Antibody Production in Avian Embryos and Young Chicks

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SUMMARY: The inoculation of killed *Salmonella pullorum* into the developing avian embryo on or after the 17th day of incubation or of live *Sal. pullorum* into young chicks evoked no immediate antibody response. Approximately 20–40 days after hatching, however, agglutinins and non-agglutinating antibodies were sometimes detected in the sera of these chicks. Maximum antibody production did not occur until the chicks were approximately 100 days old.

The intravenous inoculation of killed *Sal. pullorum* into developing embryos on or before the 15th day of incubation stimulated the production of practically no demonstrable antibody by the chicks during approximately the first 80 or 100 days after hatching. These chicks, however, showed a marked decrease in capacity to produce demonstrable antibodies in their sera after subsequent oral infection with *Sal. pullorum*.

These experimental results are discussed in relation to various theories of antibody production. It is suggested that they are evidence in support of Burnet's adaptive enzyme theory. The results are explained on the hypothesis that the introduction of antigenic material into the embryo under suitable experimental conditions enables the host cells to become adapted to the antigen, resulting in a diminished antibody response by the host, during post-embryonic life, to a subsequent challenge with similar antigenic material.

A variety of antigenic materials has been employed by earlier workers in unsuccessful attempts to demonstrate the production of antibodies by the developing avian embryo and young chick. These antigens have included diphtheria toxin (Grasset, 1929), *Clostridium sporogenes* (Weinberg & Guelin, 1936), and *Escherichia coli* (Rywosch, 1907). Rywosch claimed that the blood sera from developing embryos of 14–18 days incubation were bactericidal for *E. coli* but to a lesser extent than serum from an adult bird. It was probable that some, at least, of the bactericidal effect of embryonic serum was associated with bactericidins passively transferred from the hen to the egg. Sherman (1919*a, b*) found that complement did not appear in embryo serum until the 17th day of incubation, and that lysins for rabbit erythrocytes were not formed until the embryo was about to hatch. Polk, Buddingh & Goodpasture (1938) confirmed Sherman's results on the development of complement, and found that young chicks do not possess normal haemagglutinins and do not respond to immunization with foreign red cells until they have spontaneously developed some agglutinating capacity.

The responses of the embryo to viruses and to tumour material have also been investigated. No antibody was detected in the sera of chicks hatched from eggs infected with influenza virus (Burnet, 1941), bacteriophage

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(Beveridge & Burnet, 1946), or with washed human red cells (Burnet, Stone & Edney, 1950). Moreover, there was no demonstrable change in the subsequent response of the hatched chick to the corresponding antigen. Fox & Laemmert (1947) failed to detect antibodies to yellow fever virus in the embryo, but they observed antibody only in those chicks in which the virus persisted in the blood stream until hatching time. Response of the embryo to implanted tumour tissue did not occur before the 18th to 21st day of incubation (Murphy, 1913, 1914, 1916; Stevenson, 1917, 1918).

Although a wide variety of antigenic materials has been used in experiments to observe the capacity of the embryo and young chick to develop its own antibodies, the sera have been tested by techniques which only detect the presence of complete (divalent or multivalent) antibodies. While it is generally accepted that the mechanism for the production of complete antibodies is absent in the embryo and does not develop until later in life, it is not known whether the development of the antibody-producing mechanism passes through a stage when it can produce only incomplete antibodies in the embryo and newly hatched chick.

In the experiments recorded here, sera from embryos and young chicks have been tested for agglutinins and for non-agglutinating antibodies to *Salmonella pullorum*, to observe whether the non-agglutinating antibody was produced at an earlier stage in development than the agglutinins.

MATERIALS AND METHODS

The methods of preparing suspensions of *Sal. pullorum* and of carrying out tests for agglutinins and non-agglutinating antibodies have already been described (Buxton, 1951, 1952). Suspensions of *Sal. pullorum* for inoculation into developing embryos were washed twice in saline to remove all traces of ethanol and then resuspended to the required concentration. A fresh suspension was prepared for inoculating each batch of eggs.

