STUDIES ON ANTIBODY PRODUCTION

XIII. The Effect of Chloramphenicol on Priming in Mice*

By ANDRE CRUCHAUD,‡ M.D., and ALBERT H. COONS,§ M.D.

(*From the Department of Bacteriology and Immunology, Harvard Medical School, Boston*)

The preceding paper describes the inhibition by chloramphenicol of "priming" for the secondary antibody response by a standard dose of antigen (1). For full effect, the inhibiting drug must have been administered from the day of stimulus continuously for 10 to 14 days thereafter. This paper examines this phenomenon in more detail; the results confirm the earlier experiments and indicate that priming can be effected within 3 hours after a subcutaneous injection of antigen.

Little work has been done until recently on the effect of chloramphenicol on antibody formation. Slanetz (2) reported a decrease in the agglutinin response in rats to *Salmonella enteritidis* when they were fed chloramphenicol[1] for long periods. But neither Watson (3), nor Zhuravleva and Gorchakova (4) could demonstrate such an effect in rabbits given bacterial antigens and chloramphenicol in doses of 20 to 55 mg/kg/day. And Nathan *et al.* (5) also failed to demonstrate impairment of the agglutinin response to sheep erythrocytes in mice given 250 mg/kg/day.

These contradictory findings were resolved, in part, when Ambrose and Coons (6) recently reported that chloramphenicol in bactericidal concentrations completely inhibited the secondary antibody response in tissue culture. They found that its inhibitory action was effective only during the first stages of the response, before the synthesis of antibody was well under way, and that it was without effect later, during the period when most of the antibody was produced. This led them to suggest that chloramphenicol interferes with messenger RNA, and that the apparent insensitivity of mammalian systems was due to the greater stability of mammalian messenger. In line with this suggestion was the report by Weisberger *et al.* (7), working also with a mammalian system, reticulocyte ribosomes. They found that chloramphenicol in a concen-

tration of 0.01 μM interfered with the interaction between messenger RNA and ribosomes, preventing polyuridylic acid (poly U), for example, from directing the synthesis of polyphenylalanine. The inhibitory effect failed if the poly U was added as little as 5 minutes before the chloramphenicol. Moreover, Kučan and Lipmann (8) have found that chloramphenicol in a concentration of 0.05 μM inhibited leucine incorporation by *Escherichia coli* ribosomes more strongly when exogenous messenger RNA was added *in vitro*, than when its incorporation was directed by messenger already present on the ribosomes.

Materials and Methods

Harvard mice, fluid diphtheria toxoid, antigen injections, and antibody assays were the same as those described in the preceding paper (1). The first dose of antigen (20 Lf) was administered on day 0 of the experiment, the second (20 Lf) on day 40, and the third (20 Lf) on day 74 or 75.

The dose of chloramphenicol succinate[2] was freshly dissolved daily in 0.45 per cent NaCl. The daily dose for each mouse, whether 100, 1100, or 1500 mg/kg, was contained in 0.6 ml, given intraperitoneally every 8 hours in 0.2 ml amounts throughout the experiment.

Sera.—0.5 to 0.7 ml samples of blood were obtained from a razor cut in a tail vein. The samples were kept at room temperature for 2 hours, when clots were "rimmed" and the samples stored overnight at 4°C. They were then centrifuged at 4°C at 2900 RPM for 45 minutes. Sera were separated, immediately quick-frozen, and stored at −20°C until the time of titration.

Titration of Diphtheria Antitoxin in Sera.—Antitoxin titers were determined by the passive agglutination of sensitized tanned sheep red cells (9). Inactivated sera were titrated in twofold serial dilutions made with 1 per cent normal rabbit serum in phosphate-buffered saline at pH 7.2. The antibody titer is expressed as the logarithm to the base 2 of one-tenth the reciprocal of the serum dilution. Thus a hemagglutination titer of 1/20 is recorded as 1, 1/40 as 2, 1/80 as 3, etc.

Sera of the same experiment were titrated with the same batch of sensitized cells.

Chloramphenicol Blood Levels.—Three groups of 10 mice were injected intraperitoneally at 8-hour intervals with 1500 mg/kg/day of chloramphenicol for 3 days. These groups were bled 15 minutes, 4 hours, and 8 hours respectively after the last injection of the drug. Blood was drawn from a tail vein into microblood sugar pipettes calibrated to contain 0.2 ml. This blood was then pipetted into 4.0 ml of a 0.05 per cent saponin solution. After a few minutes, during which hemolysis was completed, the specimens from each group of 10 mice were pooled; 10 ml of 15.6 per cent trichloracetic acid was added, and, after standing for 20 minutes, the tubes were centrifuged to remove the protein precipitate.

A colorimetric determination of aromatic nitro compounds of chloramphenicaol was performed in the supernatant according to the method described by Glazko *et al.* (10) as modified in the Research Laboratories of Parke, Davis & Company.[3]

Statistical Evaluations.—The standard deviation was calculated in each group for the responders only. The significance of differences between groups was evaluated according to the "Student" t test.

