NON-SPECIFIC STIMULATION OF A NATURAL ANTIBODY.

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CONTENTS.

INTRODUCTION.

The question of non-specific formation of antibodies is of considerable theoretical and practical importance, and is closely related to the problem of "non-specific immunisation" which has attracted attention in therapeutics during recent years.

The recognised occurrence of the so-called heterogenetic or heterophile antibodies might be regarded as exemplifying the non-specific stimulation of antibody formation and might appear to be a striking exception to the basic principle of specificity in acquired immunity. Thus, a haemolytic antibody for sheep's blood (Forsmann's antibody) can be developed in high titre in the serum of the rabbit by immunising this animal with certain heterologous antigens, *e.g.* the tissues of the guinea-pig and certain other species (see Taniguchi, 1921). This heterophile antibody represents, however, not a new substance but an increased output of a natural immune body present normally in appreciable amount. Though the phenomenon may be supposed to depend on the fact that the homologous and heterologous antigens possess some common antigenic property, it shows that antibody production can be influenced by stimuli which are not apparently specific.

It has also been noted in persons vaccinated with *B. typhosus* and subsequently developing a paratyphoid infection at a time when the post-vaccine

agglutinin has practically disappeared from the blood, that the paratyphoid infection leads to a renewed production of the typhoid agglutinin (Mackie and Wiltshire, 1917). Other infections may produce the same effect (see Browning, 1918), which in this case therefore is not dependent entirely on the biological relationship of the heterologous to the homologous antigen. The reappearance of latent agglutinins has also been demonstrated under experimental conditions as the result of non-specific stimuli, *e.g.* injection of deutero-albumose and sodium nucleinate (Jaggi, 1923). Thus non-specific effects may increase the production of antibodies once the tissues have been sensitised, as it were, by immunisation with the specific antigen.

Apart altogether from substances of known antigenic nature, it has been claimed that certain other substances, even some simple chemicals, may stimulate antibody formation. Salomonsen and Madsen (1898) stated that in horses immunised with diphtheria toxin, pilocarpine injections increased the antitoxin content in the blood. According to Walbum (1921), metallic salts (in virtue of their catalytic action), *e.g.* manganous chloride, when injected intravenously, exert a marked effect in increasing the concentration of antitoxin and certain other antibodies in immunised animals. McIntosh and Kingsbury (1924) and O'Brien (1924) have failed, however, to confirm Walbum's results.

In the course of certain observations on the natural haemolytic antibody of the rabbit for sheep's red cells (referred to above) it was found that this antibody is invariably present in definitely measurable amount in the serum and that in apparently normal healthy rabbits its concentration is remarkably constant. The constancy of this antibody has also been referred to and illustrated by Taniguchi (1921) and Iijima (1923). In view of the recognised influence of certain heterologous antigens in stimulating its production, a series of experiments were carried out with the object of ascertaining whether other non-specific influences might increase the output of the natural haemolysin. While it would be unwise to generalise from either positive or negative results obtained in this way, these tests seemed a convenient method of obtaining some information regarding the possible effect of non-specific stimuli on antibody formation, and apart altogether from the question of non-specific immunisation, any information with regard to those influences that affect antibody production is of obvious interest.

The effect of intravenous injection of certain metallic salts (manganous and beryllium chloride) was first investigated and later various other substances and influences, which have been applied in "non-specific therapy" or have been referred to by experimental workers as exercising a non-specific effect on antibody formation, were tested, *e.g.* a colloidal metal (manganese), salvarsan, sodium nucleinate, various alien proteins, certain bacterial products, the turpentine abscess, and repeated bleeding.

It must be recognised that the reputed non-specific stimulation of antibodies is entirely a secondary effect by which the output of these substances

can be augmented beyond the normal production under the existing conditions, *e.g.* following the primary specific immunisation or where the particular antibody is a natural product of the tissues as in the case of the haemolysin studied in these experiments.

TECHNIQUE OF EXPERIMENTS.

