EFFECT OF CHLORAMPHENICOL ON THE PRODUCTION OF ANTIBODY TO BACTERIAL AND NON-BACTERIAL ANTIGENS

KENNETH C. WATSON, M.D.(Aberd.), *From the Department of Pathology, University of Natal, Durban, South Africa.*

One of the fundamental problems associated with the use of antibiotics in acute infectious disease states is the effect which the antibiotic may exert on the humoral response of the host to the causative organism. Depression of antibody response in this way is often quoted as one of the dangers of antibiotic therapy. Theoretically, there is an expectation that the reduction in the amount of antigen available for antibody response that is brought about by depression of multiplication of the infecting agent would lead to a diminution in the amount of antibody compared with non-treated hosts where multiplication of the causative agent is not so inhibited. Various types of host-parasite-antibiotic relationship have been investigated in an attempt to assess the effect of antibiotics on antibody production. No clear-cut pattern of response has emerged from these studies. Conflicting results have been reported which appear to depend on the type of antibiotic used, the nature of the infecting agent, and a number of variable host factors.

Our interest in this problem was aroused by the observation that the incidence of the relapse phase in typhoid fever may be considerably increased by inadequately prolonged treatment with chloramphenicol, as compared with non-treated patients or with those given an adequate course of the drug. This finding has been reported by a number of authors (El Ramli, 1950 and 1953; Smadel *et al.*, 1949). It has usually been suggested that the increased incidence of the relapse phase is secondary to a depression of antibody response as a direct result of the reduction in the amount of antigenic stimulus available. We have questioned this view, however, since in our experience the relapse phase in typhoid fever occurs late in the disease at a time when levels of agglutinating antibody may be very high (Watson, 1957). We have observed these high antibody titres in spite of the fact that the patients have been treated with chloramphenicol.

The present investigation was undertaken to try to determine the effect of chloramphenicol on the production of agglutinating antibody to bacterial antigens and on the production of Forssman-type antibody.

MATERIALS AND METHODS

Bacterial Strains and Immunization Procedures

The following strains were used to demonstrate antibody production against somatic, envelope and flagellar antigens respectively: (a) *Salmonella typhi* (0.901 strain, N.C.T.C. 5759), (b) *Escherichia coli* (5396/38 strain, which is a potent source of Vi antigen), and (c) *Salmonella typhi-murium* (N.C.T.C. 5710).

All strains were subcultured daily in heart-digest broth for 10 days before use. Suspensions for immunization were prepared by subculturing onto nutrient agar slopes, incubating for 18 hours at 37·0°C and then harvesting in sterile 0·85% saline. An alcoholized suspension of the *S. typhi* strain and a formalinized suspension of the *S. typhi-murium* strain were prepared in the usual way. The Vi-containing *E. coli* strain was not killed. All suspensions were standardized to contain approximately 20×10^8 organisms per ml. by means of opacity tubes.

Healthy adult rabbits of approximately 3,500 g. weight were injected intravenously on alternate days with a dose of 0·2 ml. of bacterial suspension for 4 injections, followed by a further 2 injections of 0·4 ml. each, in the case of the *S. typhi* and *S. typhi-murium* suspensions. In the case of the *E. coli* suspension the same doses were given, preceded by an initial intravenous injection of 0·01 ml. Six rabbits were used for each suspension. Chloramphenicol was given concurrently to 50% of the rabbits in each group by daily intramuscular injection in a dose of 25 mg. per lb. of body weight. Antibiotic injections were discontinued the day after the last immunizing injections. All rabbits were bled on the 10th day after the last injection and antibody titres were estimated.

Non-bacterial Antigen

The effect of chloramphenicol on the production of antibody to a non-bacterial antigen of the Forssman type was investigated.

Two kidneys from a freshly killed guinea pig were emulsified in 25 ml. of sterile saline in a blender. The suspension was incubated at 37·0°C for 4 hours and then kept in the cold overnight at 4·0°C. It was then centrifuged at 2,000 revs. per minute for 5 minutes to deposit gross particles. The supernatant suspension was removed and injected intravenously in 0·5 ml. amounts into each of 6 rabbits. Further intravenous injections of 1·0 ml. were given on the 4th, 7th and 10th days. Preliminary Forssman antibody titres were estimated on the rabbit sera before the injections were begun. Chloramphenicol was given intramuscularly to 3 of the rabbits as described above. Trial bleeds were performed on the 10th day after the last injection.