Various routes of inoculation were employed, particularly the intravenous and yolk-sac methods, using the techniques described by Beveridge & Burnet (1946). A 1.0 ml. all-glass syringe was used with No. 27 needle. The dosage was 0.1 ml. per embryo. In some experiments newly hatched chicks were infected orally and the dose for each chick consisted of 0.1 ml. of an 18 hr. broth culture of *Sal. pullorum* in peptone broth.

The preparation and use of anti-livetin serum for the detection of non-agglutinating antibody in sera was described earlier (Buxton, 1952).

RESULTS

Antibody production in the developing embryo

Initial experiments consisting of the inoculation of minute doses of live *Sal. pullorum* into developing embryos proved fatal on all occasions, and subsequently suspensions of killed bacteria were used.

Intravenous inoculation. Two unsuccessful attempts were made to demonstrate agglutinins and non-agglutinating antibodies in the sera of newly

hatched chicks after they had been inoculated intravenously during incubation with killed *Sal. pullorum*. In the first experiment twelve 11-day embryos each received 9×10^7 organisms, and in the second experiment a similar number of 11-day embryos each received 46×10^9 organisms. Immediately after hatching the chicks were bled from the heart and the sera tested in dilutions beginning at 1/5. In no case was any antibody demonstrable.

The observations of Sherman (1919*a*) and of Polk, Buddingh & Goodpasture (1938) that complement could not be detected in embryo serum until at least the 17th day of inoculation, and also that at about that time the embryo suddenly developed the ability to react to the implantation of foreign tissue, suggested that inoculation of bacterial antigen into the embryo on or after the 17th day of incubation might result in an antibody response which is not apparent after inoculation of antigen as early as the 11th day. Fifteen 19-day embryos were inoculated intravenously with approximately 9×10^4 killed bacteria. Eight of these embryos hatched satisfactorily and their sera were tested at intervals during the subsequent 173 days. A group of healthy control eggs from the same source was incubated and hatched with the experimental eggs. Four control chicks were kept with the experimental ones and their sera tested. All chicks were kept strictly isolated throughout the experiment, during which time no antibodies were demonstrated in the sera of the controls. During the period of observation one experimental chick received a mechanical injury and had to be killed. The results are shown in Fig. 1. As in previous trials no antibody was detected in the sera of experimental chicks immediately after hatching. At 20 days of age non-agglutinating antibody was demonstrable in five sera (1/10, 1/20, 1/40, 1/20, 1/10). On the 34th day non-agglutinating antibody (1/80, 0, 1/240, 1/240, 1/40, 0, 1/160, 0) in two of five positive experimental sera showed a well-marked zone, extending in each case from dilutions of 1/40 to 1/160. On the 72nd day the antibody had completely altered in character and all sera contained agglutinins at dilutions of about 1/125 (1/80, 1/160, 1/100, 1/160, 1/160, 1/160, 1/50). Thereafter, there was a marked fall in antibody titres. It was noticeable that at the final test on the 173rd day the non-agglutinating antibody demonstrable in all sera (1/40, 1/20, 1/40, 1/80, 1/40, 1/40, 1/40) did not show any zoning as in earlier tests.

Yolk-sac inoculation. For antibody production to occur in the young chick as a result of inoculation of bacterial antigen *in ovo*, the bacteria may have to maintain their antigenicity until such time as the defence mechanism of the chick has developed sufficiently to respond to the antigenic stimulus. Masurier, Branion & Marcellus (1946) found that in many chicks the yolk-sac was retained for a variable time after hatching. For these reasons a suspension of killed *Sal. pullorum* was inoculated into the yolk-sac of developing embryos to observe whether there was any antibody response by these embryos after hatching. Each of twelve 9-day old embryos was inoculated into the yolk-sac with approximately 23×10^5 killed bacteria. Six chicks hatched successfully and were kept isolated for a period of 123 days, during which time serum samples were tested for agglutinins and non-agglutinating antibodies. Four uninoculated control chicks from the same source of eggs were hatched and kept with

the experimental chicks. Their sera remained negative throughout the period of observation. The results of this experiment are shown in Fig. 2. As in the previous experiment, zoning of non-agglutinating antibody occurred before the peak of antibody production, which was delayed until about the 125th day after hatching. (Agglutinating antibody titres: 1/20, 0, 1/30, 1/80, 1/40, 1/5. Non-agglutinating antibody titres: 1/40, 0, 1/40, 1/375, 1/375, 1/50.)