The method of determining the concentration of the antibody in the serum was to test varying quantities of heated serum (57° C.) with fixed amounts of sheep's red cells and guinea-pig's complement, as in the usual haemolytic experiments; fresh guinea-pig's serum, from which the natural immune body for sheep's corpuscles had been removed by treatment with the homologous cells at 0° C. (1 hour), was used as complement.

The degree of activity of the haemolysin is dependent of course on the particular specimen of complement and, to some extent, on the specimen of sheep's blood used in the particular test. To ensure uniformity in comparing a series of specimens of serum obtained at different times from an experimental animal, the samples were all tested simultaneously with the same cells and complement; a quantity of about 2 c.c. of blood was obtained by bleeding from an ear vein, the serum was separated, and heated for 1½ hours at 57° C. (in sealed quill tubes) and then kept in an ice-box till tested. The haemolysin has proved stable under these conditions. A corresponding method has been used by Taniguchi and by Iijima in their work on heterophile antibody.

The test amount of sheep's blood was 0·5 c.c. of a 3 per cent. suspension of washed red cells in normal saline. After the addition of the rabbit's serum, 0·05 c.c. of complement was added (*i.e.* from 5 to 10 M.H.D.). Varying amounts of the rabbit's serum were tested, usually the following series 0·2 c.c., 0·1 c.c., 0·05 c.c., 0·025 c.c., 0·01 c.c., 0·005 c.c., 0·0025 c.c.; when necessary larger and smaller amounts were also included in the series. The results have been stated in terms of the minimum amount which produced *complete* lysis of the test blood suspension after 1 hour at incubator temperature. For purposes of recording the results in a convenient comparative form, the reciprocals of these doses, indicating the *relative* concentrations of the antibody in the serum, have been stated in the tables.

CONTROL TESTS.

As shown by comparative tests over several weeks, this natural antibody has been found to remain constant in normal healthy animals. Since the experiments in some cases involved the comparison of specimens of serum taken at intervals during a period of as long as 31 days, a number of control animals (14 in all) were examined as in the case of the various test animals. In no case has any natural increase of the antibody been observed and the stability of the antibody in the specimens of serum preserved by the method described above has been established. The content remained unchanged except in two animals in which a diminution was noted. These animals, during the period in which they were examined, lost weight, and one died later showing marked coccidiosis at autopsy.

The constancy of the antibody is further evidenced by the negative results in certain of the actual experiments and by the results of experiments in which animals were submitted to successive large daily bleedings (*v. infra*). It is noteworthy from such results that in the normal animal this antibody is maintained at a fixed concentration, even in some cases after repeated bleedings

amounting to a total withdrawal of over 100 c.c. of blood in rabbits weighing from $1\frac{1}{2}$ to 2 kilograms. This is similar to what has been noted in regard to the content of antitoxin in immunised horses after repeated bleedings, and corresponds to the "antitoxic equilibrium" described by Madsen (1923). Glenny and Sudmersen (1921) have recorded an interesting example of a constant concentration of diphtheria antitoxin in an immunised guinea-pig maintained over a prolonged period (one to two years).

EXPERIMENTS WITH METALLIC SALTS.

Manganous chloride and beryllium chloride were employed. The former is the substance that was most used by Walbum in his work. Among the various salts tested, beryllium chloride was also found to be highly effective in stimulating antibody formation (see Madsen, 1923). Thus, as a result of repeated intravenous injections of beryllium chloride (1 c.c. of a 1/1000 molecular solution per kilogram of body weight) in goats and rabbits previously immunised with *B. coli*, the agglutinin content in the serum was increased 1170 per cent. (average).

It has been found in the writer's experiments that both in the case of the manganese and beryllium salts a dose of 1 c.c. of a 1/1000 molecular solution per kilogram given intravenously is not uniformly tolerated by rabbits especially in repeated doses, and it has been necessary to use smaller doses (0·5 c.c.–0·25 c.c. of 1/1000 molecular solution in animals weighing from 1 to 2 kilograms).

