SUPPRESSION OF ANTIBODY SYNTHESIS AND PROLONGATION OF HOMOGRAFT SURVIVAL BY CHLORAMPHENICOL*

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PLATES 19 AND 20

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Previous experiments have demonstrated that chloramphenicol can inhibit protein synthesis in mammalian cell-free systems as effectively as it inhibits protein synthesis in analogous microbial systems (1, 2). Significant inhibition occurs only when protein synthesis is stimulated by adding additional template RNA to ribosomes, there being comparatively little inhibition of protein synthesis in the absence of added stimulatory RNA. It was postulated from these studies that chloramphenicol may inhibit the function of messenger RNA by preventing its attachment to ribosomes.

Recently Ambrose and Coons demonstrated that chloramphenicol also inhibits protein synthesis by intact mammalian cells *in vitro* (3). These authors demonstrated that chloramphenicol can inhibit antibody synthesis in cultures of lymph node fragments and suggested that chloramphenicol might exert its inhibitory effect by blocking the function of messenger RNA formed in response to the antigenic stimulus.

The present studies were undertaken to determine whether chloramphenicol would inhibit *de novo* protein synthesis *in vivo* following antigenic stimulation. The data demonstrate that chloramphenicol can markedly suppress the primary immune response in rabbits without substantially modifying a subsequent anamnestic response and that chloramphenicol can prolong the survival of skin homografts. The observations are in accord with the hypothesis that chloramphenicol suppresses antibody synthesis by interfering with the function of messenger RNA.

Material and Methods

White New Zealand rabbits and grey chinchilla rabbits weighing between 2 and 3 kg were maintained in individual cages on Purina rabbit chow and tap water. In addition, 2 black and white rabbits were used in homotransplantation experiments.

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Chloramphenicol[1] was prepared as a suspension with carboxymethyl cellulose in 0.75 per cent NaCl in the proportion of 1 gm of chloramphenicol and 10 mg of carboxymethyl cellulose in 3 ml of 0.75 per cent NaCl. The chloramphenicol suspension was injected intramuscularly in the desired dosage with half the daily dose being administered at 12-hour intervals. In all instances, chloramphenicol was administered 24 hours prior to initiating the antigenic stimulus.

Humoral Antibody Synthesis.—The response of rabbits to antigenic stimulation was determined in control and experimental groups as shown in Tables I to III. The effect of chloramphenicol on both the primary immune response and on the secondary response was determined.

Primary immune responses were studied in the following groups of animals: (*a*) 10 control animals on a standard diet; (*b*) 5 control animals, starved; (*c*) 9 animals receiving chloramphenicol 0.15 to 0.30 gm per kg for 10 days; (*d*) 18 animals receiving chloramphenicol 0.5 to 0.6 gm per kg for 10 to 14 days (Table II).

Anamnestic responses were determined in the following groups of animals: (*a*) 8 control animals that had a normal primary response; (*b*) 12 animals whose primary immune response had been suppressed by chloramphenicol; (*c*) 9 animals receiving chloramphenicol 0.5 gm per kg for 10 days at the initiation of the secondary response (Table III).

Antibody synthesis was induced by injecting 4 mg of alum-precipitated bovine gamma globulin (BGG) (fraction II) into each hindfoot-pad. Animals were bled from marginal ear veins at appropriate intervals; the serum was separated and stored at −20°C until assayed. Antibody assays were performed by the tanned, formalinized erythrocyte hemagglutination technique described by Daniel *et al.* (4). The amount of antibody was expressed in terms of dilution titer of the sera or as a reciprocal of the dilution titer. Serum levels of free chloramphenicol were determined on all samples by a modification of the method of Glazko *et al.* (5) previously described (6).

The animals were weighed twice weekly. Hemoglobin, hematocrit, leukocyte count, reticulocyte count, and differential counts were obtained twice weekly while the animals were receiving chloramphenicol.

The type of antibody synthesized by animals with delayed and partially suppressed immune responses was determined by ultracentrifugation of sera in sucrose gradients by the method of Edelman *et al.* (7). Determinations were performed on the sera of the 1 rabbit manifesting incomplete suppression of primary antibody synthesis and on the sera of the 4 rabbits with delayed and suppressed anamnestic responses.

Skin Homografts.—Transplants were performed using skin from rabbit ears and in all but 4 instances the transplants were made from albino rabbits to grey chinchilla rabbits or *vice versa*. The remaining transplants were exchanged between albino and black and white rabbits. Administration of chloramphenicol was begun 24 hours before transplanting the grafts and was continued in varying amounts and for varying periods as shown in Table I. Circular full thickness skin grafts, 2.5 cm in diameter, were dissected simultaneously from paired albino and grey chinchilla rabbits and exchanged. Grafts were sutured in place with 8 to 10 silk 0000 sutures. No dressings were applied. The homografts were inspected daily and survival of the grafts readily determined by gross changes consisting of hemorrhage with subsequent hardening and eschar formation (Figs. 1 *a* and 1 *b*).

