transport-related enzymes (e.g., Na^+, K^+ Mg^{2+}–ATPase) to the plasma membrane (12) may have relevance to the maintenance of normal transport mechanisms.

Summary. An amino acid transport system in HeLa cells utilizing ^{14}C-1-alpha-aminoisobutyric acid is described. Neuraminidase treatment of HeLa cells decreases the net accumulation of amino acid without alteration of the rate of efflux of preloaded cells. These studies suggest that the sialic acid residue of the external cell surface may have a significant role in amino-acid transport in human cells.

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Suppression of Antibody Synthesis by Chloramphenicol Analogs* (33928)

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We have previously demonstrated that chloramphenicol can effectively inhibit protein synthesis in mammalian systems (1, 2). Significant inhibition of mammalian protein synthesis *in vivo* occurs only in those situations where cells are being committed to newly induced protein synthesis and where new messenger RNA (mRNA) is being formed and deposited on ribosomes. The ability of chloramphenicol to inhibit newly induced mammalian protein synthesis is reflected in its ability to suppress immune responses in animals and in humans when used in pharmacologic amounts during the inductive phase of antibody synthesis (2–5).

Both the antimocrobial properties of chloramphenicol and its inhibitory effect on protein synthesis are dependent upon maintaining the steric configuration of the molecule. Jardetzky (6) demonstrated, by nuclear magnetic resonance studies, that chloramphenicol has the configuration of a pyrimidine analog. Modification of the propranediol moiety of chloramphenicol alters the steric configuration with a resultant loss of antimicrobial activity. In contrast, substituion of the *p*-nitrophenol moiety of chloramphenicol by various alkyl groups does not alter the steric configuration of the molecule and such compounds retain antimicrobial properties. A number of such chloramphenicol analogs with antimicrobial properties have been prepared but their effectiveness as inhibitors of mammalian protein synthesis has not been determined.

The present study was undertaken to compare the effectiveness of chloramphenicol and a number of chloramphenicol analogs with respect to their effectiveness in inhibiting primary antibody synthesis *in vivo*. The comparative effectiveness of these compounds in prolonging the survival of skin homografts was also determined. Certain analogs of chlo-

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ramphenicol were 4–6 times as effective as chloramphenicol in suppressing antibody synthesis *in vivo* and in prolonging skin transplants even though they were no more effective than chloramphenicol in inhibiting protein synthesis *in vitro*. In preliminary studies, the most potent of these analogs had a clinically significant immunosuppressive effect in patients with lupus glomerulonephritis (7).

Methods. Humoral antibody synthesis. Primary antibody synthesis was induced in white New Zealand rabbits by injection of 4 mg of alum-precipitated bovine gamma globulin (BGG) (fraction II) into each hind foot pad. Animals were bled from marginal ear veins at appropriate intervals; the serum was separated and stored at —20° until assayed. Antibody assays were performed by the tanned, formalinized erythrocyte hemagglutination technique described by Daniel *et al.* (8). The amount of antibody was expressed as a reciprocal of the dilution titer.

The comparative effectiveness of various chloramphenicol analogs in inhibiting the primary immune response was determined as follows. Chloramphenicol[1] was prepared as a suspension with carboxymethyl cellulose in 0.75% NaCl in the proportion of 1 g of chloramphenicol and 10 mg of carboxymethyl cellulose in 3 ml of 0.75% NaCl. Analogs of chloramphenicol were synthesized by substitution of the nitro group in the *p*-nitrophenol moiety of chloramphenicol and were known to be effective antimicrobial agents. The CH_3SO_2 analog (WIN 5063-2, Thiocymetin) was furnished by the Sterling-Winthrop Research Institute, Rensselaer, New York; the CF_3SO_2 analog (SKF 7682) by Smith, Kline & French Laboratories, Philadelphia, Pennsylvania; and the aceto analog (D-threoacetomycetin) by Warner-Lambert Research Institute, Morris Plains, New Jersey. The analogs were suspended in carboxymethyl cellulose as described above. The drugs were injected intramuscularly in the desired dosage with half the daily dose being administered at 12-hr intervals. Injection of chloramphenicol and its analogs was begun on the same day that the antigen was injected; and the drugs were administered for 12 days.