Antibody Titrations

Antibacterial agglutinating antibody was titrated with fresh saline suspensions of living bacteria washed from 18-hour agar slope cultures (Felix and Pitt, 1934). Serial dilutions of the sera were incubated with the suspensions for 2 hours at 37·0°C in 3 by $\frac{1}{2}$ inch tubes. The tubes were then stood at room temperature for a further 24 hours before reading.

Forssman antibody was titrated with serial doubling dilutions of serum in 0·5 ml. amounts. Aliquot volumes of a 2·0% suspension of thrice-washed sheep erythrocytes suspended in 0·85% saline were added together with an excess of complement. Tubes were incubated at 37·0°C for 4 hours and the end point was taken as the last tube showing clear sparkling haemolysis.

RESULTS

(a) *Effect of chloramphenicol on antibody response to bacterial antigens*

In Table I are detailed the titres obtained from different rabbits immunized with the varying antigens. The figures show that in no case is there evidence of a reduction in the amount of antibody produced by the rabbits which received

TABLE I. EFFECT OF CHLORAMPHENICOL ON THE PRODUCTION OF ANTIBODY TO VARYING BACTERIAL ANTIGENS

Type of antibody	*Serum agglutinating titres (reciprocal values)*	
	With chloramphenicol	*Without chloramphenicol*
'O' antibody	Rabbit (1)—10,240 Rabbit (2)— 5,120 Rabbit (3)— 5,120	Rabbit (4)—10,240 Rabbit (5)— 5,120 Rabbit (6)— 5,120
Vi antibody	Rabbit (1)— 5,120 Rabbit (2)— 5,120 Rabbit (3)— 5,120	Rabbit (4)— 5,120 Rabbit (5)— 5,120 Rabbit (6)—10,240
'H' antibody	Rabbit (1)— 1,280 Rabbit (2)— 1,280 Rabbit (3)— 1,280	Rabbit (4)— 640 Rabbit (5)— 1,280 Rabbit (6)— 1,280

supplements of chloramphenicol under the experimental conditions described. We are concerned here with final titres after a full course of immunization and not with the rate of antibody production. It is possible that chloramphenicol may have an effect in delaying the production of antibody, as compared with non-treated animals, but without affecting the final titres. It is, for example, our impression that patients with typhoid fever treated with chloramphenicol in the first few days of illness exhibit a delay in antibody production. Similarly, Smadel (1954) has shown that antibody production in human infections with *Rickettsia orientalis* is delayed by early chloramphenicol therapy.

(b) *Effect of chloramphenicol on production of antibody to non-bacterial antigen*

Table II shows the response to heterophile-type Forssman antigen in the two groups of experimental animals. Here again there appears to be no difference in the response of the two groups, the titre differences varying by only one tube. The dosage of chloramphenicol employed in both investigations is comparable to that used in human infections. The findings would also indicate that chloramphenicol in these dosages has little if any effect on the antibody-forming reticulo-endothelial cells. It is known, however, that large doses of antibiotics may exert a toxic effect on reticulo-endothelial tissues.

TABLE II. EFFECT OF CHLORAMPHENICOL ON THE PRODUCTION OF ANTIBODY TO FORSSMAN-TYPE ANTIGEN

Titres after immunization

With chloramphenicol	*Without chloramphenicol*
Rabbit (1)—1,280 (16)	Rabbit (4)—2,560 (32)
Rabbit (2)—1,280 (16)	Rabbit (5)—1,280 (16)
Rabbit (3)—1,280 (8)	Rabbit (6)—1,280 (16)

Reciprocal values of preliminary titres before immunization are shown in parentheses

DISCUSSION

The findings reported here regarding the effect of chloramphenicol on the production of antibody to non-bacterial antigen are essentially in agreement with most previous reports on the subject. Giunchi *et al.* (1953), for example, studied the action of penicillin, streptomycin, chloramphenicol, oxytetracycline and chlortetracycline on the production of haemagglutinins in rats injected with group-0 Rh-negative human erythrocytes. The antibody titres in these animals did not differ significantly from the contol group. Such results might be expected on *a priori* grounds if the antibiotic agents are not given in doses which will depress reticulo-endothelial cell function. One of the few reports describing a diminution of antibody production to non-bacterial antigen is that of Stevens (1953) using radio-iodinated bovine gamma globulin.