The effect of varying the dose and duration of administration of chloramphenicol on the survival of the skin homografts was determined in 24 animals and compared with the survival of skin homografts in control groups consisting of 19 animals. The following groups of animals were studied: (*a*) 12 control animals on a standard diet; (*b*) 7 control animals, starved; (*c*)

[1] Chloramphenicol was generously supplied by Parke, Davis and Co., Detroit.

6 animals receiving 0.15 to 0.30 gm per kg of chloramphenicol daily for 10 days; (*d*) 16 animals receiving 0.5 to 0.6 gm per kg of chloramphenicol daily for 11 to 14 days; (*e*) 2 animals receiving chloramphenicol 0.6 gm per kg daily for 21 days (Tables I and IV). In 21 of the animals, the ability of chloramphenicol to modify both circulating antibody synthesis and homograft survival was determined simultaneously by injecting BGG when the homografts were transplanted (Table 1).

TABLE I

The Effect of Chloramphenicol on Homografts Survival and on Primary Antibody Response

Homograft survival					Primary immune response
Chloramphenicol			Graft Survival	Day Rejected after chloramph. discontinued	
Amount	Duration	Mean Serum Levels			Anti-BGG titer (max)
gm/day	*days*	*μg/ml*	*days*		
0.6	21	12.9	>27*	>5	0
0.6	21	11.0	>28*	>7	0
0.6	14	20.2	20	6	0
0.6	14	17.5	>26*	>12	0
0.5	12	12.1	12	0	0
0.5	12	8.7	17	5	0
0.5	12	4.7	22	10	0
0.5	12	11.5	18	6	0
0.5	12	16.2	21	9	0
0.5	12	12.9	25	13	0
0.5	12	10.5	46	34	0
0.5	12	13.8	18	6	Not done
0.5	12	8.7	16	4	" "
0.5	12	11.4	14	2	" "
0.5	12	13.8	16	4	0
0.5	12	8.5	26	14	0
0.5	12	16.4	24	12	0
0.5	12	6.4	20	8	0
0.5	12	10.8	Not done	—	1/160 (20 days)
0.5	12	11.1	" "	—	0
0.6	12	17.5	" "	—	0
0.3	10	7.2	15	5	0
0.3	10	4.8	15	5	1/40 (20 days)
0.15	10	1.9	9	0	1/40 (14 days)
0.15	10	4.2	8	0	1/160 (11 days)
0.15	10	4.1	10	0	1/160 (10 days)
0.15	10	7.5	10	0	1/320 (8 days)
0.25	10	Not done	Not done	—	1/320 (11 days)
0.25	10	" "	" "	—	1/40 (20 days)
0.25	10	" "	" "	—	1/1280 (11 days)

* Died with homograft intact.

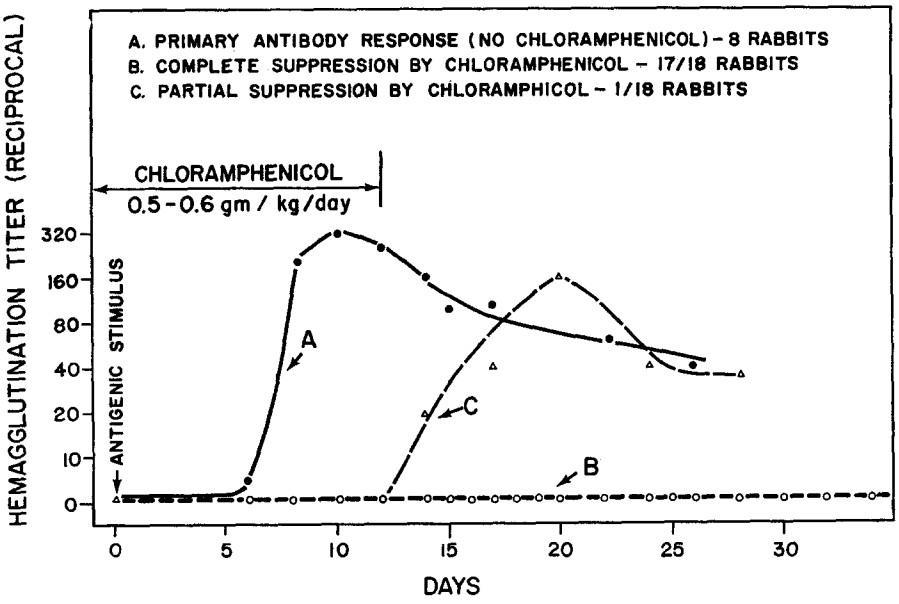
RESULTS

Effect of Chloramphenicol on Primary Antibody Response.—The effects of chloramphenicol on the primary immune response are summarized in Tables I and II and in Text-fig. 1. Normal antibody responses were obtained in 10

