Immunology, 1965, **9**, 311.

The Transmission of Passive Immunity to *Escherichia coli* from Mother to Young in the Domestic Fowl (*Gallus domesticus*)

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(*Received 7th January* 1965)

Summary. The serum antibody responses of laying hens that had received a single intravenous injection of dead *E. coli* bacteria, have been compared with antibody levels in normal birds. The passage of immune antibodies into the yolk and into the sera of the hatched chicks has been observed and no substantial difference was found between progeny of a number of inoculated and control birds.

Some evidence is presented which suggests that a selective concentration of $7S\gamma_2$-globulin occurs during the process of yolk formation.

INTRODUCTION

Antibodies produced in response to a variety of antigens have been shown to pass through the yolk of the egg to the developing chick (Sooter, Schaeffer, Gorrie and Cockburn, 1954). Most of these antigens were either micro-organisms pathogenic to this species (Buxton, 1952), or products of bacteria which are normally pathogenic to other species (Jukes, Fraser and Orr, 1934).

This paper records the production of antibodies in the sera of laying hens after inoculation with *E. coli* 02:K1 serotype, the passage of these antibodies to yolk, and their subsequent appearance in the sera of newly hatched chicks. An attempt has also been made to characterize the antibodies measured in these experiments.

MATERIALS AND METHODS

Experimental birds. A group of Rhode Island Red cross-bred hens and a pure-bred Rhode Island Red cock were penned together and provided with trap nests. The freshly laid eggs were alternately placed in individual bags and incubated, or used to determine yolk antibody levels.

Bacterial inoculum. E. coli 02:K1 was grown on nutrient agar in Roux flasks for 24 hours. After harvesting, the bacteria were washed three times with normal saline and boiled for 10 minutes to inactivate the capsular antigens. The suspension was diluted to match Brown's tube number 2 (approximately 10^7 organisms per ml).

One ml of this suspension was injected intravenously into each of four hens, four other birds received 1 ml of normal saline. This procedure did not affect normal egg laying nor reduce egg fertility.

Sera. Sera were obtained from blood samples taken at regular intervals during the course of the experiment. Adult birds were bled from a wing vein, and blood was obtained from the chicks by cardiac puncture. The sera were frozen at $-20°$ until required. No preservatives were used.

Egg yolk extraction. A modification of the ethylene dichloride–ether method of Schmittle and Millen (1948) was used. The intact yolk was washed with 0·15 M NaCl and thoroughly agitated before removal of the yolk sample.

Treatment of serum and yolk with 2-mercaptoethanol. Serum or yolk extract was mixed with an equal volume of 0·2 M 2-mercaptoethanol in normal saline and incubated at 37° for 1 hour. The controls were serum or yolk mixed with normal saline. Titrations were carried out immediately after incubation. 2-Mercaptoethanol causes the dissociation of macroglobulins and thus inactivates γ_1M-globulin antibodies (Deutsch and Morton, 1957). There is also some indication that the disulphide groups of γ_1A globulins are similarly reduced by this agent (Leddy, Freeman, Luz and Todd, 1962).

Starch block electrophoresis. The technique of Kunkel and Slater (1952) was used.

Indirect haemagglutination and antiglobulin tests. The indirect haemagglutination test was done with chicken erythrocytes treated with an alkaline extract of *E. coli* (Buxton and Thomlinson, 1961).

Anti-avian serum was prepared in a rabbit by a series of intravenous injections of normal chicken serum. The rabbit was bled 10 days after the last intravenous injection.

The serum was inactivated at 56° for ½ hour and absorbed twice with a large volume of washed, packed chicken erythrocytes.

For the anti-globulin haemagglutination test, the absorbed and inactivated serum was diluted 1:100 with normal saline; this avoided non-specific agglutination.

RESULTS

ANTIBODY TITRES IN THE SERUM AND EGG YOLK OF LAYING BIRDS

Fig. 1 shows the serum and egg yolk antibody titres of two birds injected with *E. coli*. Fig. 2 shows the serum and egg yolk antibody titres of two control birds injected with saline.