The primary immune response was determined in 19 control animals, 7 of which were starved for 12 days without demonstrable diminution of antibody response. The comparative effect of chloramphenicol analogs on the immune response was determined as follows: chloramphenicol was administered to 20 animals; the CH_3SO_2 analog to 15 animals; the CF_3SO_2 analog to 12 animals; and the aceto analog to 10 animals. Chloramphenicol was administered in a dosage of 0.6 g/kg per day; the CH_3SO_2 analog was given in a dosage of 0.1 g/kg per day; the CF_3SO_2 analog and the aceto analog were given in a dosage of 0.15 g/kg per day. The amount of chloramphenicol administered (0.6 g/kg per day) was necessary to maintain bacteriostatic blood levels in rabbits (2). The analogs were given in reduced amounts because larger doses produced anorexia, loss of hair, inanition, and death due to unknown causes. The animals tolerated chloramphenicol and the analogs in the doses employed without difficulty.

Skin homografts. Rabbit ear skin transplants were performed and the effect of chloramphenicol and its analogs in prolonging survival of the grafts was determined. Circular full-thickness skin grafts, 2.5 cm in diameter were dissected simultaneously and exchanged between albino and grey chinchilla rabbits. Administration of chloramphenicol or its analogs was begun 24 hr prior to skin transplantation and continued for a period of 12 days. The amounts of chloramphenicol and chloramphenicol analogs administered were the same as those used for suppressing the humoral response. Eighteen animals received chloramphenicol, 15 animals received the CH_3SO_2 analog, 6 animals received the CF_3SO_2 analog, 10 animals received the aceto analog (Fig. 3). Homograft survival in these animals was compared with that obtained in 20 control animals. The homografts were inspected daily and survival of the graft was determined by gross changes consisting

[1] Chloramphenicol used in these experiments was generously supplied by Parke, Davis & Co., Detroit, Michigan.

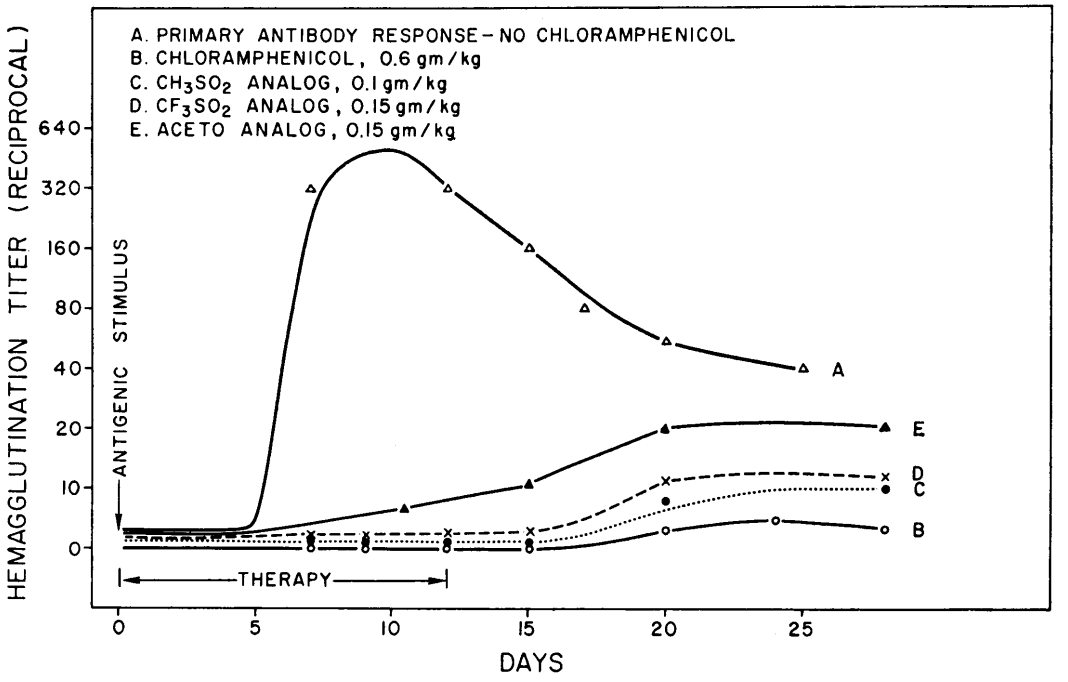

FIG. 1. Suppression of primary immune responses by chloramphenicol and by chloramphenicol analogs: the analogs were given for a 12-day period after antigenic stimulation with bovine gamma globulin. Approximately the same degree of suppression of the primary immune response was obtained with chloramphenicol and with all the analogs of chloramphenicol studied. However, the analogs were as effective as chloramphenicol when given in much smaller amounts and on a weight per kilogram basis were 4–6 times as effective as chloramphenicol in suppressing the immune response. Despite the suppressed primary response, these animals subsequently exhibited a normal anamnestic response.