TABLE II

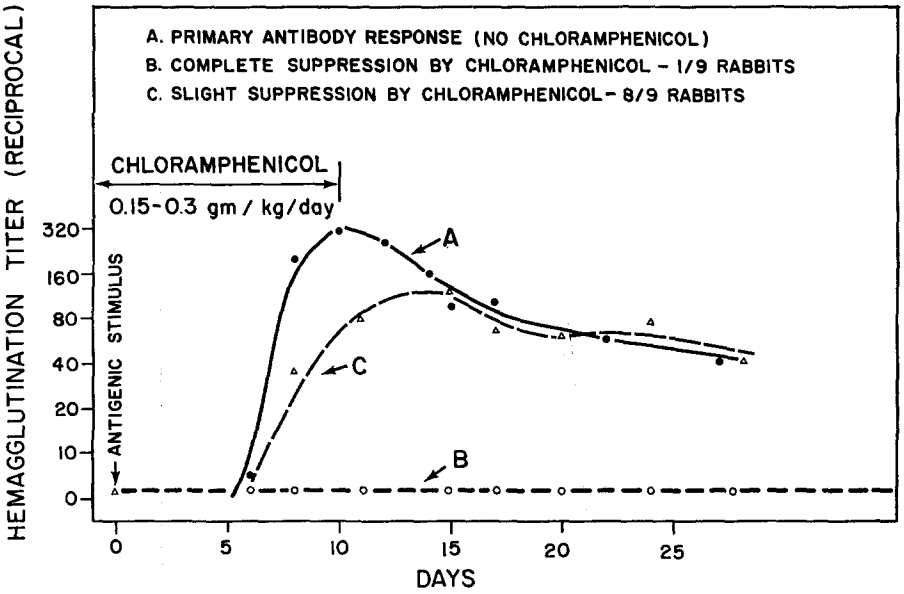
The Effect of Chloramphenicol on the Primary Immune Response

	No. of rabbits	Primary immune response
No chloramphenicol (normal diet)	10	Normal
No chloramphenicol (starved)	5	Normal
Chloramphenicol, 0.15–0.30 gm/kg, 10 days	9	Complete suppression (1) Slight suppression (8)
Chloramphenicol, 0.5–0.6 gm/kg, 10–14 days	18	Complete suppression (17) Partially suppressed and delayed (1)



TEXT-FIG. 1. Suppression of primary antibody response with chloramphenicol. Complete suppression of the primary antibody response was observed in 17 out of 18 rabbits receiving chloramphenicol in a dosage of 0.5 to 0.6 gm daily for 12 days (*B*). Average values calculated from the geometric mean of all values are plotted. Only one of the 18 rabbits developed measurable circulating antibody (*C*). Compared to the normal primary immune response (*A*), this response was delayed and slightly suppressed. Ultracentrifugation studies in sucrose gradients demonstrate that the initial antibody formed in the delayed response (*C*) is 19S in type.

rabbits on a standard diet, with circulating antibody detectable on the 6th day after injection of BGG and a maximum titer on the 10th day (mean titer 1/320, range 1/80 to 1/640). Since animals receiving chloramphenicol in the dosage employed in these experiments frequently lost weight, antibody formation was determined in an additional control group of 5 animals who were starved for a 10 day period. These animals also manifested normal antibody responses. No



TEXT-FIG. 2. Effect of smaller doses of chloramphenicol on the primary immune response. Smaller doses of chloramphenicol (0.15 to 0.3 gm per kg daily for 10 days) were almost completely ineffective in suppressing antibody response in 9 rabbits. Complete suppression of detectable circulating antibody was observed in only 1 rabbit (*B*). A slight suppression and a slight delay in appearance of antibody was demonstrable in 8 of the 9 rabbits (*C*) when compared to the normal primary immune response (*A*).

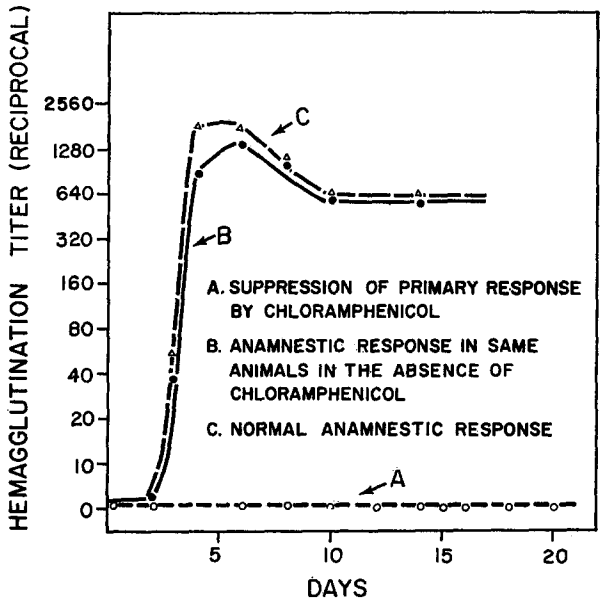
antibody formation was detectable over a 4 week period in 17 of 18 rabbits receiving chloramphenicol in a dosage of 0.5 to 0.6 gm per kg for 10 to 14 days (Text-fig. 1, *B*). The serum levels of free chloramphenicol in these animals ranged from 4.7 to 20.2 μg per ml with a mean value of 12.6 μg per ml. A delayed and partially suppressed primary antibody response occurred in 1 of the 18 rabbits (Text-fig. 1, *C*). In this animal, antibody formation was first detected on the 14th day and a maximum titer (1/160) was obtained on the 20th day. The mean serum chloramphenicol level in this rabbit was 10.8 μg per ml.