Fig. 1. Antibody production by young chicks which had been inoculated intravenously with killed *Sal. pullorum* on the 19th day of incubation.

Fig. 2. Antibody production by young chicks which had been inoculated into the yolk-sac with killed *Sal. pullorum* on the 9th day of incubation.

Antibody production in the young chick

Intraperitoneal infection. Ten 4-day healthy chicks were inoculated intraperitoneally with an 18 hr. broth culture of *Sal. pullorum*. Each chick received a dose of approximately 2×10^7 organisms in 0.1 ml. of diluted culture. Three chicks died within a few days of inoculation and the sera from the remainder were tested at intervals for agglutinins and non-agglutinating antibodies. A 4th chick died from a generalized infection of *Sal. pullorum* soon after the 102nd day of the experiment. The results presented in Fig. 3 show that, as in the case of chicks which had received killed bacteria *in ovo*, non-agglutinating antibody appeared first and rose to a higher titre than agglutinins. (Agglutinating antibody: 1/80, 1/80, 1/60, 1/1920, 1/40, 0, 1/1280. Non-agglutinating antibody: 1/100, 1/320, 1/160, 1/3840, 1/160, 0, 1/8320.) Two chicks showed marked zoning of agglutinins on the 55th and 102nd days of the experiment. A similar experiment was repeated on four more chicks which survived intraperitoneal infection and the results are similar to those in Fig. 3, namely that non-agglutinating antibody appeared first and increased to a higher titre than the agglutinins.

Oral infection. Each of ten chicks received a dose of approximately 2×10^7 live *Sal. pullorum* at one day of age, and their subsequent antibody production is shown in Fig. 4. In general, antibodies consisted mainly of the non-agglutinating type and maximum titres were lower than those resulting from intraperitoneal inoculation of live bacteria. Nevertheless, the pattern of antibody response was similar to the previous experiment in two respects, namely, that non-agglutinating antibody was detected earlier than agglutinins and that it

reached higher titres than the latter. (Agglutinating antibody: 1/20, 1/5, 0, 1/5, 0, 1/10, 0, 0, 1/20, 0. Non-agglutinating antibody: 1/40, 1/40, 1/20, 1/10, 1/100, 1/50, 1/10, 1/20, 1/40, 1/80.)

Fig. 3. Antibody production by young chicks which had been inoculated intraperitoneally with live *Sal. pullorum* on the 4th day after hatching.

Fig. 4. Antibody production by young chicks which had been infected orally with *Sal. pullorum* on the 1st day after hatching.

The effect of inoculation of antigen in ovo on the subsequent development of antibodies in the chick

Inoculation of living antigen intraperitoneally or orally into newly hatched chicks resulted in antibody production which was only demonstrable after a period varying from 11 to 30 days following inoculation. Similarly, the inoculation of killed antigen into the developing embryo under suitable experimental conditions also resulted in antibody production in the chicks which hatched from these eggs. After intravenous inoculation into the embryo on the 19th day of incubation, agglutinins were not uniformly produced by all the hatched chicks until they were at least 20–30 days old. During this lag phase between the time the embryos were inoculated and the time of appearance of antibodies in the hatched chicks' sera, the necessary conditions for the production of detectable antibodies must have been developed. In their theory on the development of marker recognition in embryonic life and its relationship to antibody production, Burnet & Fenner (1949) were of opinion that following a generalized non-fatal infection of the embryo *in utero* by a pathogenic micro-organism, the animal after birth would be incapable of responding by antibody production to injection or infection with the same type of micro-organism. Thus, if developing embryos were inoculated intravenously with killed *Sal. pullorum* and after hatching the chicks were again subjected to contact with this antigen, the subsequent antibody production would be less than in chicks which had not previously been inoculated *in ovo*. The testing of this possibility was the object of the following experiments.