The results show that whereas the serum antibody response of the *E. coli* injected birds was anamnestic, there was no corresponding increase in yolk antibody titre. In addition the range of yolk antibody titres was similar in birds receiving either *E. coli* or saline.

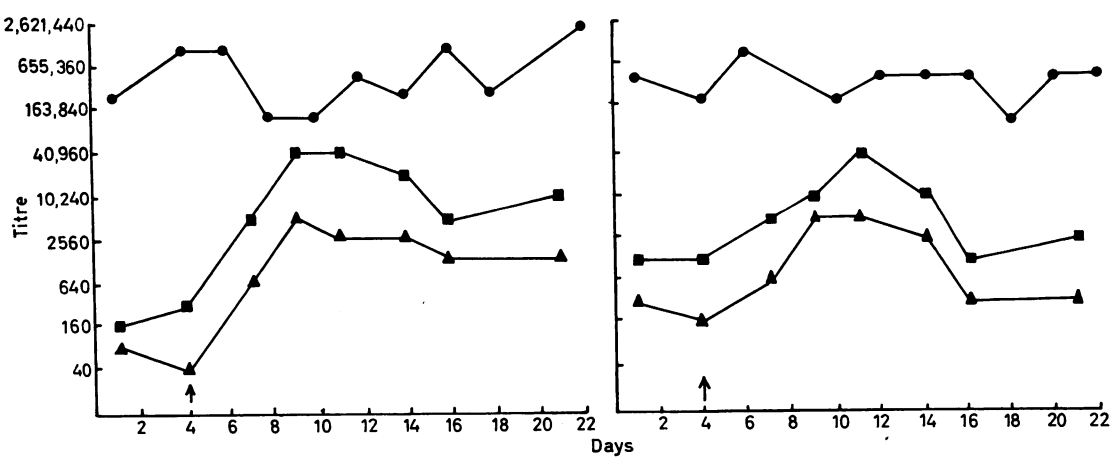

FIG. 1. The serum antibody responses of two birds injected with *E. coli* and the antibody content of the yolks of their eggs. ▲, Haemagglutinin titre of serum; ■, antiglobulin haemagglutinin titre of serum; ●, antiglobulin haemagglutinin titre of yolk; arrows indicate injection of killed *E. coli*.

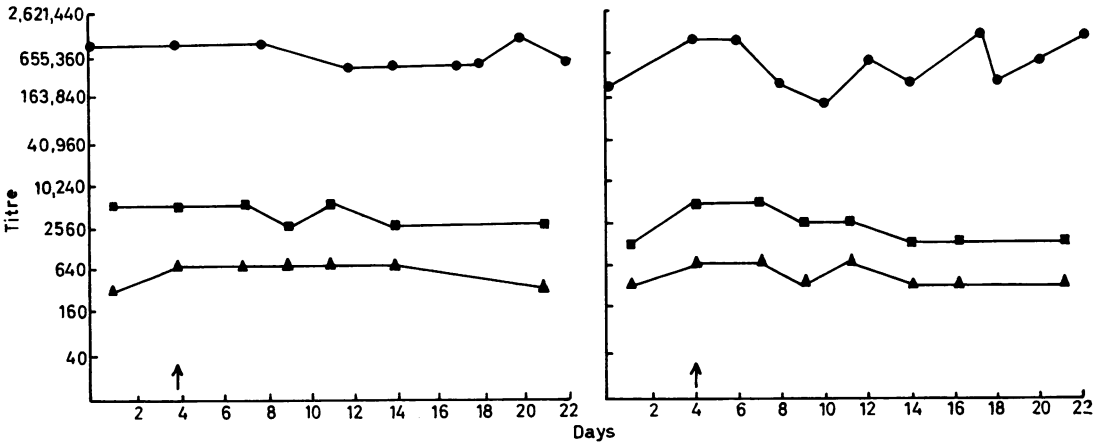

FIG. 2. The serum antibody levels of two control birds and the antibody content of the yolks of their eggs. ▲, Haemagglutinin titre of serum; ■, antiglobulin haemagglutinin titre of serum; ●, antiglobulin haemagglutinin titre of yolk; arrows indicate injection of saline.