In contrast, 8 of 9 animals receiving chloramphenicol in a dosage of 0.15 to 0.3 gm per kg for 10 days exhibited a relatively insignificant suppression of the

TABLE III

The Effect of Chloramphenicol on the Anamnestic Response

	No. of rabbits	Anamnestic response
No chloramphenicol (normal primary immune response)	8	Normal
No chloramphenicol (primary suppressed by chloramphenicol)	12	Normal
Chloramphenicol (normal primary immune response), 0.5 gm/kg/day, 10 days	9	Slight delay and suppression (5) Delayed and partially suppressed (4)

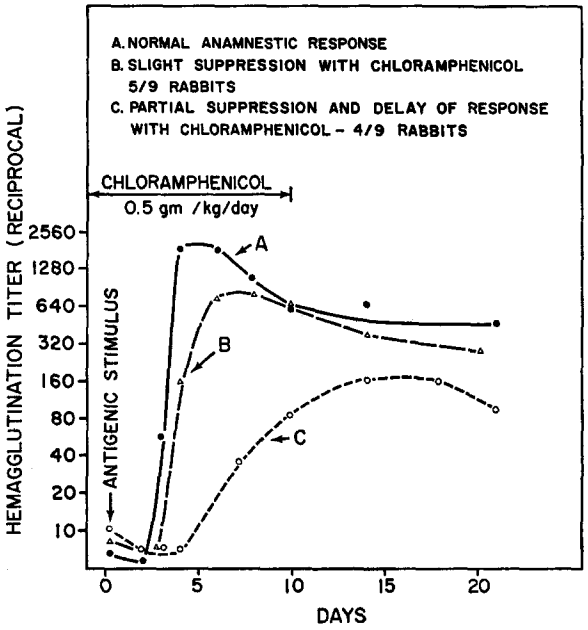


TEXT-FIG. 3. Normal anamnestic response after suppression of primary response with chloramphenicol. The primary immune response was completely suppressed by chloramphenicol in 12 rabbits (*A*). These same 12 rabbits were given booster doses of antigen after 10 to 12 weeks and exhibited an anamnestic response (*B*) which was indistinguishable from that obtained in animals whose primary immune response had not been inhibited by chloramphenicol (*C*).

primary antibody response (Tables I and II, Text-fig. 2). Complete suppression of antibody formation was observed in only 1 of the 9 rabbits. This animal received 0.3 gm per kg of chloramphenicol per day for 10 days and had a mean serum-free chloramphenicol level of 7.2 μg per ml. The mean serum level of

free chloramphenicol in all animals of this group was 5.9 μg per ml (range 1.9 to 7.5 μg per ml).

Twelve animals which had a complete suppression of the primary immune response with chloramphenicol were challenged with a second injection of BGG to determine whether the resulting antibody synthesis, in the absence of chloramphenicol, would be primary or accelerated in type (Table III). The second



TEXT-FIG. 4. The effect of chloramphenicol on anamnestic responses. Administration of chloramphenicol in a dosage of 0.5 gm per kg per day for 10 days delayed and partially suppressed the anamnestic response in 4 of 9 rabbits (*C*). Only slight suppression of response occurred in 5 of the 9 rabbits (*B*). The normal anamnestic response in 8 rabbits is shown in curve *A*.

antigenic stimulus was given 10 to 12 weeks after the initial injection of antigen, at a time when all chloramphenicol had been excreted. Each of these animals responded with a typical anamnestic response. Circulating antibody was detectable by the 3rd day with a peak value (mean titer 1/1280, range 1/640 to 1/2560) between the 4th and 6th days (Text-fig. 3, *B*). The anamnestic response in these animals was almost identical with that obtained in 8 control animals (Text-fig. 3, *C*).

