
III	47,0±7,5	78,0±3,7	182,4±12,4	20 921±5 344 ($P>0,05$)	10 397±2 736 ($P>0,05$)
IV	34,0±1,3	75,4±0,7	142,5±11,8	6 256±2 436 ($P<0,01$)	7 552±1 852 ($P<0,05$)
V	46,0±2,0	79,0±2,5	131,2±2,2	32 013±3 018	13 120±1 312

synthesizing 19S-antibodies. For example, when chloramphenicol was injected 72 h after antigen, the number of cells synthesizing macroglobulin hemolysins was only 19% of that in the control, whereas the number of cells synthesizing 7S-antibodies was reduced to 57%.

Chloramphenicol thus acts differently on cells synthesizing 19S- and 7S-antibodies. On the latter it has either an inhibitory or no effect, whereas the number of cells synthesizing 19S-antibodies changed variously depending on the time of injection of chloramphenicol relative to the time of immunization. If given at early stages (simultaneously with antigen), this antibiotic had a stimulant effect, but if given later (72 h after immunization), it had an immunodepressive action. Meanwhile the total number of lymphoid cells in the spleen and the weight of the immunocompetent organs were unchanged by the action of chloramphenicol (Table 1).

In the modern view chloramphenicol acts on protein synthesis at the translation level. Its inhibitory effect is based on the formation of incompletely built molecules of ribosomal protein, known as chloromycetin particles [2]. In accordance with these ideas, inhibition of antibody formation by chloramphenicol can be explained. At the same time, it is evident that the compound is most effective if injected late, for it is only thus that the antibiotic can be expected to be present in the antibody-forming cells in an effective concentration.

Differences in the sensitivity of cells synthesizing 19S- and 7S-antibodies to chloramphenicol can be explained on the assumption that, despite their equal immunologic competence, they are different cell populations. Cells synthesizing 19S-antibodies are converted into immunocompetent cells by direct transformation without preceding mitoses [6], while cells synthesizing 7S-antibodies, on the other hand, pass through a series of successive mitotic divisions before commencing antibody synthesis. Chloramphenicol does not inhibit DNA and RNA synthesis in concentrations which almost completely inhibit the formation of polypeptides [1]. It can therefore be assumed that the small doses of chloramphenicol used in these experiments did not completely block the autosynthetic interphase of cells synthesizing 7S-antibodies, and under these conditions their mitotic division continued, so that the concentration of antibiotic in the cells decreased with each division, and at the time of observation it was below threshold. By contrast, protein (antibody) synthesis in cells forming 19S-antibodies, which were in the heterosynthetic interphase, was considerably inhibited.

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