of hemorrhage, hardening, and eschar formation.

Results. Suppression of the primary immune response. The effect of chloramphenicol and chloramphenicol analogs on the primary immune response in rabbits immunized with BGG is shown in Fig. 1. Control animals exhibited a normal immune response with maximal titers being obtained 6–12 days after antigenic stimulation (Fig. 1A). Similar results were obtained with control animals fed a normal diet and control animals starved for a 12-day period. Chloramphenicol markedly delayed and suppressed the primary immune response when administered in a dosage of 0.6 g/day for a period of 12 days (Fig. 1B). No humoral antibody was detectable in these animals until 20 days after antigenic stimulation and the titers which did develop were markedly reduced. Similar inhibition of the primary immune response was obtained with all of the analogs tested. However the analogs were as effective as chloramphenicol when administered in much smaller dosages and on a weight per kilogram basis were 4–6 times as effective (Fig. 1C, D, and E). The CH_3SO_2 analog (Thiocymetin) was the most effective of the analogs tested and it was as effective as chloramphenicol when administered in a dosage of 0.1 g/kg per day for a period of 12 days. The dosages employed in these experiments were the optimal amounts of these drugs needed to obtain immunosuppression. Lesser doses of chloramphenicol or its analogs were only partially effective in suppressing the immune response and larger doses caused anorexia, weight loss, inanition, and often death due to unknown causes. There were no adverse effects with the dosages employed in these experiments

and none of the animals lost a significant amount of weight. No hematologic abnormalities developed in the animals receiving either chloramphenicol or its analogs. Specifically none of the animals developed anemia, leukopenia, or thrombocytopenia.

Immunosuppression was obtained in the above experiments when administration of either chloramphenicol or the chloramphenicol analogs was begun on the same day that the antigen was injected. However, significant immunosuppression was also obtained if administration of chloramphenicol analogs was delayed for several days after antigenic stimulation. Four groups consisting of 8 rabbits in each group were employed in these studies. The BGG was injected into the hind foot pads of all animals on day zero. In the first group administration of the CH_3SO_2 analog was begun 2 days after antigenic stimulation and in the other groups administration of the analog was instituted 3, 4, or 5 days after antigenic stimulation. In all instances the drug was given for a period of 12 days.

The effect of delayed administration of the CH_3SO_2 analog of chloramphenicol (Thiocymetin) on the primary immune response is shown in Fig. 2. When administration of the analog was begun 2 days after antigen stimulation, immunosuppression was essentially the same as that obtained when therapy was instituted simultaneously with the antigenic stimulation. When therapy was instituted 3 days after antigenic stimulation significant delay in appearance and suppression of antibody titer was observed in all 8 animals. When therapy was begun 4 days after antigenic stimulation, 5 of the 8 animals exhibited significant immunosuppression; but 3 animals developed a normal immune response. When therapy was begun 5 days after antigenic stimulation, 6 of the 8 animals exhibited a normal immune response; 2 of the animals however, still manifested a significant suppression of the immune response.

The effect of chloramphenicol and chloramphenicol analogs on survival of skin homografts. The effect of chloramphenicol and chloramphenicol analogs in prolonging the survival of skin transplants is shown in Fig. 3. Control animals exhibited prompt rejection of the homografts in 6–9 days (mean survival, 7 days). The rejection time was the same in 13 animals receiving a normal diet and in 7 animals who received no food for the duration of the experiment. Eighteen animals receiving chloramphenicol in a dosage of 0.6 g/kg for a 12-day period exhibited prolongation in the survival of skin homografts (mean survival, 21.7 days). Fifteen animals receiving the CH_3SO_2 analog in a dosage of 0.1 g/kg for 12 days also exhibited prolonged survival of skin homografts (mean survival, 27.2 days). Animals receiving the CF_3SO_2 analog or the aceto analog in a dosage of 0.15 g/kg for 12 days manifested modest prolongation of homograft survival (mean survival, 15 and 17 days, respectively).