The Effect of Chloramphenicol on the Anamnestic Response.—The effect of chloramphenicol on the anamnestic response is summarized in Table III and in Text-fig. 4. Chloramphenicol administration was begun 24 hours before injec-

tion of the second antigenic stimulation. Two types of responses were noted. Four of 9 rabbits receiving chloramphenicol in a dosage of 0.5 gm per kg for 10 days exhibited a delayed and partially suppressed anamnestic response when challenged with a second antigenic stimulus (Text-fig. 4, *B*). Circulating antibody was not apparent until the 7th day compared with appearance of antibody on the 3rd day in control animals (Text-fig. 4, *A*). Peak antibody titers were found on the 16th or 17th day compared with the peak on the 4th day in control animals. The mean maximum titer was 1/160 (range 1/80 to 1/320) in animals receiving chloramphenicol compared to 1/1280 (range 1/640 to 1/2560) in control animals. Five of the 9 rabbits receiving chloramphenicol exhibited a slight delay and suppression in the anamnestic response Text-fig. 4, *C*). The

TABLE IV

Survival of Skin Homografts in Rabbits Receiving Chloramphenicol

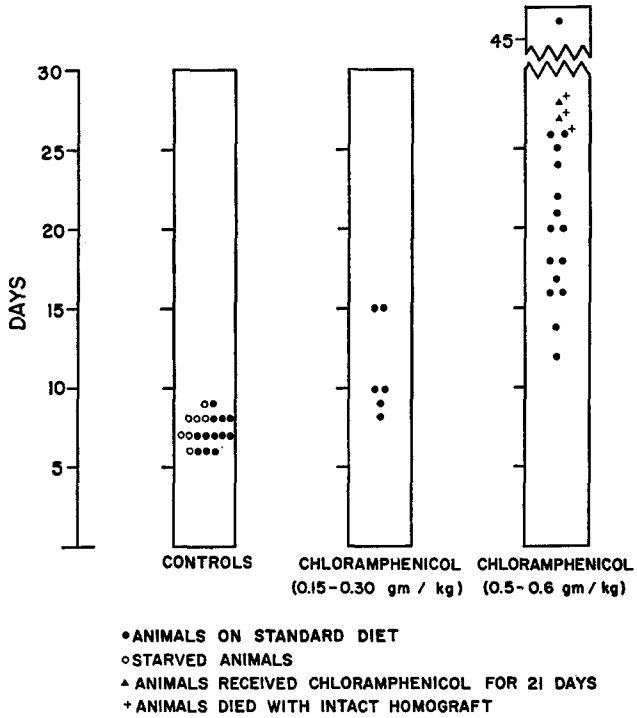
	No. of animals	Survival of homograft	
		Range	Mean
		days	*days*
No chloramphenicol (standard diet)	12	6–8	7
No chloramphenicol (starved)	7	6–9	7.5
Chloramphenicol, 0.15–0.30 gm/kg/day, 10 days	6	8–15	11
Chloramphenicol, 0.5–0.6 gm/kg/day, 11–14 days	16	12–46	22
Chloramphenicol, 0.6 gm/kg/day, 21 days	2*	27–28*	27.5

* These animals died without rejecting the homograft.

serum level of free chloramphenicol in these animals ranged from 2.5 μg per ml to 8.2 μg per ml with a mean value of 5.5 μg per ml. There was no significant difference in serum chloramphenicol levels in the two groups of animals.

The effect of Chloramphenicol on Survival of Skin Homografts.—The effect of chloramphenicol on the homograft reaction is summarized in Tables I and IV and in Text-fig. 5. Twelve animals on a normal diet rejected skin transplants within 6 to 8 days (mean, 7 days). Seven animals that were starved for 10 days and had weight losses comparable to those observed in animals receiving chloramphenicol, rejected skin grafts in 6 to 9 days (mean, 7.5 days). Sixteen rabbits receiving chloramphenicol in a dosage of 0.5 to 0.6 gm per kg for 10 to 14 days rejected their skin grafts in 12 to 46 days[2] (mean 22 days). The serum level of free chloramphenicol in these animals ranged from 4.7 to 20.2 μg per ml with a mean value of 15.8 μg per ml. Two rabbits which received chloramphenicol in the dosage of 0.6 gm per kg for 21 days died without rejecting the homograft. Death was due to a ruptured viscus in both instances. Most animals rejected

[2] One animal died with severe diarrhea on the 26th day with the homograft intact.



TEXT-FIG. 5. Summary data on survival of skin homografts in rabbits. All control animals rejected skin grafts in 6 to 9 days (mean, 7 days). Animals receiving chloramphenicol 0.15 to 0.30 gm per kg for 12 days rejected skin grafts in 8 to 15 days (mean, 11 days). Animals receiving chloramphenicol 0.5 to 0.6 gm per kg for 12 days rejected skin grafts in 12 to 46 days (mean, 22 days).