With bacterial antigens, however, the situation is less clear, certain reports indicating an apparent diminution in antibody produced by antibiotic-treated animals, as compared to control groups. Other workers, however, have been unable to confirm these findings. Ceccarini (1947) found that penicillin had an unfavourable effect on agglutinin production against *S.typhi* in man, and De Marchi *et al.* (1949) reported similar observations in the rabbit. Green *et al.* (1951) on the other hand found no difference in agglutinin titres in patients given TAB vaccine with or without penicillin. Similarly, Ferrata and Olivari (1950) reported that chloramphenicol had no effect on antibody production against *S.typhi* in rabbits. Other reports with chloramphenicol and *S.typhi* in different types of experimental animal seem to support these findings.

The reasons for these conflicting observations are uncertain. Variations in the type of antibiotic, the type of infecting agent and host factors may all play a part singly or in combination. With regard to host factors some interesting findings relating to the effect of diet have been reported. Slanetz (1953), for instance, found that when rats and mice were fed on a diet supplemented with either chlortetracycline or oxytetracycline for a short period, there was an increase in antibody production against *S.enteritidis*. When, however, these supplements were added for a long-term period, there was a depression of antibody response. Such depression could be counteracted by the addition of thiamine or yeast to the diet. Stevens (1953) measured the rate of antigen disappearance in rabbits as an indication of antibody response, using radio-iodinated bovine gamma globulin as antigen. He found that oxytetracycline, chlortetracycline, penicillin and dihydrostreptomycin all produced a marked delay in antigen removal. The response, however, was not constant, and was not observed at all in certain rabbits. Stevens also found that X-irradiation or restriction of the protein content of the diet produced a more marked effect than antibiotics in delaying antigen removal. The mode of action of antibiotic in diminishing antibody production in dietary-restricted animals is problematical and it is not at all certain that this is necessarily a constant effect. It may be that there is a failure of protein absorption from the gut. On the other hand, the action of certain antibiotics such as penicillin appears to result in a failure of protein synthesis. It has been suggested, for example, that penicillin interferes with certain enzyme systems such as ribonuclease and galactosidase. It may be, then, that the antibiotic effect is exerted at a cellular level and that a reduction of antibody formation is aggravated by a lack of 'building blocks' which results from protein deficiency. Edsall (1955) has pointed out the difficulty in translating these results to the situation which exists within the human host. Our own experience, however, in a non-European community where the average protein intake is low, does not suggest that antibody formation, at least in the case of antibody against *S.typhi* is less than that found in better-fed communities.

Some attention has also been given to the production of antitoxin in the presence of antibiotics. One of the few reports is that of Ramon *et al.* (1951), who found no differences in antibody titres to staphylococcal toxoid in penicillin- or streptomycin-treated animals as compared with control animals.

One important aspect of this problem has received little consideration, namely, whether antibiotic agents may result in qualitative changes in the type of antibody produced. This may be a factor of some importance for, if antigenic changes resulted from antibiotic therapy, they might lead to the production of antibody which does not possess the protective power of the normal-type antibody. Servant (1951), for example, has shown that treatment of certain strains of *S.typhi* and *E.coli* with dihydrostreptomycin may lead to a decrease in the amount of extractable polysaccharide of as much as 300% compared with the non-treated organisms. Similarly

Farhi and Lamensans (1956) found that strains of *S.typhi* adapted to grow in chloramphenicol at a concentration of 3.0 μg per ml. became less susceptible to agglutination by anti-Vi sera and more susceptible to agglutination with anti-'O' sera. They also found that the antibodies produced in response to chloramphenicol-modified organisms were less protective than those produced against the parent strains. This problem is at present under investigation in our laboratory.

SUMMARY

Under the experimental conditions described it was not possible to demonstrate any quantitative effect on antibody production as a result of administration of chloramphenicol to rabbits. Antibody production was measured against the following bacterial antigens, (a) Vi antigen (*E.coli* strain), (b) 'O' antigen (*S.typhi* 0.901), and (c) 'H' antigen (*S.typhi-murium*).

Similarly no depression of antibody production against Forssman-type antigen (guinea-pig kidney) was detected.

It is suggested, however, that antibiotic agents may lead to a qualitative change in antibody due to alteration of antigenic structure in the case of bacterial antigens.

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