Twelve experiments were carried out with the manganese salt. The results have been variable. In certain experiments in which repeated injections were given, a marked increase of the antibody content was noted even up to twenty times its original concentration (Tables I and II). In Rabbit 134 (Table I) a progressive increase was observed; in Rabbit 2 A (Table II) a decrease occurred followed by a marked increase. Such results seem to afford a striking example of how antibody production may be influenced by a non-specific stimulus. In other instances, however, the increase was less marked, not more than twice the original value, and in some the content remained unchanged (Table III).

Table I.

Rabbit 134.

Day of experiment	Injection	Concentration of antibody
1	0·25 c.c. *M*/1000 $MnCl_2$, $4H_2O$ intraven.	5—before injection
4	Ditto	—
5	—	10
6	0.25 c.c. *M*/1000 $MnCl_2$, $4H_2O$ intraven.	—
8	Ditto	—
13	—	100
14	—	100

Note. In the tables the figures representing the relative concentration of the antibody are the reciprocals of the minimum haemolytic doses.

Table II.

Rabbit 2 A.

Day of experiment	Injection	Concentration of antibody
1	0·25 c.c. *M*/1000 $MnCl_2$, $4H_2O$ intraven.	100—before injection
7	Ditto	10 „ „
15	Ditto	10 „ „
21	—	1000

Table III.

Rabbit 402.

Day of experiment	Injection	Concentration of antibody
1	0·25 c.c. *M*/1000 $MnCl_2$, $4H_2O$ intraven.	20—before injection
3	0·35 „ „ „ „	20 „ „
5	0·35 „ „ „ „	20 „ „
8	0·35 „ „ „ „	20 „ „
12	0·4 „ „ „ „	—
15	0·4 „ „ „ „	20 „ „
19	0·4 „ „ „ „	20 „ „
25	—	20

It has been noted also in an experiment in which repeated doses were given at short intervals that a marked and progressive reduction of the concentration occurred (Table IV) and this animal died two days after the last injection, apparently due to poisoning by the chemical.

Table IV.

Rabbit 410.

Day of experiment	Injection	Concentration of antibody
1	0·25 c.c. *M*/1000 $MnCl_2$, $4H_2O$ intraven.	10—before injection
3	0·35 „ „ „ „	2 „ „
7	0·35 „ „ „ „	2 „ „
9	—	1

Experiments were also carried out in which single doses only were given and the antibody content was estimated at intervals following the injection. In one test in which specimens were taken at intervals of a few hours, a marked decrease was noted in the earlier samples while after 20 hours the content was restored to the original value. Table V shows how the increase may be merely a temporary one; four days after a single dose the concentration fell to the original figure.

Table V.

Rabbit 441.

Day of experiment	Injection	Concentration of antibody
1	0·5 c.c. *M*/1000 $MnCl_2$, $4H_2O$ intraven.	3·3—before injection
2	—	10
3	—	10
4	—	3·3

The results in the various experiments with manganous chloride may be summarised as follows.

Alteration in Antibody Content.

Repeated injections	
Expt. 1	Increase— × 10
,, 2	Nil
,, 3	Decrease, then return to original value
,, 4	Progressive decrease
,, 5	Progressive increase— × 20
,, 6	Nil
,, 7	Increase— × 10
,, 8	Increase— × 2
Single injection	
Expt. 9	Nil
,, 10	Decrease, then return to original value
,, 11	Increase— × 3·3
,, 12	Increase— × 2

The best results were obtained by repeated injections, the increase being progressive or observable after the last dose, but there has been no uniformity, the effects varying with different animals. Certain animals responded well, while others failed to react.

Six experiments were carried out with beryllium chloride in which single injections were given. With this substance distinct effects were noted after a single dose. Table VI (Rabbit 430) shows an immediate increase occurring after 2 hours.

Table VI.

Rabbit 430.

Hours following injection	Injection	Concentration of antibody
—	0·25 c.c. *M*/1000 $BeCl_2$, $4H_2O$	20—before injection
2	—	100
4	—	100
24	—	100

Madsen has commented on the rapidity of the increase in antitoxin following an injection of manganous chloride. In another experiment, however, with the beryllium salt the increase did not occur till the third day (Table VII).