RESULTS

Experiment 1: Study of the Primary Response to Diphtheria Toxoid.—

Fecsik, Butler, and Coons (11) showed that most mice injected subcutane-

---

[2] Kindly supplied by Dr. C. A. McDonald of Parke, Davis & Co., Detroit.

[3] These determinations were kindly done by Dr. Anthony J. Glazko of Parke, Davis & Co.

ously with 10 Lf diphtheria toxoid do not make a detectable primary antibody response after 10, 24, or 30 days, and that those responding have a low titer of antitoxin. Our first experiment studied the response of mice to 20 Lf; it was carried out as a preliminary investigation to see whether the earlier results were reproducible with twice the dose of antigen.

Four groups of 10 mice each were injected with 20 Lf of diphtheria toxoid. Groups I, II, and III were bled 10, 25, and 40 days later respectively. Group IV received another injection of 20 Lf of diphtheria toxoid 40 days after the first one and was bled 10 days later.

TABLE I

Antibody Response of Normal Mice to 20 Lf of Diphtheria Toxoid

Group	Time of bleeding after injection of diphtheria toxoid	No. of responders	Average Ab titer	
			All animals	Responders
	days			
I	10	2/10 (20 per cent)	0.4	2.0 —
II	25	5/9 (55 per cent)	2.0	3.6 ±0.89
III	40	6/10 (60 per cent)	2.2	3.7 ±0.082
IV	50* (secondary response)	9/9 (100 per cent)	10.3	10.3 ±1.66

* A second injection of diphtheria toxoid was given on day 40.

Results.—The sera of 38 animals were analyzed. The results are recorded in Table I. It is to be noted that in both *primary* groups, bled 25 and 40 days after the injection of diphtheria toxoid, responders had titers between 3 and 5, whereas animals bled during the *secondary* response had titers between 7 and 12.

It is thus clearly established that in our animals the primary response differs from the secondary response in (a) the number of responders, (b) the range of values of individual titers, and (c) the average titers. These results confirm those of Fecsik *et al.* (11). The greater number of responders found in our experiment during the late period of the primary response is apparently due to the use of 20 Lf of diphtheria toxoid instead of 10 Lf.

Experiment 2: Effect on Priming of Low and Middle Doses of Chloramphenicol Administered before and after Antigen.—

Two groups of 10 mice each were injected for 10 days with 100 and 1100 mg/kg/day of chloramphenicol respectively, starting 5 days before and finishing 5 days after the administration of diphtheria toxoid. A control group of 10

mice received saline for the corresponding period. Forty days after the first injection of antigen, all animals were injected with a second dose of diphtheria toxoid. They were bled 10 days later.

At the end of treatment with chloramphenicol the weight loss was 6.0 per cent in the group injected with 1100 mg/kg/day and 1.0 per cent in the group injected with 100 mg/kg/day. The weight of the control mice increased 7.3 per cent. There were no deaths in the three groups.

SECONDARY RESPONSE	
No. of Responders	Average Ab. Titer
9/9	7.4 ±2.2
9/9	11.4 ±2.3
10/10	12.0 ±0.8

-5 0 5   40   50 Days
      ↑   ↑   ↑
   DT I   DT II   Bled

■■■ Chloramphenicol 1100 mg/kg/day for 10 days
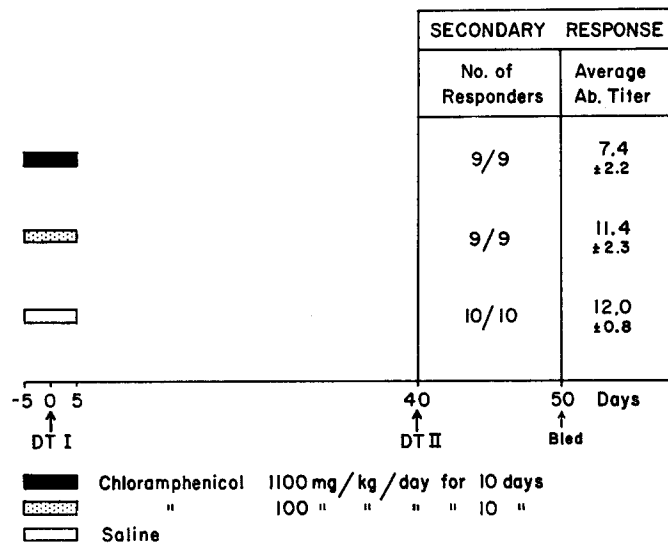▨▨▨   "   100 "   "   "   " 10 "
▭▭▭ Saline

Fig. 1. Effect of chloramphenicol for 5 days before and 5 days after first injection of antigen. DT I = diphtheria toxoid, 20 Lf subcutaneously, first injection; DT II = diphtheria toxoid, 20 Lf subcutaneously), second injection.

Results.—The sera of all animals were analyzed. The results are recorded in Fig. 1.