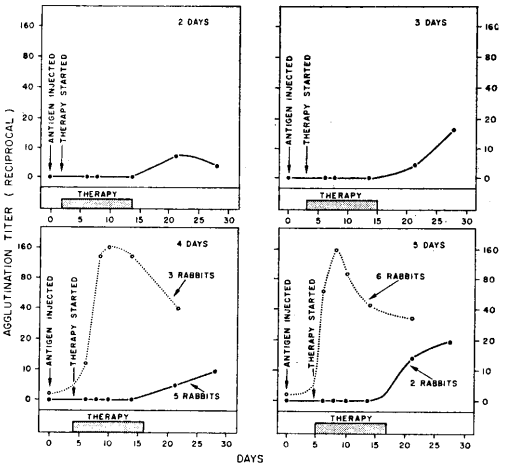

FIG. 2. The effect of delayed therapy with a chloramphenicol analog (Thiocymetin) on primary immune responses: four groups of 8 animals each were immunized with BGG and the immunosuppressive effect of Thiocymetin was determined when therapy was begun 2–5 days after antigenic stimulation. Significant immunosuppression was obtained in all animals receiving Thiocymetin for a 12-day period starting either 2 or 3 days after antigenic stimulation. Normal immune responses occurred in 3 of 8 rabbits when Thiocymetin therapy was started 4 days after antigenic stimulation and in 6 of 8 rabbits when Thiocymetin therapy was started 5 days after antigenic stimulation. Significant immunosuppression was obtained in 5 of 8 rabbits started on Thiocymetin 4 days after antigenic stimulation and in 2 of 8 rabbits started on Thiocymetin 5 days after antigenic stimulation.

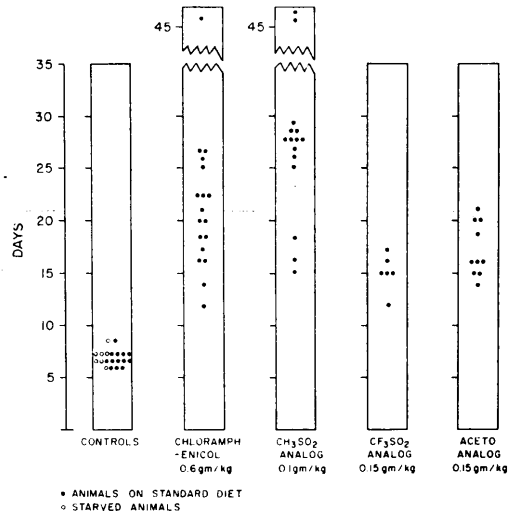

FIG. 3. Comparison of the effect of chloramphenicol on the survival of skin homografts in rabbits with that obtained with a number of chloramphenicol analogs: the methyl sulfonyl analog of chloramphenicol (Thiocymetin) was as effective as chloramphenicol in prolonging homograft survival even when administered in much smaller amounts.

The effect of delaying the institution of therapy with the CH_3SO_2 analog on prolongation of skin homograft survival in rabbits is shown in Table I. In these experiments, injection of the CH_3SO_2 analog of chloramphenicol was begun 2, 3, 4, or 5 days after homotransplantation. Essentially, the same survival of skin homografts was obtained if administration of the drug was begun 2, 3, or 4 days after transplantation as was obtained if administration of the drug was begun the day before transplantation.

Discussion. The data demonstrate that the chloramphenicol analogs employed in these experiments are considerably more effective than chloramphenicol in suppressing the primary immune response and in prolonging the survival of skin homografts in rabbits. The results are in accord with the observations that maintenance of the steric configuration of the molecule which is necessary for the antimicrobial effects of chloramphenicol may also be necessary to suppress antibody formation.