First experiment. A total of forty-five embryonated eggs laid by known healthy Brown Leghorns was divided into three groups of 15 eggs each. After

9 days of incubation one group of eggs was inoculated in the yolk-sac with approximately 25×10^4 killed *Sal. pullorum* organisms suspended in 0.1 ml. saline. After 15 days of incubation the second group of eggs was inoculated intravenously with a similar dose. The third group of 15 embryonated eggs was kept as uninoculated controls. After hatching, there was available a total of 10 chicks in the yolk-sac inoculated group, 8 chicks in the intravenously inoculated group, and 7 chicks in the control group. The groups were kept strictly isolated in a separate chick-rearing room and all groups were

Fig. 5. Average antibody production in groups of chicks which had been inoculated intravenously or into the yolk-sac *in ovo* with killed *Sal. pullorum*, and later infected orally with *Sal. pullorum* 34 days after hatching.

maintained under identical conditions of management. When the chicks were 27 days old their sera were tested for agglutinins and non-agglutinating antibodies. All were negative at 1/5 and upwards.

When 34 days old, each chick was infected orally with 0.1 ml. of an 18 hr. broth culture of *Sal. pullorum*. Subsequently the chicks were tested for antibodies at 54, 68, 90 and 118 days old. These tests showed (Fig. 5) the average serum titre for each group of chicks. By the 68th day (i.e. 34 days after infection) the average titres of agglutinins (1/80, 1/150, 1/2250, 1/20, 1/2000, 1/500, 1/20) and non-agglutinating antibodies (1/160, 1/300, 1/2500, 1/80, 1/2250, 1/640, 1/80) in the control group had risen to approximately 1/700 and 1/850 respectively, while the average serum titre for non-agglutinating antibodies (1/80, 1/1280, 1/80, 1/10, 1/160, 1/10, 1/10, 1/40) was only about 1/200 in the group which had previously been inoculated intravenously with antigen on the 15th day of incubation. There was little difference between the control group and the group previously inoculated with antigen into the yolks-sac on the 9th day of incubation, except that the non-agglutinating antibody titre of

the control group was lower than that in the latter group. The results showed that under these experimental conditions the inoculation of bacterial antigen intravenously into developing embryos on the 15th day of incubation decreased the capacity of the hatched chicks to produce demonstrable antibodies after subsequent infection with live organisms at 34 days of age.

Second experiment. The apparent diminution of antibody response in hatched chicks to infection after preliminary intravenous inoculation *in ovo* with killed homologous antigen, might depend largely on the developmental stage of the antibody-producing cells in the embryo at the time of inoculation. The only immune responses ever observed in the avian embryo have been to the implantation of foreign tissue during the 18th to 21st days of incubation. This, together with the appearance of complement on or after the 17th day, suggested on the basis of Burnet's hypothesis that during these last 4 or 5 days of incubation the balance of intracellular enzyme systems in the embryo becomes less labile and, therefore, more able to respond to the implantation of foreign tissue. At an earlier stage in development, however, the embryo may be able to adapt itself to the introduction of bacterial antigen and the demonstrable response of the hatched chick to subsequent contact with the same antigen might thereby be decreased. The following experiment was carried out in an attempt to confirm this view.

A group of 19 chicks was used; 6 had been inoculated with killed *Sal. pullorum* on the 15th day of incubation, 6 on the 20th day, and the remaining 7 had been inoculated on both the 15th and 20th days of incubation. The inoculum was given intravenously and the dosage in each case was approximately 25×10^4 killed bacteria suspended in 0.1 ml. saline. The chicks were kept strictly isolated. On the 45th day after hatching, each chick was infected orally with 0.1 ml. of an 18 hr. broth culture of live *Sal. pullorum*. Subsequently the sera were tested for agglutinins and non-agglutinating antibodies. The average serum titres for each group of chicks are shown in Table 1. There

Table 1. *The average antibody production of groups of chicks inoculated intravenously with killed Salmonella pullorum during incubation and subsequently infected orally with live Sal. pullorum when 54 days old*

The figures in the table are the reciprocals of the antibody titre. A=agglutinins; NA=non-agglutinating antibody.