Both experimental groups responded to the second injection of diphtheria toxoid. However, the average antibody titer was 7.4 in mice injected with 1100 mg/kg/day of chloramphenicol, while the average of animals injected with 100 mg/kg/day was 11.4. The average titer of the control groups was 12.0. The difference between titers of 7.4 on the one hand and 11.4 and 12.0 on the other hand is significant ($P < 0.01$); that between titers of 11.4 and 12.0 is not.

Experiment 3: Effect on Priming of High Doses of Chloramphenicol Administered for 10 Days, Starting on the Day of Sensitization (Fig. 2).—

Two experimental groups of 20 and 30 animals each were injected intra-peritoneally with 10 mg of chloramphenicol 1 hour before the administration

of diphtheria toxoid, and chloramphenicol, 1500 mg/kg/day, was continued for 10 days. A control group of 10 mice was injected with saline.

The first experimental group was bled 4 days after the second injection of antigen; the second experimental and the control groups were bled after 10 days. The weight loss in the experimental groups at the end of chloramphenicol treatment was 6.3 per cent. Control animals lost 1.5 per cent. The death rate in both experimental groups was 10.0 per cent during the administration of chloramphenicol; there were no deaths in the control group.

	SECONDARY RESPONSE		
	No. of Responders	Average Ab. Titer	
		all animals	responders
	4/20 (20%)	1.4	6.7 ±0.5
	9/23 (39%)	3.3	8.4 ±2.0
	10/10 (100%)	8.7	8.7 ±2.2

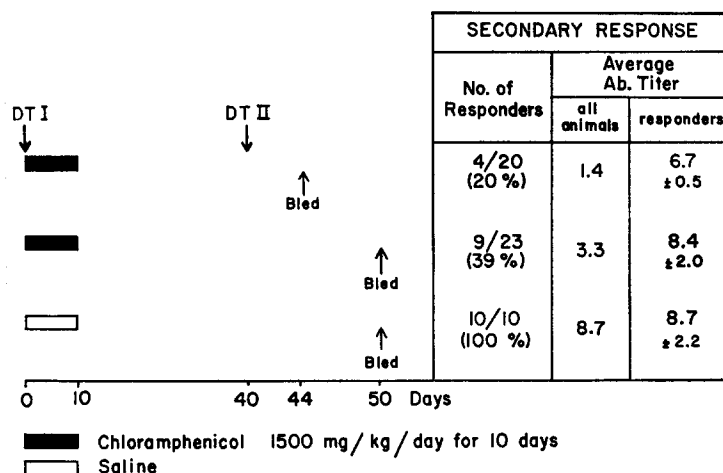Chloramphenicol 1500 mg/kg/day for 10 days
Saline

Fig. 2. Chloramphenicol's effect on priming when given for 10 days after the first dose of antigen. Abbreviations as in Fig. 1.

Results.—The sera of 53 animals were analyzed. The results are recorded in Fig. 2.

These data show that by 4 days after the injection of the second dose of antigen, the potential responders had not had time to initiate an immune response. Chloramphenicol in a dose of 1500 mg/kg/day inhibits priming in about two-thirds of the animals. The animals in which priming has not been inhibited by the drug develop a secondary immune response with antibody titers similar to those of the control group.

Experiment 4: Effect on Priming of Delayed Administration of High Doses of Chloramphenicol.—

Part I (Fig. 3).—Five experimental groups and two control groups of 15 animals each were used in this experiment. All groups were injected at the same time with diphtheria toxoid. In addition, the five experimental groups received 1500 mg/kg/day of chloramphenicol starting 6, 12, 24, 48, and 72 hours respectively after the administration of antigen and continuing for 15

days after the injection of antigen. One of the control groups was injected intraperitoneally with 10 mg of chloramphenicol 10 and 2 hours prior to injection of diphtheria toxoid. Then chloramphenicol was continued as in experimental groups. In the second control group, chloramphenicol was replaced by saline. All groups received a second injection of diphtheria toxoid 40 days after the first, and were bled 10 days later. A third injection of diphtheria toxoid was administered 34 days after the second, and the mice were again

	SECONDARY RESPONSE			TERTIARY RESPONSE	
	No. of Responders	Average Ab. Titer		No. of Responders	Average Ab. Titer
		all animals	responders		
72 Hrs.	11/13 (85%)	8.4	10.0 ±1.7	13/13	12.0 ±1.4
48	10/15 (66%)	5.5	8.3 ±2.3	14/14	10.9 ±1.2
24	5/11 (45%)	3.1	6.8 ±3.0	10/10	11.8 ±2.1
12	6/12 (50%)	3.9	7.8 ±2.5	12/12	10.6 ±0.8
6	6/14 (43%)	3.4	7.8 ±3.5	13/13	9.0 ±1.0
	1/11 (9%)	0.5	6.0 ±0	11/11	8.7 ±1.5
	11/15 (73%)	6.7	9.1 ±2.8	15/15	9.5 ±2.2