Although the peak titres of haemagglutinating antibody were relatively high (1 : 2560) in birds receiving *E. coli*, no haemagglutinating antibody was found in the yolks at the lowest dilution tested (1:12·5 approx.). Indeed, in most instances, no complete 'O' haemagglutinins to a variety of *E. coli* serotypes have been detected in the yolks of eggs laid by clinically normal birds. The significance of this finding may be considered in the light of 2-mercaptoethanol treatment of the serum (see below).

ANTIBODY TITRES IN THE SERUM OF HATCHED CHICKS

Fig. 3 shows the decline of passively acquired antibody in the progeny of the birds receiving *E. coli*, and control birds.

The chicks were divided arbitrarily into three groups, according to the time that the eggs from which they were hatched, were laid. These groups (I, II and III) correspond to the maternal serum pre-antibody peak (days 0–5), antibody peak (days 5–13), and post-antibody peak (days 13–19) respectively.

There was no significant difference between the anti-globulin haemagglutinin titres of newly hatched chicks from the experimental or the control groups (Fig. 3). Furthermore, as there was no apparent difference between the initial antibody titres of the three groups

TABLE 1

THE MEAN ANTIGLOBULIN HAEMAGGLUTININ TITRES OF ALL THE HATCHED CHICKS FROM HENS WITH SERUM PRE-ANTIBODY PEAKS (GROUP I) ANTIBODY PEAKS (GROUP II), POST-ANTIBODY PEAKS (GROUP III)

Day	Group I		Group II		Group III	
	Infected	Control	Infected	Control	Infected	Control
0	218(6)	217(5)	192(5)	160(4)	294(5)	128(7)
5	N.T.	N.T.	313(4)	288(4)	N.T.	N.T.
7	233(5)	128(4)	N.T.	N.T.	512(3)	169(5)
12	N.T.	N.T.	24(3)	N.T.	N.T.	N.T.
14	N.T.	N.T.	N.T.	13(3)	N.T.	N.T.
18	5(4)	14(4)	N.T.	N.T.	N.T.	N.T.

The figures in parentheses indicate the number of chicks tested. Decreases in these figures are the result of death from cardiac puncture.