Age of chicks (days)	Age of embryos when inoculated (days of incubation)					
	15		15 + 20		20	
	A	NA	A	NA	A	NA
61	21	45	6	48	32	88
67	32	213	16	50	462	3082
74	25	30	18	40	3840	6400
82	15	20	18	180	5120	25600
88	30	60	20	70	960	3840
95	15	20	18	35	1600	3840
102	nt	nt	17	60	1280	5120
109	20	30	20	80	640	1280

nt=not tested.

is a striking contrast between the titres of those chicks which were originally inoculated on the 20th day of incubation, and those inoculated on the 15th or 15th and 20th days. The results of this and the previous experiment indicate that in order to demonstrate Burnet's hypothesis of embryonic adaptation to antigenic material, it is necessary to inoculate bacterial antigen intravenously into the embryo before the 20th day of incubation. The 15th day of incubation was found satisfactory.

Third experiment. It was considered possible that under these conditions the degree of adaptation might depend on the size of the initial dose of antigen inoculated *in ovo*, and that a dose in excess of the optimum might result in some of the inoculum remaining antigenically unaltered until the embryo had reached the stage in development when it could respond to the antigenic stimulus by the production of antibodies. This might be observed by either relatively high antibody titres in the hatched chicks when compared with uninoculated controls, or titres might be higher in those chicks after they had been subsequently challenged with a further dose of antigen than in ones which received only the initial dose *in ovo*.

Preliminary experiments showed that the inoculation of a sufficiently large dose of killed bacteria was toxic to the embryo. For this reason four groups of embryos were inoculated and each received a dose of 25×10^7 , 25×10^6 , 25×10^5 , or 25×10^4 killed bacteria intravenously on the 12th day of incubation. The total number of chicks hatched from these four groups were 14, 10, 12 and 12 chicks, respectively. When the chicks were 35 days old their sera were tested for agglutinins, and half the number of chicks in each group was infected orally with 0.1 ml. of an 18 hr. broth culture of *Sal. pullorum*. The uninfected chicks were isolated. A total of 16 control chicks, uninoculated *in ovo*, were also divided into two groups, one group being infected when 35 days old. The sera from all chicks were tested for agglutinins during the subsequent 80 days. The results are shown in Fig. 6. Uninfected control chicks remained negative. There was no significant difference between the average titres of the groups of experimental chicks infected when 35 days old, and these titres were markedly lower than the corresponding average titres of the control infected chicks. The group receiving *in ovo* inoculation of 25×10^5 organisms responded with an average maximum titre of 1/10 on the 36th day after infection. These results show that the intravenous inoculation of killed *Sal. pullorum* into the developing embryo on the 12th day of incubation did not result in subsequent agglutinin production by the hatched chicks, and that such chicks when later challenged orally with live *Sal. pullorum* produced markedly lower agglutinin titres than control infected chicks. Moreover, the range of doses of killed organisms inoculated *in ovo*, resulted in no significant difference between the antibody response of the different groups of chicks.

DISCUSSION

The experimental results have shown that the inoculation of killed *Sal. pullorum* into the avian embryo on the 11th day of incubation failed to stimulate the production of demonstrable antibodies in the newly hatched chicks;

and that similar inoculation of antigen on the 12th day of incubation did not result in agglutinin production by the chicks at least during the first 100 days after hatching. After the oral infection of 4-day chicks with *Sal. pullorum*, antibodies were not detected until the chicks were 20–40 days old; maximum antibody production did not occur after intraperitoneal inoculation until the chicks were approximately 100 days old. These latter results correspond to those of Norton, Wolfe & Crowe (1950) who used crystalline beef albumin or undiluted beef serum as antigen, and who found that there was some correlation between the size of spleens in the young chicks and their capacity to produce demonstrable antibodies.

