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Influence of Chloramphenicol and Cetophenicol on Antibody Formation in Mice (33427)

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Chloramphenicol has been shown to inhibit both the specific primary antibody response and priming for a subsequent secondary response, as well as to inhibit induced protein synthesis in mammalian cell-free systems (1–4). The influence of chloramphenicol on various expressions of the immune response and on mammalian cell function has recently been reviewed (5). Cetophenicol, an antibiotic analog of chloramphenicol in which the nitro group in the para position of chloramphenicol is replaced by an acetyl group (6), has an antibacterial spectrum identical with that of chloramphenicol (7). Cetophenicol, like chloramphenicol, has been shown to inhibit protein synthesis *in vitro* (4). In this initial report the inhibition of antibody formation by chloramphenicol is demonstrated at substantially lower than usually required doses of antibiotic, made possible by use of the technique of localized hemolysis in agar (8). Further, the two antibiotic analogs are shown to differ qualitatively in their opposite effects upon the increase in numbers of hemolysin-forming spleen cells induced by bacterial endotoxin (9, 10). Chloramphenicol suppresses and cetophenicol enhances this elevation in numbers of antibody-forming cells, whereas both antibiotics suppress the specific primary response to foreign erythrocytes. Studies on priming for a subsequent secondary response to specific antigen, to be reported separately, reveal the same distinction between the two analogs: enhancement, instead of suppression, of priming by cetophenicol despite inhibition of the primary response equivalent to that produced by chloramphenicol.

Materials and Methods. Female CD-1 mice (Charles River), weighing 20–22 g, were injected with sheep red blood cells, i.v., at a dose of 10^8 cells, or with *Salmonella abortus equi* Boivin endotoxin (Difco), i.p., at a dose of 10μg. Chloramphenicol (D-threo-2-[2, 2-dichloroacetamido]-1-[*p*-nitrophenyl]-1,3-propanediol) or cetophenicol (D-threo-1-[*p*-acetylphenyl]-2-[2, 2-dichloroacetamido]-1, 3-propanediol) was administered, i.p., at doses of 1 or 2 mg, 3 times/day (approx 150 or 300 mg/kg/day). Treatment with antibiotic was given 6 and 2 hr before and 2 hr after injection of red cells or endotoxin, and was repeated the next day at 4-hr intervals. The following day, 42 hr after immunization or endotoxin treatment, spleens were harvested for assay. Numbers of hemolysin-forming spleen cells were determined by the technique of localized hemolysis in agar (8) using sheep red blood cells and 1/5 of the cell suspension of the whole spleen, as previously described

TABLE I. Influence of Chloramphenicol and Cetophenicol on Numbers of Hemolysin-Forming Spleen Cells 42 hr after Injection of Sheep Red Blood Cells (sRBC) or Endotoxin (ET).

Treatment	No. of hemolysin-forming spleen cells (mean ± SE)		
	Expt. A	Expt. B	Expt. C
None	8 ± 4.4	1 ± 0.6	0 ±(—)
Chloramphenicol, only	5 ± 1.6	—	—
Cetophenicol, only	—	1 ± 0.7	—
sRBC, only	72 ± 7.7	46 ± 3.6	54 ± 7.3
+ chloramphenicol	24 ± 9.4[b]	—	5 ± 0.8[c]
+ cetophenicol	—	19 ± 1.2[c]	5 ± 1.7[c]
ET, only	33 ± 1.6	41 ± 1.5	48 ± 2.0
+ chloramphenicol	18 ± 4.1[a]	—	17 ± 0.6[c]
+ cetophenicol	—	66 ± 7.8[a]	86 ± 3.3[c]

[a] Significantly different from appropriate control at $p < 0.05$.
[b] Significantly different from appropriate control at $p < 0.01$.
[c] Significantly different from appropriate control at $p < 0.001$.

(9, 10). Individual assays employed control and experimental groups of 5 mice each. Mean ± standard error was computed for each group and significance of difference between means based upon p values is given in Tables I–IV. Solutions for injection were prepared daily in nonpyrogenic saline and rigorous precautions to avoid contamination with extraneous endotoxins were observed.

Results. The data in Table I establish that chloramphenicol inhibited the antibody-forming cell response to specific antigen, sheep red blood cells, at unit doses of the antibiotic of 1 mg (150 mg/kg/day). In terms of antibacterial potency, cetophenicol is approximately one-half as active as chloramphenicol *in vitro* and *in vivo* (7) and the data in Tables I, II, and III were obtained using equivalent antibacterial doses, i.e., 2 mg unit doses of cetophenicol (300 mg/kg/day). The two antibiotics are compared at equal absolute doses in Table IV. As shown in Table I, cetophenicol, too, suppressed the response to the specific antigen. Additional experiments using higher doses of both antibiotics demonstrated more profound inhibition of the response to sheep red blood cells. Neither antibiotic when given alone significantly modified normal background counts found in untreated mice, whether injected for 2 days, as in Table I, or for 4 days, as in other experiments, before assay. In contrast to their common inhibition of the response to the specific antigen, chloramphenicol also inhibited the increase in hemolysin-forming spleen cells found 42 hr after injection of endotoxin whereas cetophenicol enhanced this response (Tables I, II, IV). These opposite influences disappeared when both antibiotics were injected into the same animals (Table II). As shown in Table III, chloramphenicol inhibited the heightened response found when mice are injected with both sheep red blood cells and endotoxin (9, 10), but cetophenicol did not. A comparison of the two antibiotics both at 1 and 2 mg unit doses is given in Table IV. The stimula-

TABLE II. Influence of Combined Treatment with Chloramphenicol and Cetophenicol on Numbers of Hemolysin-Forming Spleen Cells 42 hr after Injection of Endotoxin (ET).