0   40   50   74   84   Days
DT I   DT II Bled   DT III   Bled

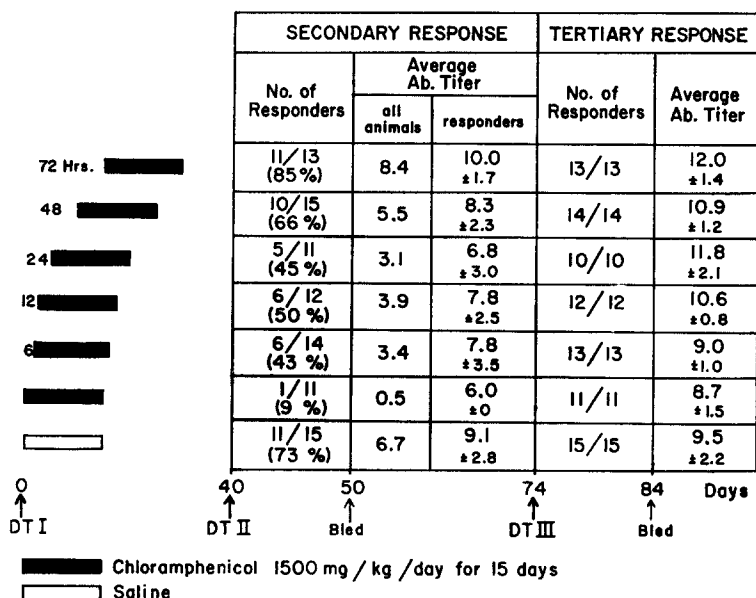▮▮▮▮ Chloramphenicol 1500 mg / kg /day for 15 days
☐☐☐☐ Saline

FIG. 3. The critical nature of timing of chloramphenicol in inhibiting priming of the secondary antibody response. DT I, DT II, DT III = 1st, 2nd, and 3rd injections of diphtheria toxoid, 20 Lf subcutaneously.

bled 10 days later. The average weight loss of the five experimental groups and of the first control group, all of which received chloramphenicol, was 3.8 per cent. The death rate for these six groups was 15.5 per cent. The second control group (saline) showed a weight gain of 6.2 per cent and had no deaths.

Results, secondary response: The sera of 91 animals were analyzed. The results are recorded in Fig. 3.

Both groups in which 72 and 48 hours had elapsed between the injection of antigen and the administration of chloramphenicol had many which responded. Antibody titers referred to them were 10.0 and 8.3 respectively, that is to say, not significantly different from the titer of 9.1 found in the untreated control group ($P > 0.3$ and $P > 0.15$). In the groups where chloramphenicol was started 24, 12, and 6 hours after diphtheria toxoid, the percentage responding

was similar in all three groups, but lower than in the former two groups. The average antibody titers were lower whether referred to all animals or only to those responding; in the latter case they were not significantly different from the average titer of the responsive mice in the untreated control group ($P > 0.1$; $P > 0.3$; $P > 0.3$). In the group where chloramphenicol was started before antigen only one responded, with a modest antibody titer of 6.0. In the untreated control group, 4 animals of 15 had no antibody. Although a few non-responders to the second stimulation were expected in untreated animals (11), there were more in this group than in any of our other experiments.

Results, teritary response: The sera of 89 animals were analyzed; one of them was discarded because of non-specific hemagglutination in the control tube.

All animals responded to the third injection of diphtheria toxoid. In the two groups where chloramphenicol had been started 3 days and 1 days respectively after diphtheria toxoid, the titers of 12.0 and 12.8 were significantly higher than the titer 9.5 of the untreated control group ($P < 0.001$ and $P < 0.01$). The differences between the other groups and the untreated control group were not significant.

Part II (Fig. 4).—When the results of Part I were known, it was decided to investigate, with larger groups of mice, whether antigen administered less than 6 hours before chloramphenicol still allowed antibody formation. Two experimental groups each contained 35 animals and two control groups of 15 and 10 animals respectively. All animals were injected with diphtheria toxoid at the same time. In addition, the two experimental groups received chloramphenicol, 1500 mg/kg/day, for 15 days. The drug was started 1 or 3 hours after the administration of antigen. In the first control group, 10 mg of chloramphenicol was injected 10 and 2 hours prior to diphtheria toxoid. The drug was then continued as in the experimental groups. In the second control group, chloramphenicol was replaced with saline. A second and a third injection of diphtheria toxoid were administered 40 and 74 days after the first one, and the mice were bled 10 days after these injections. The average weight loss in the two experimental groups and in the first control group all of which received chloramphenicol, was 8.0 per cent. The death rate was 9.4 per cent. The animals of the second control group, though receiving no chloramphenicol, lost 2.3 per cent of their initial weight, but there were no deaths.

Results, secondary response: The sera of 68 animals were analyzed, 7 of which were discarded because of non-specific hemagglutination in the control tubes. The results are recorded in Fig. 4.