Fig. 6. Average agglutinin production of groups of chicks which had been inoculated intravenously with different doses of killed *Sal. pullorum* on the 12th day of incubation, and subsequently infected orally with *Sal. pullorum* on the 35th day after hatching.

The first appearance of detectable agglutinins at relatively long intervals after administration of antigenic material is an indication of the time taken for the development of the antibody producing mechanism. There is some evidence, however, that the host reacts to the presence of antigen before the production of agglutinins. This reaction may take the form of the production of non-agglutinating antibody alone, or diminution of the hosts' ability to produce agglutinins after subsequent contact with the homologous antigen. The absence of agglutinins in the serum of young chicks which have previously been inoculated with bacterial antigen does not necessarily imply a total lack of antibody response by the host. On the contrary, the presence or absence of agglutinins may be only an indication of the state of development of the host's cells responsible for the production of antibodies. Burnet & Fenner

(1948) believed that the type of antibody produced might differ according to the type and stage of specialization of the cells responsible for its production. Brandt, Clegg & Andrews (1951) examined in detail the quantitative and qualitative change of serum globulin fractions during the young chick's development to maturity, and the results give some indication of the rate at which maturation of the antibody-producing cells proceeds. They found that the values for globulin varied according to the age of the bird and that they increased from 0.74 g./100 ml. in 4- to 7-week-old chicks to 1.3 g./100 ml. in laying hens. The values for albumin and β -globulin showed little variation. Thus, in the young chick a proportion of the globulin-producing cells is immature. In such cells which are undergoing development, the pattern of the balance existing between the intracellular enzymes may be in various stages of development towards the final form. According to Burnet & Fenner's theory, the lability of intracellular enzymes is lost as the young host matures, and is later replaced by a permanent balance which remains throughout adult life. Until this final balance is reached it seems improbable that contact between the cell and foreign antigen will stimulate the production of complete antibody molecules (i.e. agglutinins) in which each molecule possesses two or more combining sites.

Although the intravenous inoculation of bacterial antigen into developing embryos on or before the 15th day of incubation did not stimulate the production of antibodies in the hatched chick, the capacity of these chicks later in life to produce demonstrable antibodies, when challenged again with similar bacterial antigen, was diminished. In the opinion of Burnet & Fenner (1949) '...the primary units (adaptive enzymes) on which antibody production is based, are modifications of enzyme systems primarily adapted to specific adaptation to one or other of the self-marker components of the body cells. When and only when, the specific adaptations occur, the marker is enzymatically destroyed without disturbance of intracellular equilibrium'. These primary units (adaptive enzymes) may be the only form of response which the embryo or newborn is capable of producing. Although the presence of primary units in sera would not be demonstrable by the customary *in vitro* techniques for the demonstration of antibodies, they may be responsible for the decreased capacity of the host to produce demonstrable antibodies to the particular antigen later in life, provided that the pattern of the enzymes can be adapted to fit the configuration of the substrate (i.e. the antigenic components of *Sal. pullorum*).

Burnet *et al.* (1950) were unable to demonstrate antibodies in chick embryos after inoculation *in ovo* of either influenza virus or bacteriophage. Moreover, the inoculation of these antigens did not alter the response of the hatched chicks to subsequent immunization with similar antigens. These results are at variance with those reported here using a bacterial antigen. Burnet and colleagues did not inoculate their experimental materials intravenously into the developing embryo; with *Sal. pullorum* the intravenous route was the only one which yielded any response by way of a diminished ability of the young chick to produce antibodies later in life. On the basis of the theory already