Treatment	No. of hemolysin-forming spleen cells (mean ± SE)
None	1 ± 0.4
ET, only	33 ± 1.3
+ chloramphenicol	15 ± 2.0[a]
+ cetophenicol	72 ± 3.0[a]
+ chloramphenicol + cetophenicol	34 ± 1.2

[a] Significantly different from control at $p < 0.001$.

TABLE III. Influence of Chloramphenicol and Cetophenicol on Numbers of Hemolysin-Forming Spleen Cells 42 hr after Injection of Sheep Red Blood Cells with Endotoxin (sRBC + ET).

Treatment	No. of hemolysin-forming spleen cells (mean ± SE)
None	3 ± 3.0
sRBC + ET	139 ± 8.1
+ ET + chloramphenicol	43 ± 5.2[a]
+ ET + cetophenicol	221 ±51.2

[a] Significantly different from control at $p < 0.001$.

tory influence of endotoxin on numbers of hemolysin-forming cells was inhibited by chloramphenicol and enhanced by cetophenicol, with greater effect at the higher doses in both cases.

Discussion. The application of the technique of localized hemolysis in agar has made possible a demonstration of the *in vivo* immunosuppressive effect of chloramphenicol at low dose over a brief period of time, avoiding the toxicity which usually attends such experiments (5). There is no need for restriction to foreign red blood cells as antigen, for additional studies using diphtheria toxoid, and erythrocytes coated with diphtheria toxoid as indicator cells, have yielded entirely similar results with the same chloramphenicol regimen.

TABLE IV. Influence of Dose of Chloramphenicol and Cetophenicol on Numbers of Hemolysin-Forming Spleen Cells 42 hr after Injection of Endotoxin (ET).

Treatment	No. of hemolysin-forming spleen cells (mean ± SE)
None	2 ± 0.7
ET, only	35 ± 1.7
+ chloramphenicol (1 mg)	14 ± 0.9[b]
(2 mg)	9 ± 0.5[b]
+ cetophenicol (1 mg)	75 ± 5.2[a]
(2 mg)	124 ± 4.3[b]

[a] Significantly different from control at $p < 0.01$.
[b] Significantly different from control at $p < 0.001$.

The distinction between chloramphenicol and cetophenicol in their opposite effects upon the increase in numbers of preexisting hemolysin-forming cells induced by endotoxin is significant in a number of directions. It is of interest that these two analogs, having the same antibacterial spectrum (7) and a common inhibitory effect upon mammalian protein synthesis (4), should differ both in the particular cell proliferative system described here and identically in their opposite influences on priming, to be reported separately. It is reasonable to suppose that their common suppression of the initial response to the specific antigen, sheep red blood cells, derives from their common ability to interfere with *de novo* protein synthesis at the ribosome (4). However, the findings reported here for endotoxin-stimulated animals suggest that: (a) there is an additional site of action at which the two antibiotics act oppositely, further supported by the data in Table II; (b) the influence of endotoxin does not follow the same pathway as that initiated by the specific antigen. For this second point, it has already been suggested from a variety of experimental approaches that there is good reason to believe that the modification by endotoxin of the numbers of hemolysin-forming cells may not be attributable to cross-reacting antigens (9–12). The present findings with cetophenicol strongly support this view, for an explanation of the stimulatory effect of endotoxin based solely on the presence of cross-reacting antigens would anticipate that cetophenicol would suppress the response to endotoxin as it does the response to the specific antigen, and this is not found. A clue to the mechanism of endotoxin stimulation, and its escape from the protein synthesis inhibition of cetophenicol, may be the sensitivity of circumstances dependent upon newly formed mRNA, and the insensitivity of those where informational RNA is already bound to the ribosome, to the action of these antibiotics in studies in cell-free systems (4). Our recent observation (12) that FUDR inhibits the response of mice to sheep erythrocytes but is without effect upon the he-

molysin-forming cell response to endotoxin appears also relevant.

Studies on the effects of these and additional analogs upon antibody formation and priming and their implications for the mechanisms of the immune response will be reported separately.

Summary. The immunosuppressive effect of chloramphenicol *in vivo* has been demonstrated in mice at low dose in short-term experiments by application of the technique of localized hemolysis in agar. The antibiotic suppressed the increase in numbers of hemolysin-forming spleen cells found 42 hr after immunization with sheep red blood cells or treatment with endotoxin. Cetophenicol, an analog of chloramphenicol having the same antibacterial spectrum and ability to inhibit *de novo* protein synthesis, also suppressed the response to the specific antigen, sheep erythrocytes. In contrast, however, cetophenicol enhanced the proliferation of hemolysin-forming cells induced by endotoxin. These opposite influences disappeared when the two antibiotics were injected into the same endotoxin-stimulated mice. Neither analog by itself modified the normal background of antibody-forming cells found in unstimulated mice. The implications of these findings for the modes of action of the analogs and for the influence of endotoxin are discussed.

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Variation in Intestinal Transport of L-Valine in Relation to Age* (33428)

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The active transport of amino acids by the small intestine has been demonstrated both in vivo (1–3) and *in vitro* (4–6). Although the general age and/or weight range of the animals used in these studies is stipulated, no information is available on the effect of the age or weight of the animal on amino acid transport. It has been reported that the muscle/plasma ratio of α-aminoisobutyric acid is decreased with age both in the rat (7) and in man (8). The present paper reports the observation of decreasing intestinal transport of amino acids as typified by L-valine with increasing age in rats.

Methods. Weanling male Wistar rats were used as the experimental animal. The rats

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