In the group in which chloramphenicol was started 3 hours after diphtheria toxoid, 23 out of 28 animals formed antibodies. The average titer of the whole group was 7.8 and the titer of the untreated control group was 9.4. If the titer of the experimental group is taken as the average of responders only, there is no difference from the titer of the untreated animals.

In the group in which only 1 hour had elapsed between antigen injection and the beginning of chloramphenicol treatment, only 10 out of 24 animals responded. The average titer for the whole group was 3.3, but responders made a complete immune response with an average titer of 7.8, which is not significantly different from the titer of the control group ($P > 0.2$).

Results, tertiary response: The sera of 66 animals were analyzed and 6 of them were discarded because of non-specific hemagglutination in the control tubes. In the group which received chloramphenicol 3 hours after diphtheria toxoid, all animals responded to the third injection of antigen with a titer of 13.3. The

	SECONDARY RESPONSE			TERTIARY RESPONSE		
	No. of Responders	Average Ab. Titer		No. of Responders	Average Ab. Titer	
		all animals	responders		all animals	responders
3 hrs. ▉	23/28 (82%)	7.8	9.4 ±1.9	26/26 (100%)	13.3	13.3 ±0.62
▉	10/24 (42%)	3.3	7.8 ±2.7	24/25 (96%)	13.6	14.2 ±1.13
▭	8/9 (89%)	8.3	9.4 ±2.1	9/9 (100%)	13.2	13.2 ±1.46

```
O        40        50              74        84        Days
↑        ↑         ↑               ↑         ↑
DT I     DT II     Bled            DT III    Bled
```

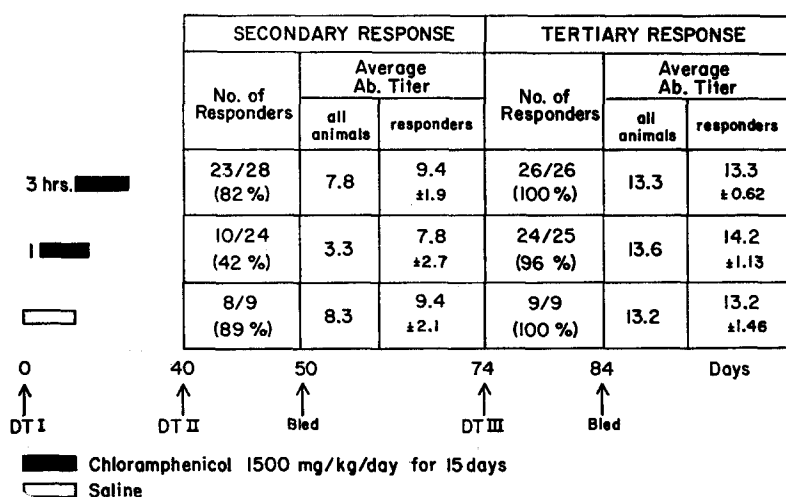▉ Chloramphenicol 1500 mg/kg/day for 15 days
▭ Saline

FIG. 4. Further examination of the time of administration in its relation to the inhibition of priming. Abbreviations as in Fig. 3.

average titer of untreated animals was 13.2. There was one non-responder in the group of animals which had received chloramphenicol 1 hour after the second injection of antigen. The average titer for the whole group was 13.6; or referred to the responders only, 14.2. The titers of both experimental groups are very close to the titer of the control group and the slight differences are not significant. The average titers are apparently higher in Part II than in Part I of Experiment 4 because the standard end-point of the batch of sensitized red cells was higher in Part II than in Part I.

Part III (Fig. 5).—The results obtained in Part I with the drug control group, which received chloramphenicol for 15 days beginning 10 and 2 hours before diphtheria toxoid, are in close agreement with those obtained by Butler and Coons (1). However, an additional group of 15 mice was treated in the same way as this control group, while 10 untreated control mice received saline in place of chloramphenicol. Chloramphenicol-treated mice lost 1.4 per cent of

their initial weight; there were no deaths in this group. Untreated control mice gained 0.5 per cent.

Results: The sera of 23 animals were analyzed. The results are recorded in Fig. 5.

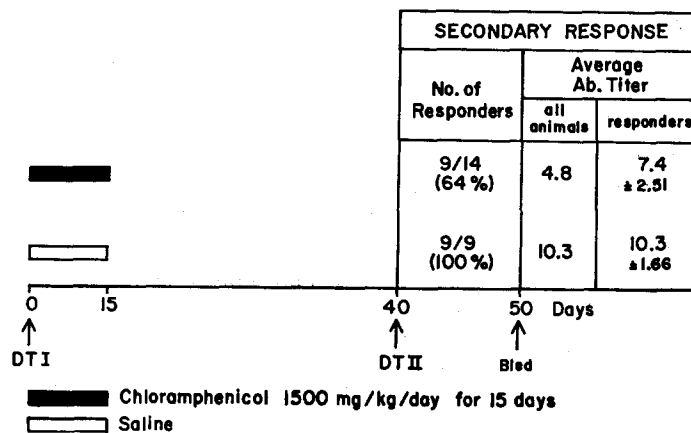There were a surprisingly large number of responders in the group treated

SECONDARY RESPONSE		
No. of Responders	Average Ab. Titer	
	all animals	responders
9/14 (64%)	4.8	7.4 ±2.51
9/9 (100%)	10.3	10.3 ±1.66

0   15   40   50 Days

↑   ↑   ↑
DT I   DT II   Bled

■■■■ Chloramphenicol 1500 mg/kg/day for 15 days
☐☐☐ Saline

FIG. 5. Prolongation of chloramphenicol administration for 15 days.