stated, it is reasonable to expect that early contact between developing cells of the embryo's reticulo-endothelial system and unaltered antigen are essential conditions for the demonstration of this phenomenon, and that these conditions would be maintained provided that the inoculations were made intravenously. The detection of antibody in Burnet's experiments required the production by the embryo of virus antihaemagglutinins and phage-inactivating antibodies. Each of these may be considered as complete types of antibody in that they appear as the result of an adult host's response to contact with the respective antigens and are the products of the stimulation of the mature reticulo-endothelial system. Under the experimental conditions described, if the embryos had responded by producing the equivalent of non-agglutinating bacterial antibodies or even primary units of antibody (adapted enzymes) these would not have been observed by the *in vitro* tests employed. A further difference between the experimental procedure of Burnet and those reported here is that in the former embryos were inoculated with living particles (virus, bacteriophage) and in the latter they received killed *Sal. pullorum*. Basset (1951) and others emphasized that the intracellular existence of virus and bacteriophage may differ fundamentally in the process of antibody production from that which occurs with bacteria. Particles containing enzymes may be more resistant to enzyme attack than dead particles (Sevag, 1951). In embryos in which enzyme systems are only partially developed the host may be unable to respond as effectively to a stimulus from living antigen as it can from dead antigen. In addition, the enzyme systems of living organisms such as viruses, adapt themselves to their environment (Monod, 1949; Hinshelwood, 1946) so that the cellular enzymes of the young host, which are still in the developmental stage, must respond not only to the stimulus of the original inoculation of living particles, but also to possible adaptation of the particles themselves to their environment in the host. In contrast to the living antigenic materials used by Burnet, the intravenous inoculation of killed bacteria into the embryo resulted in a relatively sudden contact between the host's cells and antigen which is less resistant to attack by cellular enzymes. In addition, dead particles of antigen will be altered only as a result of responsive action by the host's cells which will not be modified by any enzyme activity of the antigen itself.

The experimental results have been discussed in relation to Burnet & Fenner's adaptive enzyme theory of antibody production. Pauling (1940) and Haurowitz (1949), however, are of opinion that antibody production can proceed only so long as antigen remains intact within the host's cells. Confirmation of this hypothesis can only follow the use of techniques which detect the presence of minute quantities of antigen *in vivo*. Limited evidence in favour of this theory has been produced by Herdegen, Halbert & Mudd (1947), who worked with *Shigella paradysenteriae*, and by McMaster & Kruse (1951), who used labelled globulin as antigen. Moreover, it is considered by some that long-lasting immunity associated with virus diseases (e.g. smallpox, yellow fever, measles) may be due to the multiplication in the host of variants to these viruses which do not produce pathological symptoms. The evidence

from experiments with *Sal. pullorum*, however, appears to conform more nearly to the adaptive enzyme theory of Burnet, since the inoculation of antigen into the embryo on or before the 15th day of incubation not only failed to stimulate the production of antibodies in the hatched chick, which according to Pauling and Haurowitz suggests that the antigen had been destroyed, but it resulted in a subsequent diminished ability of the chick to produce antibody, possibly because of the adaptation of the host's intracellular enzymes to the antigen.