Immunosuppression was obtained with chloramphenicol in dosages of 0.6 g/kg for a 12-day period. In comparison, the analogs which have equivalent antimicrobial activity produced similar effects when administered in dosages of 0.1–0.15 g/kg. It is known that chloramphenicol is rapidly inactivated in rabbits and that it is necessary to give 0.6 g/kg daily to maintain blood levels which are required for antimicrobial activity in man (2). Conceivably the analogs may not be as readily inactivated and as a result more sustained levels would be obtained with smaller doses. However, Kunin and Finland (9) demonstrated that following oral administration, comparable blood levels of antimicrobial activity were obtained with both chloramphenicol and the CH_3SO_2 analog. Accordingly, it does not seem likely that the increased effectiveness of the CH_3SO_2 analog is due to higher or more sustained blood levels.

Although the analogs were 4–6 times as effective as chloramphenicol in suppressing the immune response, inhibition of protein synthesis was obtained in cell-free systems with equimolar amounts of these compounds (1). The difference in the sensitivity between the cell-free systems and the *in vivo* systems remains unexplained. In view of the evidence that comparable antimicrobial blood levels are achieved with chloramphenicol and Thiocymetin, a cellular mechanism may be involved.

TABLE I. Prolongation of Homograft Survival by a Chloramphenicol Analog:[a] Effect of Delayed Administration of the Drug.

Analog administered (from transplant; days)	Survival of homograft		
	No. of animals	Range (days)	Mean (days)
Before			
1	14	19–47	28
After			
2	4	19–24	21
3	6	15–25	20
4	8	14–36	20
5	8	9–17	11

[a] The CH_3SO_2 analog of chloramphenicol (Thiocymetin) was employed in these experiments. 0.1 mg/kg was administered intramuscularly for a period of 12 days. Injections were begun as indicated in column 1.

Both chloramphenicol and its analogs were effective in suppressing primary immune responses when administration of these drugs was begun 3 or more days after antigenic stimulation. This indicates that these compounds do not suppress antibody synthesis by interfering with cellular instruction which occurs during the inductive phase of antibody synthesis. The ineffectiveness of these compounds when given late in the inductive phase is in accord with observations that in mammalian systems the drugs interfere with the function of specific mRNA synthesized in response to antigenic stimulation and that the drugs have no effect on protein synthesis once mRNA–ribosomal complexes are formed and the initial events of protein synthesis are allowed to proceed (1, 2). Recent experiments in our laboratory indicate that chloramphenicol may interfere with the function of mRNA by preventing the formation of polyribosomes (10). In these experiments, mRNA attaches to monosomal particles but in the presence of chloramphenicol, polyribosome formation was prevented.

The immunosuppressive effect of chloramphenicol is obtained in humans with pharmacologic doses of the drug (4). The immunosuppressive effect of the CH_3SO_2 analog (Thiocymetin) is sufficiently greater than chloramphenicol so that it may have clinical usefulness in diseases in which immunologic mechanisms may be implicated. Preliminary studies have now been performed with Thiocymetin in patients with lupus glomerulonephritis and indicate that this analog of chloramphenicol may indeed have clinical application (7). In 4 of 6 patients with lupus glomerulonephritis, serum complement levels returned to normal, anti-DNA and antinuclear factor titers fell, L-E preparations became negative and kidney function stabilized following therapy with Thiocymetin. In addition it was demonstrated that glomerular-bound gamma globulin disappeared after a single course of therapy. The most provocative observation in these studies is that improvement in these parameters has now lasted for up to 3 years following a single course of therapy. Such sustained improvement suggests that Thiocymetin may have interfered with an autoimmune process or possibly have induced a state of tolerance.

Summary. In previous studies we showed that the immunosuppressive effects of chloramphenicol are related to its ability to inhibit protein synthesis in cells being committed to newly induced protein synthesis. In the present study a number of chloramphenicol analogs were 4–6 times as effective as chloramphenicol in suppressing the primary immune response and in prolonging the survival of skin homografts in rabbits. These analogs have an antimicrobial effectiveness equivalent to that of chloramphenicol. The dissociation between the antimicrobial and immunosuppressive effectiveness of these compounds is probably related to structural modifications and cellular mechanisms rather than to higher or more sustained levels of these antibiotics. The most effective analog of chloramphenicol with respect to immunosuppression was the CH_3SO_2 derivative (Thiocymetin). Preliminary studies with this compound in patients with lupus glomerulonephritis indicate that this analog of chloramphenicol may have some clinical application.

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