Table VII.

Rabbit 432.

Day of experiment	Injection	Concentration of antibody
1	0·25 c.c. *M*/1000 $BeCl_2$, $4H_2O$ intraven.	20—before injection
2	—	20
3	—	100

The results with beryllium chloride have also been inconstant and are summarised as follows:

Alteration in Antibody Content.

Single injection	
Expt. 1	Increase— × 5
,, 2	Nil
,, 3	Increase— × 5
,, 4	Increase after 2 hours— × 5
,, 5	Progressive decrease
,, 6	Nil

Colloidal Manganese.

A commercial and a laboratory preparation have been used[1].

The injection of certain colloidal metals has been advocated and applied in the treatment of various infections. The question arises as to whether these preparations exercise some influence on the immunity mechanism (see Petersen, 1922). It has been suggested by Steabben (1925) from results of experiments with various colloids that the antibody response to a specific antigen may be increased by the simultaneous injection of a colloid substance.

Three experiments were carried out with colloidal manganese injected intravenously and in each an increased concentration of the antibody was noted. The preparations contained a certain amount of glucose but in two parallel experiments intravenous injections of the amounts of glucose present in the quantity of the preparation used produced no increase. Table VIII shows one of the results with colloidal manganese.

Table VIII.

Rabbit 485.

Day of experiment	Injection	Concentration of antibody
1	1 c.c. colloidal manganese intraven.	10—before injection
7	Ditto	20 „ „
14	Ditto	50 „ „
20	—	100

Salvarsan.

It has been suggested that salvarsan may exert a stimulating effect on antibody formation and it has been stated that experimentally this substance may augment the production of agglutinins (Nicolas, Gourmont and Gate, 1912, and Walker, 1920). McIntosh and Kingsbury (1924) have discussed this question in a recent paper but have not observed any definite alteration in antibody content in immunised animals following salvarsan injections.

In two experiments repeated injections of novarsenobillon were followed by an increase in the content of the natural haemolysin, 2- and 4-fold respectively. Table IX shows one of these results.

Table IX.

Rabbit 463.

Day of experiment	Injection	Concentration of antibody
1	0·08 gm. novarsenobillon intraven.	10—before injection
8	0·1 „ „ „	10 „ „
16	0·1 „ „ „	20 „ „
18	—	40
28	—	40

[1] Prepared according to the formula of Martindale and Westcott (1922), *Extra Pharmacopoeia*, I. 358.

SODIUM NUCLEINATE.

The appearance of latent agglutinins has been noted after injections of this substance (Jaggi, 1923).

In four experiments in which successive doses of sodium nucleinate were given intravenously, an increase of the haemolysin was observed in three instances, while in the fourth animal there was a progressive decrease. Table X shows the result in an experiment in which a 5-fold increase occurred after the first injection and was maintained subsequently during the experiment though the concentration was not further increased. Table XI gives the result with another animal in which a 4-fold augmentation followed the first injection while the repeated doses led to a progressive decrease.

Table X.

Rabbit 48.

Day of experiment	Injection	Concentration of antibody
1	1 c.c. 5 % soln. sod. nucleinate intraven.	20—before injection
6	Ditto	100 „ „
11	Ditto	100 „ „
15	Ditto	100 „ „
19	—	100

Table XI.

Rabbit 492.

Day of experiment	Injection	Concentration of antibody
1	1 c.c. 5 % soln. sod. nucleinate intraven.	5—before injection
8	Ditto	20 „ „
14	Ditto	5 „ „
24	Ditto	2 „ „
31	—	2

FOREIGN PROTEINS.

(*Normal Serum, Albumose, and Milk.*)

As is well known the parenteral injection of native proteins and "protein split products" has been extensively applied in non-specific therapy.

In the case of a rabbit injected subcutaneously with successive doses of normal horse serum a very marked increase in the antibody was noted, the final content being 30 times greater than the original (Table XII).