TABLE V

Hematologic Changes during Chloramphenicol Therapy

	Mean initial values	Mean values during therapy
Hematocrit	37 per cent	30 per cent
Reticulocyte count	5.6 per cent	3.6 per cent
Leukocyte count	8750 cells/mm^3	9550 cells/mm^3
Lymphocytes (absolute count)	3850 cells/mm^3	5750 cells/mm^3
Granulocytes (absolute count)	4885 cells/mm^3	2960 cells/mm^3

the homografts 6 to 13 days after chloramphenicol was discontinued. In only one instance was the graft rejected while the animal was receiving chloramphenicol. Graft rejection in control animals and graft survival in chloramphenicol-treated rabbits are illustrated in Figs. 1 *a* and 1 *b* and Figs. 2 *a* and 2 *b*.

Six rabbits which received chloramphenicol in a dosage of 0.15 to 0.30 gm per kg for 10 days rejected skin grafts in 8 to 15 days (mean 11 days). Serum levels of free chloramphenicol ranged from 1.9 to 7.5 μg per ml in these animals with a mean value of 5.9 μg.

Most of the animals were moderately anorectic while receiving chloramphenicol. Changes in weight ranged from −0.4 to +0.15 kg with an average loss of 0.15 kg in the animals receiving 0.5 to 0.6 gm per kg of chloramphenicol. With the exception of the 2 animals who died after receiving chloramphenicol for 21 days and the 1 animal who died with fulminating diarrhea, all animals rapidly regained their appetites and appeared to be normal in health.

TABLE VI

Type of Antibody Synthesized in Animals with Delayed Responses

Primary response			Secondary response		
Day	19S	7S	Day	19S	7S
	per cent	*per cent*		*per cent*	*per cent*
15	100	—	7	15	85
20	82	18	15	5	95
26	41	59	18	5	95
			21	2	98

The type of antibody synthesized by animals with delayed and partially suppressed immune responses was determined by ultracentrifugation of sera in sucrose gradients. Hemagglutination titers were plotted against the distance of antibody sedimentation through the gradient and the relative percentage of antibody calculated by estimating the area under the 7S and 19S portions of the curve obtained.

In the 1 animal with a delayed primary response, the initial antibody was of the 19S variety. In the 4 animals with delayed secondary responses, the initial antibody response was of the 7S variety.

Hematologic and Serum Protein Changes with Chloramphenicol.—In the dosage employed, chloramphenicol usually produced a modest fall in hematocrit, reticulocytes, and granulocytes without any significant change in the total leukocyte count (Table V). Studies performed by ultracentrifugation in sucrose gradients on sera from the 1 animal whose primary immune response was not completely suppressed, revealed a normal primary type of response (8) with respect to the sequence of appearance of 19S and 7S antibodies, the initial antibody being of the 19S variety (Table VI). The initial antibody response in the 4 animals whose anamnestic response was partially delayed and suppressed by chloramphenicol was the 7S variety (Table VI).

DISCUSSION

The data demonstrate that chloramphenicol can suppress primary hemagglutinating antibody synthesis *in vivo* without affecting the ability of the animals

subsequently to develop a normal anamnestic response. Although complete suppression of the primary antibody response was observed, it is possible that some antibody synthesis occurred which was not detectable by the assay technique employed. The subsequent prompt development of a normal anamnestic response indicates that cells were prepared for antibody synthesis during the period that chloramphenicol suppressed the synthesis of circulating antibody. These findings are in accord with the hypothesis that chloramphenicol inhibits *de novo* mammalian protein synthesis by blocking the function of messenger RNA without directly affecting DNA or RNA synthesis (2, 3).

Antibody formation implies that messenger RNA capable of directing the synthesis of specific protein must be formed and deposited on ribosomes. In the preceding report (2) it was demonstrated that chloramphenicol probably prevents the attachment of messenger RNA to ribosomes and thereby prevents its function in cell-free systems. This may be the mechanism by which chloramphenicol also suppresses antibody formation *in vivo*. The results of these experiments are in accord with the observations of Ambrose and Coons (3) on the inhibition by chloramphenicol of antibody synthesis by intact mammalian cells *in vitro*. These findings indicate that the inhibitory effect of chloramphenicol on *de novo* protein synthesis may be applicable to mammalian cells *in vivo* as well as to cell-free systems and intact cells *in vitro*. Although there is a possibility that the results are due to reversible hypoplasia of antibody-producing cells, no clear evidence of such hypoplasia was found in histologic sections of spleen, lymph nodes, gastrointestinal tract, pulmonary lymph follicles, or marrow of 4 animals receiving chloramphenicol in a dosage of 0.5 gm per kg. In particular there was no cellular degeneration such as is seen with the use of irradiation, alkylating agents, or purine analogs.