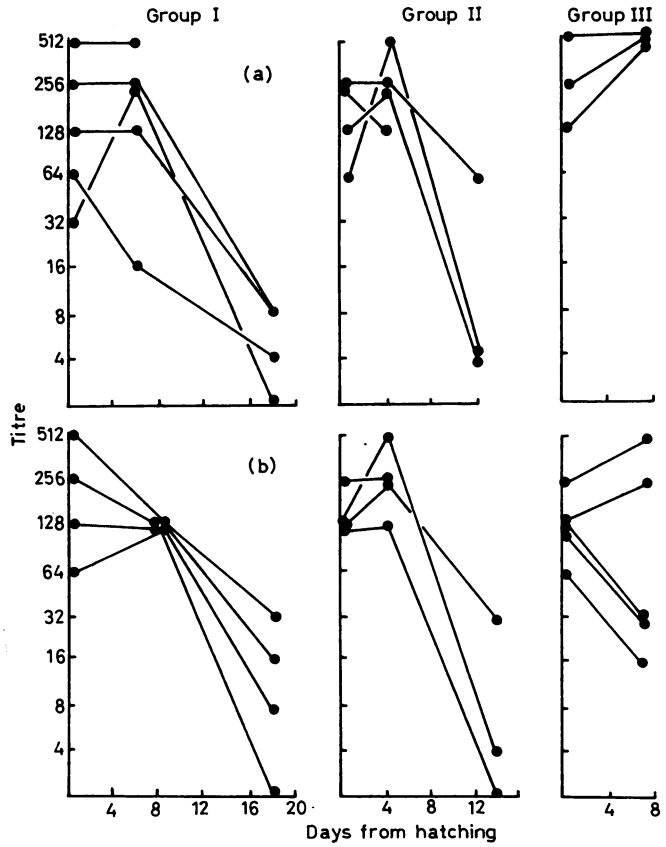

FIG. 3. The decline of passively acquired antibody of the progeny of (a) birds receiving *E. coli*, and (b) the control birds. ●, Antiglobulin haemagglutinin titre of chick sera. Group I = Chicks from hens with serum pre-antibody peak (days 0–5); group II = chicks from hens with serum antibody peak (days 5–13); group III = chicks from hens with serum post-antibody peak (days 13–19).

of chicks, no relationship existed, apparently, between the newly hatched chick antibody levels and the time of lay. This point is illustrated further by Table 1 which gives the mean antiglobulin haemagglutinin titres of all the hatched chicks.

Finally the rate of disappearance of this antibody in chicks derived from both experimental and control groups was very similar (Fig. 3).

2-MERCAPTOETHANOL TREATMENT OF SERA AND YOLKS

The effect of 2-mercaptoethanol treatment of the serum of a bird injected with *E. coli* is shown in Fig. 4(a), that of a control bird in Fig. 4(b). In both cases there was a decline in the antiglobulin haemagglutinin titres, but complete disappearance of the haemagglutinins occurred only in the serum of the control bird.

It was assumed that two types of antibody were present, since in the fowl the 2-mercaptoethanol sensitive antibody represents predominantly 19S, γ_1-macroglobulin, while the resistant antibody is 7Sγ_2-globulin (Benedict, Brown and Hersh, 1963). This type of haemagglutinin was revealed following treatment with 2-mercaptoethanol in the serum of the infected bird only after inoculation.

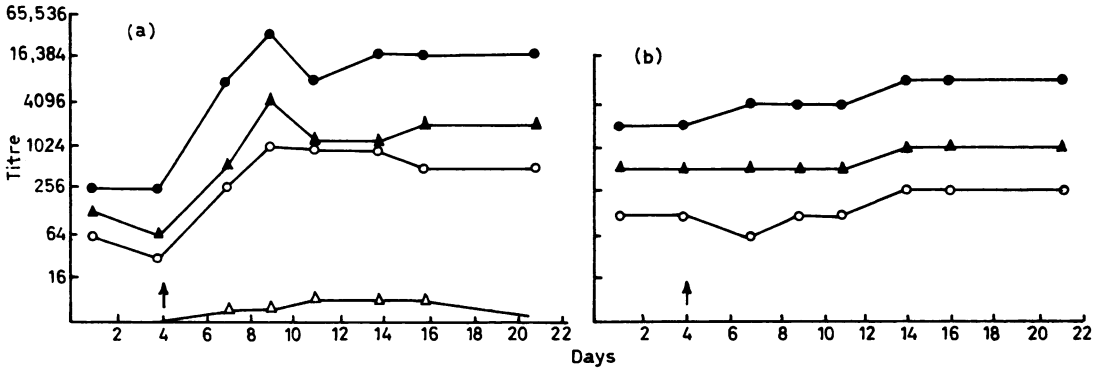

FIG. 4. 2-Mercaptoethanol digestion of serum antibodies of (a) a bird receiving *E. coli*, and (b) a control bird. ●, Antiglobulin haemagglutinin titre of serum before digestion; ▲, antiglobulin haemagglutinin titre of serum after digestion; ○, haemagglutinin titre of serum before digestion; △, haemagglutinin titre of serum after digestion; the arrow indicates the injection of killed *E. coli* in (a) and the injection of saline in (b). After digestion there was no detectable haemagglutinating antibody in the serum of the control bird.

Incomplete antibodies are demonstrable in the sera of all clinically healthy adult birds, and 2-mercaptoethanol fails to digest them to any great extent.