TABLE II

Concentration of Chloramphenicol in the Blood of Mice after the Last of 10 Injections

Group	Time of bleeding after last injection of chloramphenicol	Chloramphenicol equivalents per ml of whole blood
		μg
I	15 to 30 min.	333
II	4 hrs.	15
III	8 hrs.	6

Each mouse had received 500 mg/kg body weight every 8 hours for 10 injections before sampling was begun.

with chloramphenicol. However, the average antibody titer obtained in this group, referred either to all animals or to responders only, shows a highly significant difference from the average titer of the untreated group. Moreover, among the 9 responders, only 5 had a titer belonging clearly to the range encountered during the secondary response, while 4 had a titer within the limits of the primary response.

Chloramphenicol Blood Levels.—

The results presented in Table II show that 15 to 30 minutes after the last of 10 injections of 1500 mg/kg/day of chloramphenicol for 3 days (500 mg

every 8 hours), the concentration of the drug was 333 μg chloramphenicol equivalents/ml whole blood, a result consistent with the data of Thompson, Dunn, and Winder (12). The drug level dropped rapidly thereafter and had decreased to 6 μg/ml of chloramphenicol equivalents after 8 hours.

DISCUSSION

The inhibitory effect of chloramphenicol on priming of the antibody response depends on a prompt start, a long period of administration, and high dosage. The fact that a subsequent secondary response can be inhibited indicates that the phenomenon of priming exists. The following discussion is divided into two sections, the first addressed to the experiments themselves, the second, to the broader implications of the results.

The administration of chloramphenicol must start not later than an hour or so after the first injection of antigen. These initial experiments do not allow very precise timing because the antigen was not injected intravenously. However, it is clear from Experiments 3 and 4 (See Figs. 3 and 4) that within an hour or 2 after the subcutaneous injection of a relatively small dose of antigen (20 Lf = 56 μg) the process of priming had continued beyond the point at which it could be inhibited.

The duration of the treatment is also important. It is obvious (Fig. 1) that no effect is produced by treatment which lasted only 5 days after antigen injection. Presumably enough antigen persisted to prime some cells after the drug was stopped. Continuation for 10 days in maximum dosage (1500 mg/kg daily) had a distinctly inhibitory effect (61 per cent). Butler and Coons (1) gave the drug for 12 days and found 90 per cent inhibition, while the administration here for 15 days produced various results from 90 per cent (Fig. 3) to 36 per cent (Fig. 5).

Finally, the dose most effective, 1500 mg/kg daily, is the maximum dose tolerated for 15 days. It can be seen that the blood level obtained even on our schedule of injections every 8 hours drops precipitously between doses, reaching 15 μg of chloramphenicol equivalents by the 4th hour, and only 6 μg by the 8th hour. Thompson, Dunn, and Winder (12) found that these units were higher by a factor of more than 1.5 than the level of microbiologically active chloramphenicol. Hence our blood levels were about 1 μg at the mid-point between two doses, and the minimum level was <0.4 μg. That this is a borderline concentration is indicated by the fact that from 10 to 40 per cent of the animals responded in spite of it. It is *below* the range of biologically active concentrations (20 μg/ml) which inhibits the secondary antibody response *in vitro* (6) but *in* that found by Weisberger *et al.* (7) (0.3 μg/ml) to prevent peptide synthesis in a cell-free system. Earlier reports of the relative insensitivity of mammalian systems (*e.g.* references 13, 14) are perhaps due to the fact that mammalian messenger RNA is relatively more stable and that

chloramphenicol interferes only with new messenger. Moreover, it appears that priming is more sensitive to chloramphenicol than is the induction of a secondary response (1). Indeed, bacteria and the cell-free systems derived from them require ranges from 10 to 50 μg/ml for inhibition (15–18) more like the secondary response in tissue culture.

The results of these experiments are epitomized in Fig. 3, which shows the steadily increasing inhibition produced by chloramphenicol as the administration was begun progressively closer to the first dose of antigen. It is evident that the process of priming, whatever it entails, begins immediately after contact with antigen, and that its initial step is essentially complete in from 48 to 72 hours. There are evidently subsequent steps as well, requiring 3 to 4 weeks before the process reaches its maximum level (11), with which chloramphenicol probably does not interfere since its administration for the first 15 days of the period has no apparent effect once the first 48 hour period is past.