Chloramphenicol was more effective in suppressing the primary immune response than in suppressing the anamnestic response *in vivo*. Ambrose and Coons, however, were able to obtain complete inhibition of the secondary antibody response with low levels of chloramphenicol *in vitro*. The reasons for this discrepancy are not clear. Chloramphenicol may be more effective *in vitro* because more intimate contact with proliferating cells is possible and because the absence of detoxification mechanisms permits a more sustained level than is possible *in vivo*. Other factors such as the rapidity of protein synthesis and the number of cells involved in synthesis may account for the relative ineffectiveness of chloramphenicol in inhibiting the anamnestic response *in vivo*. Conceivably higher levels of serum chloramphenicol might have been more effective in suppressing the secondary response.

Fahri and Lamensans published a preliminary report alleging that chloramphenicol inhibits antibody synthesis in rabbits (10). These studies have been justly criticized by Schwartz and André (11) on the grounds that the observed effects were minimal and that one tube differences in agglutination titers were considered significant. Fahri and Lamensans used 0.065 gm of chloramphenicol

per kg body weight in their studies. According to our data this dosage is far below the minimal effective amount required to suppress antibody synthesis. Optimum results in our experiments were obtained when 0.5 gm per kg of chloramphenicol were employed. Dosages of chloramphenicol between 0.15 and 0.3 gm per kg were almost completely ineffective in suppressing antibody responses.

The mechanism of action of chloramphenicol in suppressing immune responses differs markedly from that of other immunosuppressive drugs thus far studied (11–16). Purine and pyrimidine analogs, folic acid analogs, actinomycin, and nitrogen mustard all inhibit cell growth and suppress antibody formation. Interference with nucleic acid synthesis appears to be the predominant action of these drugs and probably the means by which they suppress antibody synthesis (17). Despite the difference in mechanism of action, the effects of chloramphenicol and 6-mercaptopurine (6 MP) on antibody synthesis are similar in many respects. Thus 6 MP in a dosage of 6 mg per kg blocks the primary antibody response without affecting the secondary response. 6 MP also prolongs homograft survival as long as the drug is administered. The chief difference in the effect of 6 MP compared to chloramphenicol lies in the ability of 6 MP to produce a state of tolerance when sufficiently large doses (18 mg per kg) are administered to rabbits (16). Probably this effect of 6 MP is related to its ability to interfere with the synthesis of nucleoproteins controlling cellular information. Chloramphenicol does not directly affect nucleoprotein synthesis and therefore it would not seem likely that tolerance could be achieved with this drug. However, relatively prolonged survival of homografts after chloramphenicol was discontinued in some of the animals suggests that some degree of tolerance may have been achieved by an as yet undetermined mechanism.

The duration of chloramphenicol administration was important in suppressing antibody synthesis in relation to persistence of antigen. The primary immune response was completely inhibited by administering chloramphenicol for 10 to 12 days, possibly because adequate antigenic stimulation did not persist beyond this period. Homograft rejection tended to occur 6 to 13 days after chloramphenicol was discontinued, probably because the antigenic homograft was still present to stimulate an immune response. In all instances in which 0.5 gm of chloramphenicol per kg was used, suppression of the primary immune response was observed in association with prolonged survival of homografts. However, in no instance did a rise in humoral antibody titer occur following graft rejection. The prolongation of homograft survival by chloramphenicol is in accord with the prevailing concept that homograft rejection is mediated by an immune mechanism, and the effectiveness of chloramphenicol in prolonging homograft survival indicates that synthesis of protein is involved in the rejection phenomenon.

On a weight basis, the amount of chloramphenicol administered to these rabbits was in the order of 15 to 20 times that ordinarily given to humans.

However, the ability of rabbits to detoxify or excrete chloramphenicol appears to be much greater than that of humans. Thus, it should be noted that the serum levels of free chloramphenicol attained in these rabbits were approximately 2 to 4 times the therapeutic levels ordinarily attained in humans. It seems quite likely that some of the higher chloramphenicol levels represent an excess of the amount necessary to obtain inhibition. For example, inhibition of the primary immune response was obtained with serum levels of free chloramphenicol only slightly greater than therapeutic blood levels in humans. Furthermore, no greater survival of homografts was observed with serum chloramphenicol levels of 16 to 20 μg per ml than could be obtained with serum levels of 8 to 15 μg per ml. It is apparent, however, that better results were obtained with 0.5 gm of chloramphenicol per kg than with 0.15 to 0.3 gm per kg and that serum levels of free chloramphenicol above 8 μg per ml were in general necessary for optimum results.

The ability of chloramphenicol to suppress antibody synthesis by inhibiting *de novo* protein synthesis adds another drug to the immunosuppressive compounds which may have usefulness in studying the mechanisms of antibody synthesis. Combined use of these drugs may shed further light on the processes involved and may have some therapeutic applications.