The two sera (Fig. 4) were further analysed by horizontal starch-block electrophoresis. In the control serum all the haemagglutinin activity appeared in the γ_1 region and was 2-mercaptoethanol sensitive. In the infected serum haemagglutinating antibody activity also appeared in the γ_2 region. This smaller peak of antibody activity was not reduced in titre by this treatment.

No alteration in the antiglobulin haemagglutinin titre of the yolk or of the newly hatched chicks was demonstrable after incubation with 2-mercaptoethanol.

DISCUSSION

The process of yolk formation in the laying bird involves the preferential transfer of serum globulins across the follicular epithelial cells of the ovum (Patterson, Younger, Weigle and Dixon, 1962). The concentration of the yolk proteins which are mainly β- and γ-globulins, is so much higher than the globulin fraction of the adult serum that the mechanism of transfer of passive immunity may be compared to the mammalian species, in which selective transfer of γ-globulins is consistently encountered (Brambell, 1958). The presence of a macroglobulin type antibody (haemagglutinin) in the adult serum and its absence in the yolk and in the neonatal chick suggests that a comparison might be made with placental type transmission of immunity. The absence of γ_1M-globulins in the neonatal human has been well documented (Franklin and Kunkel, 1958; Gitlin, Rosen and Michael, 1963), whilst there are reports of selective transfer and concentration of 7Sγ_2-globulin by the placenta to the foetus (Freda, 1962; Gitlin, Kumate, Urrusti and Morales, 1964).

In a parallel series of experiments, using *E. coli* serotypes other than 02 : K1, it was found that a good correlation existed between the presence and titre of 'O' antiglobulin haemagglutinins in the yolk and in the corresponding adult sera. An explanation for the presence of high titred 7Sγ_2 antibodies in yolk in all these experiments, might be that the secretory cells which line the yolk-sac are capable only of dealing with the molecular configuration of the 7Sγ_2-molecule (Brambell, 1963).

Buxton (1952) recorded that hens orally infected with live *Salm. pullorum* laid eggs with high titres of agglutinating and non-agglutinating antibodies. Not only was his method for detection of antibodies different from that used in this paper (he employed an incomplete bacterial agglutination test), but possibly more important, the time sequence of egg laying and yolk examination makes it possible that 7Sγ_2-globulins were being measured at that stage of his experiment.

It has been shown that the temporal sequence of antibody production in the mouse to parenteral inoculation of avirulent *Salm. typhi-murium* is such that 19S complete antibodies are quickly produced and after 1 month are replaced by 7S antibodies which persist over a longer time period (Turner, Jenkin and Rowley, 1964). In the birds used in these experiments the presence of 2-mercaptoethanol digestible serum antibodies to *E. coli* 02 : K1, resembles antibody responses to vaccination of the human with this enteric group of organisms (Smith and Eitzman, 1964). The single intravenous injection of dead culture produced a slight 7S haemagglutinin response, but the antigenic stimulus appeared to be insufficient to induce the appearance of this type of antibody in the yolk. The rate of fall in titre of these circulating antibodies in the chick further suggests that they are passively acquired. If it is assumed that the half-life of γ-globulin in the chick is 72 hours (Patterson *et al.*, 1962) an initial titre of 1 : 512 will decline to 1 : 4 in 21 days (Fig. 3).

Since the hatched chick takes 4 days to absorb the yolk-sac the globulins made available in this period would appear to be present in quantities sufficient for immediate catabolism. This would explain the absence of an immediate exponential decline in antibody levels (Fig. 3).

ACKNOWLEDGMENTS

I wish to thank Professor A. Buxton for helpful advice in the design of this experiment, Professor D. L. Hughes, in whose department the work was carried out, and Dr D. Allan who read the manuscript, and I am grateful to them for their criticism and discussion. Miss B. K. Oates rendered valued technical assistance. This work was supported by a grant from the Egg Marketing Board.

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