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REFERENCES

- BASSET, J. (1951). Fièvre aphteuse. Le vaccin de Waldmann et sa posologie. Origine des anticorps et mécanisme de l'immunité. *Bull. Acad. vét. Fr.* **24**, 21.
- BEVERIDGE, W. I. B. & BURNET, F. M. (1946). The cultivation of viruses and rickettsiae in the chick embryo. *Spec. Rep. Ser. med. Res. Coun., Lond.* no. 256.
- BRANDT, L. W., CLEGG, R. E. & ANDREWS, A. C. (1951). The effect of age and degree of maturity on the serum proteins of the chicken. *J. biol. Chem.* **191**, 105.
- BURNET, F. M. (1941). Growth of influenza virus in the allantoic cavity of the chick embryo. *Aust. J. exp. Biol. med. Sci.* **19**, 291.
- BURNET, F. M. & FENNER, F. (1948). Genetics and immunology. *Heredity*, **2**, 289.
- BURNET, F. M. & FENNER, F. (1949). *The Production of Antibodies*. Monograph, Walter and Eliza Hall Inst., Melbourne: Macmillan and Co. Ltd.
- BURNET, F. M., STONE, J. D. & EDNEY, M. (1950). The failure of antibody production in the chick embryo. *Aust. J. exp. Biol. med. Sci.* **22**, 291.
- BUXTON, A. (1951). Transfer of bacterial antibodies from the hen to the chick. *Nature, Lond.* **168**, 657.
- BUXTON, A. (1952). On the transference of bacterial antibodies from the hen to the chick. *J. gen. Microbiol.* **7**, 268.
- FOX, J. P. & LAEMMERT, H. W. (1947). Cultivation of yellow fever virus: observations on infection of developing chick embryo. *Amer. J. Hyg.* **46**, 21.
- GRASSET, E. G. (1929). Recherches sur la sensibilité du tissu embryonnaire aux antigènes. Assais d'immunisation comparée de l'embryos de poulet et de la poule adulte. *C.R. Soc. Biol., Paris*, **101**, 1102.
- HAUROWITZ, F. (1949). Biological problems and immunochemistry. *Quart. Rev. Biol.* **24**, 93.
- HERDEGEN, M., HALBERT, S. P. & MUDD, S. (1947). Persistence of antigen at the site of inoculation of vaccine emulsified in oil. *J. Immunol.* **56**, 357.
- HINSHELWOOD, C. N. (1946). *The Chemical Kinetics of the Bacterial Cell*, pp. 284. Oxford: Clarendon Press.
- MASURIER, H. E. LE, BRANION, H. D. & MARCELLUS, F. M. (1946). Observations on unabsorbed yolk in chicks. *Poult. Sci.* **25**, 300.
- MCMASTER, P. D. & KRUSE, H. (1951). The persistence in mice of certain foreign proteins and azoprotein tracer-antigens derived from them. *J. exp. Med.* **94**, 323.
- MONOD, J. (1949). The growth of bacterial cultures. *Annu. Rev. Microbiol.* **3**, 371.
- MURPHY, J. B. (1913). Transplantability of tissues to the embryo of foreign species. Its bearing on questions of tissue specificity and tumour immunity. *J. exp. Med.* **17**, 482.

- MURPHY, J. B. (1914). Factors of resistance to heteroplastic tissue-grafting. Studies in tissue specificity. III. *J. exp. Med.* **19**, 513.
- MURPHY, J. B. (1916). The effect of adult chicken organ grafts on the chick embryo. *J. exp. Med.* **24**, 1.
- NORTON, S., WOLFE, H. R. & CROWE, J. R. (1950). Effect of injections of soluble antigens on the spleen size and antibody production in chickens. *Anat. Rec.* **107**, 133.
- PAULING, L. (1940). A theory of the structure and process of formation of antibodies. *J. Amer. chem. Soc.* **62**, 2640.
- POLK, A., BUDDINGH, G. J. & GOODPASTURE, E. W. (1938). Experimental study of complement and haemolytic amboceptor introduced with chick embryos. *Amer. J. Path.* **14**, 71.
- RYWOSCH, M. (1907). Über Hämolyse und Baktericidie des embryonalen Hühnerblutes. *Zbl. Bakt.* **44**, 468.
- SEVAG, M. G. (1951). *Immuno-catalysis*. 2nd ed. Springfield (Illinois): Charles C. Thomas.
- SHERMAN, H. W. (1919*a*). Antibodies in the fetus. *J. infect. Dis.* **24**, 1.
- SHERMAN, H. W. (1919*b*). Antibodies in the chick. *J. infect. Dis.* **25**, 256.
- STEVENSON, H. N. (1917). Tumour immunity in the chick embryo. *J. Cancer Res.* **2**, 245, 449.
- STEVENSON, H. N. (1918). Growth of tumours in the chick embryo. *J. Cancer Res.* **3**, 63.
- WEINBERG, M. & GUELIN, A. (1936). Recherche sur l'immunité active de l'embryon. *C.R. Soc. Biol., Paris*, **122**, 1229.

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