Turning now to the immunological implications of these experiments, it is necessary to discuss priming. In 1953, Stevens (19) reported experiments in which rabbits were irradiated with 500 R 40 hours before an intravenous injection of 0.25 to 0.4 mg of bovine γ-globulin(BGG)/kilo. Three months later they were challenged with 0.15 to 0.3 mg BGG/kg. They made a primary response in contrast to non-irradiated controls, which made a secondary response. X-Radiation had prevented priming. White has found the same (20).

In 1955 Leduc et al. (21) suggested, on the basis of the marked difference in the number of cells engaged in the first and in subsequent responses to antigen, that two encounters with antigen were necessary before any antibody synthesis could occur. It was suggested that some necessary event takes place between the two exposures to specific stimulation; the few cells which synthesize antibody after a single injection of antigen have chanced to experience such an event before the antigen concentration will have fallen too low for a second hit to occur. Sercarz and Coons (22) elaborated this suggestion, assigning non-commital labels to the postulated stages. The cell in the normal state was designated the x cell; the primed cell was called the y cell, and the cell stimulated to multiply, differentiate, and synthesize antibody, the z cell. Although the steps in antibody formation have been divided into induction and production, (e.g. Sterzl, reference 23) it seems a better analogy with induced enzyme formation to restrict the term "induction" to the triggering of the y cell:

$$x \xrightarrow{\text{priming}} y \xrightarrow{\text{induction}} z \text{ (production)}$$
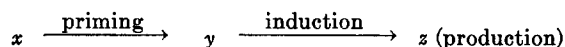
Perkins and Makinodan (24) recently studied the responses of spleen cells transferred from mice to isologous x-irradiated recipients. Some of the donors had been primed with sheep erythrocytes, some had not. They investigated

the response to an injection of sheep RBC of the recipients at various time intervals after transfer. They found that recipients of primed cells maintained a steady level of responsiveness for the first 9 days after transfer whereas recipients of normal (unprimed) cells rapidly lost the responsiveness they possessed on the day of transfer. Perkins and Makinodan offered the interpretation that the normal population was multipotential and responsive to other stimuli, such as erythropoietin or other antigens, whereas the primed cell was not so diverted. They called the responsive cells "potentially competent (PC)," and divided them into "at least two" compartments, PC_1 or multipotent, unprimed cells, and PC_2, or primed cells, specifically responsive. Clearly this scheme is identical with ours, $x = PC_1$, $y = PC_2$.

If such a scheme is correct, an additional unknown step, possibly related to differentiation, is required in antibody formation. It is this step, or a series of them, which is blocked in some way by chloramphenicol, 6-mercaptopurine (6-MP),[4] triethylenethiophosphoramide, and ethylenediaminetetraacetate at levels which do not interfere with the induction step; *i.e.*, the secondary response.[5]

SUMMARY

Young adult mice were primed with 20 Lf (56 μg) of diphtheria toxoid and given a second injection of the same size 40 days later. This procedure produces a reproducible secondary response which can be used as a standard. Chloramphenicol in maximum dosage prevents the unknown process by which the animal is primed for the second response. To be fully inhibitory, the drug must be given from the hour of the first antigen injection in maximum dosage for 2 weeks. A delay of 48 hours in starting the drug allows completion of the priming process, and shorter delays produce partial inhibition. Hence the initiation of priming is a rapid process sensitive to chloramphenicol. Subsequent changes in the cell population necessary for the full development of priming are not sensitive to chloramphenicol.

The secondary antibody response is not inhibited in mice by chloramphenicol at the doses employed.

[4] The prevention of priming would make it easier to establish immune paralysis with a large dose of antigen. This is evidently the role played by both x-ray and 6-MP in the establishment of "tolerance" (25, 26).

[5] It is true that La Plante *et al.* (27) found that 6-MP suppressed the secondary response in rabbits at dosage levels twice those effective here in preventing priming, and Ambrose and Coons (6), in work mentioned above, have shown that chloramphenicol interferes with the secondary response at levels of about 20 μg/ml in tissue culture; this is a higher level than the probable average blood level maintained on the maximum dosage used in the mouse experiments reported in this paper.

BIBLIOGRAPHY

1. Butler, W. T., and Coons, A. H., Studies on antibody production. XII. The effect of various drugs upon priming of the antibody response, *J. Exp. Med.*, 1964, **120**, 1051.

2. Slanetz, C. A., The influence of antibiotics on antibody production, *Antibiot. and Chemotherapy*, 1953, **3**, 629.

3. Watson, K. C., Effect of chloramphenicol on the production of antibody to bacterial and non-bacterial antigens, *S. Afr. Med. J.*, 1958, **32**, 684.

4. Szuravleva, E. D., and Gorchakova, I. P., The effects of antibiotics on the formation of antibodies, *J. Microbiol. Epidemiol. and Immunobiol.*, 1959, **30**, 14.

5. Nathan, H. C., Bieber, S., Elion, G. B., and Hitchings, G. H., Detection of agents which interfere with the immune response, *Proc. Soc. Exp. Biol. and Med.*, 1961, **107**, 796.