SUMMARY

Chloramphenicol suppresses primary antibody synthesis *in vivo* without affecting the ability to develop a normal anamnestic response. Chloramphenicol also prolongs homograft survival in rabbits. The survival of homografts is related to the duration as well as to the amount of chloramphenicol administered. The mechanism of action of chloramphenicol in suppressing immune responses is correlated with its ability to inhibit protein synthesis in proliferating mammalian cells. These observations suggest that the inhibitory effect of chloramphenicol on protein synthesis may be applicable to mammalian cells *in vivo* as well as to cell-free systems and to intact mammalian cells *in vitro*.

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EXPLANATION OF PLATES

PLATE 19

FIG. 1 *a*. Rejection of skin homografts in 2 control animals on the 7th day. Grafts exchanged between albino and grey chinchilla rabbits.

FIG. 1 *b*. Beginning rejection of skin homograft in animal on left and continued survival of skin homograft in animal on right after 18 days. Grafts exchanged between albino and grey chinchilla rabbits. Both animals received chloramphenicol 0.5 gm per kg daily for 12 days.

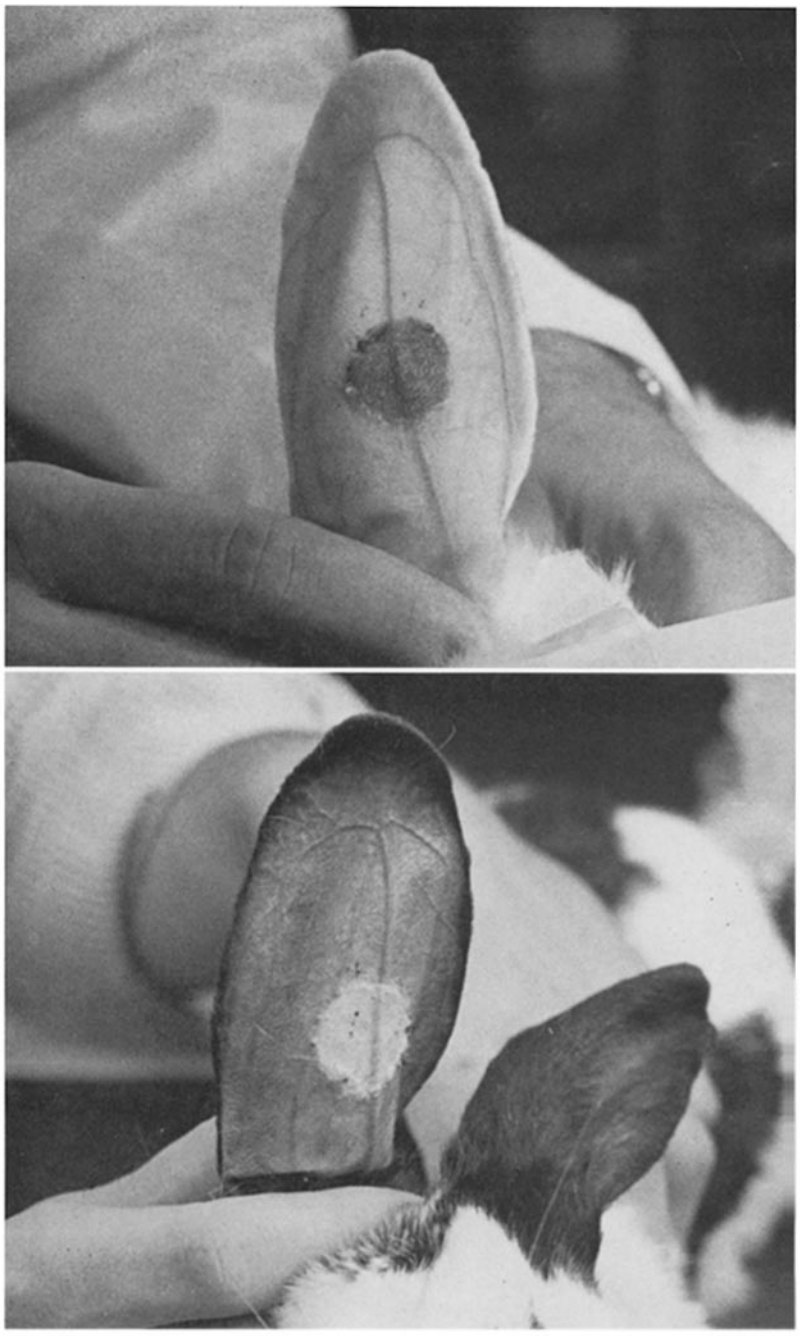
1 *a*

1 *b*

(Weisberger *et al.*: Suppression of antibody synthesis)

PLATE 20

FIGS. 2 *a* and 2 *b*. Continued survival of homografts in rabbits after 21 days. Grafts exchanged between albino and black and white rabbits. Both animals received chloramphenicol 0.5 gm per kg daily for 12 days.



2 *a*

2 *b*

(Weisberger *et al.*: Suppression of antibody synthesis)