6. Ambrose, C. T., and Coons, A. H., Studies on antibody production. VIII. The inhibitory effect of chloramphenicol on the synthesis of antibody in tissue culture, *J. Exp. Med.*, 1963, **117**, 1075.

7. Weisberger, A. S., Armentrout, S., and Wolfe, S., Protein synthesis by reticulocyte ribosomes. I. Inhibition of polyuridylic acid-induced ribosomal protein synthesis by chloramphenicol, *Proc. Nat. Acad. Sc.*, 1963, **50**, 86.

8. Kučan, Z., and Lipmann, F., Differences in chloramphenicol sensitivity of cell-free amino acid polymerization systems, *J. Biol. Chem.*, 1964, **239**, 516.

9. Stavitsky, A. B., Micromethods for the study of proteins and antibodies. I. Procedure and general application of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells, *J. Immunol.*, 1954, **72**, 360.

10. Glazko, A. J., Wolf, L. M., and Dill, W. A., Biochemical studies on chloramphenicol (chloromycetin). I. Colorimetric method for the determination of chloramphenicol and related nitro compounds, *Arch. Biochem. and Biophysics*, 1949, **23**, 411.

11. Fecsik, A. I., Butler, W. T., and Coons, A. H., Studies on antibody production. XI. Variation in the secondary response as a function of the length of the interval between two antigenic stimuli, *J. Exp. Med.*, 1964, **120**, 1041.

12. Thompson, D. E., Dunn, M. C., and Winder, C. V., Comparison of the action of chloramphenicol (chloromycetin) and penicillin G against relapsing fever in mice, *J. Infect. Dis.*, 1950, **86**, 110.

13. LePage, G. A., Effects of chloramphenicol on incorporation of glycine 2-C^{14} into mammalian tumor cell proteins and purines, *Proc. Soc. Exp. Biol. and Med.*, 1953, **83**, 724.

14. Yunis, A. D., and Harrington, W. J., Patterns of inhibition by chloramphenicol of nucleic acid synthesis in human bone marrow and leukemic cells, *J. Lab. and Clin. Med.*, 1960, **56**, 831.

15. Gale, E. F., and Folkes, J. P., The assimilation of amino-acids by bacteria. XV. Action of antibiotics on nucleic acid and protein synthesis in *Staphylococcus aureus*, *Biochem. J.*, 1953, **53**, 493.

16. Wisseman, C. L., Smadel, J. E., Hahn, F. E., and Hopps, H. E., Mode of action of

chloramphenicol (C.A.). I. Action of chloramphenicol on assimilation of ammonia and synthesis of proteins and nucleic acids in *Escherichia coli*, *J. Bact.*, 1954, **67**, 662.

17. Spiegelman, S., Protein and nucleic acid synthesis in subcellular fractions of bacterial cells, *in* Recent Progress in Microbiology, Symposium of the 7th International Congress of Microbiology, L. Tunevall, editor Stockholm, 1958, Stockholm, Almqvist & Wiksell, 1959, 81.

18. Hopkins, J. W., Amino acid activation and transfer to ribonucleic acids in the cell nucleus, *Proc. Nat. Acad. Sc.*, 1959, **45**, 1461.

19. Stevens, K. M., Antigen retention in the rabbit, *J. Exp. Med.*, 1953, **97**, 247.

20. White, R. G., cited by White, R. G., in Factors affecting the antibody response, *Brit. Med. Bull.*, 1963, **19**, 207.

21. Leduc, E. H., Coons, A. H., and Connolly, J. M., Studies on antibody production. II. The primary and secondary responses in the popliteal lymph node of the rabbit, *J. Exp. Med.*, 1955, **102**, 61.

22. Sercarz, E., and Coons, A. H., The exhaustion of specific antibody producing capacity during a secondary response, *in* Mechanisms of Immunological Tolerance, (M. Hasek, A. Lengerova, and M. Vojtiskova, editors), Prague, Publishing House of the Czechoslovakian Academy of Science, 1962, 73.

23. Sterzl, J., Quantitative and qualitative aspect of the inductive phase of antibody formation, *J. Hyg. Epidemiol. Microbiol. and Immunol.*, 1963, **7**, 301.

24. Perkins, E. H., and Makinodan, T., Relative pool size of potentially competent antibody-forming cells of primed and non-primed spleen cells grown in *in vivo* culture, *J. Immunol.*, 1964, **92**, 192.

25. Schwartz, R. A., and Dameshek, W., The role of antigen dosage in drug-induced immunologic tolerance, *J. Immunol.*, 1963, **90**, 703.

26. Nachtigal, D., and Feldman, M., Immunological unresponsiveness to protein antigens in rabbits exposed to x-irradiation or 6-mercaptopurine treatment, *Immunology*, 1963, **6**, 356.

27. LaPlante, E. S., Condie, R. M., and Good, R. A., Prevention of secondary immune response with 7-mercaptopurine, *J. Lab. and Clin. Med.*, 1962, **59**, 542.