Table XII.

Rabbit 5.

Day of experiment	Injection	Concentration of antibody
1	5 c.c. normal horse serum subcut.	3·3—before injection
7	6 c.c. „ „ „	3·3 „ „
14	10 c.c. „ „ „	20 „ „
21	—	100

The horse is one of those species whose tissues yield heterophile antigen, and the serum of such animals is known to possess a similar property though in a lesser degree (Orudschiew, 1913).

In two further tests, rabbits were injected with the serum of the ox (whose tissues are devoid of heterophile antigen). *In both instances* the first injections produced a marked increase (4-fold) but the repeated doses led to a decrease below the original concentration (Table XIII). A similar effect has been noted with sodium nucleinate (*v.* Table XI).

Table XIII.

Rabbit 486.

Day of experiment	Injection	Concentration of antibody
1	10 c.c. ox serum (fresh and unheated)	5—before injection
8	Ditto	20 ,, ,,
14	Ditto	10 ,, ,,
21	—	5

According to O'Brien (1924) peptone injections produce no effect on the concentration of diphtheria antitoxin in immunised animals. Jaggi noted the stimulation of typhoid agglutinin by deutero-albumose.

Four experiments were carried out in which intravenous injections of an albumose preparation (prepared from ox flesh) were given. In only one of these did any increase of the antibody result (Table XIV).

Table XIV.

Rabbit 129.

Day of experiment	Injection	Concentration of antibody
1	1 c.c. 10 % soln. albumose intraven.	2·5—before injection
4	—	10

In one animal three subcutaneous injections of 10–15 c.c. sterilised cow's milk were given at weekly intervals. There was a marked depression of the immune body, followed by restoration to the original content seven days after the last injection, but no increase.

BACTERIAL CULTURES AND CERTAIN OTHER BACTERIAL PRODUCTS.

It has been stated that certain bacteria contain heterophile antigen and produce a marked augmentation of the anti-sheep haemolysin of the rabbit. Thus Iijima has shown how *B. dysenteriae* (Shiga) acts in this way and his results have been confirmed in this investigation (Table XV).

Table XV.

Rabbit 21.

Day of experiment	Injection	Concentration of antibody
1	Graded doses of *B. dysenteriae* (Shiga) vaccine on 1st, 7th, and 14th days	10—before injection
7		5 ,, ,,
14		500 ,, ,,
21	—	1000

In another experiment in which a preparation of the exo-toxin of this organism was used no effect was observed.

Killed cultures of certain other organisms have been tested and also certain

other bacterial preparations. The experiments and results may be stated as follows:

		Alteration in antibody concentration
B. typhosus ...	Three intravenous injections at weekly intervals of graded doses of heat-killed saline suspension from an agar culture	Decrease
B. paratyphosus B. ...	Ditto	Increase— × 2 (1 week after second dose)
B. proteus "X19" ...	Ditto	Increase— × 2 (1 week after second dose)
B. dysenteriae (Y), Expt. 1	Ditto	Decrease at first, followed by × 10 increase after second dose
,, Expt. 2	Ditto	Nil
,, Expt. 3	Ditto	Increase— × 2 (1 week after second dose)
B. suipestifer ...	Ditto	Nil
B. diphtheriae ...	Ditto	Nil
Tuberculin ...	0·2 c.c., 0·5 c.c., 0·75 c.c. "Old Tuberculin" intravenously	Nil

The only marked effect noted was in one of the experiments with *B. dysenteriae* (Y). The second experiment with the same organism showed a much lesser effect, and in a third animal no change occurred. As in the case of the Shiga type of dysentery bacillus this may be a heterophile antigen effect.

BACTERIAL INFECTIONS.

Two animals were inoculated intravenously with a bovine strain of the tubercle bacillus and died in 27 and 28 days respectively. Specimens of serum taken at intervals, including samples when the animals were moribund, showed no variation in the M.H.D. as compared with that before the inoculation.

Similarly, two rabbits inoculated with *B. suipestifer* and dying after seven days of a general infection with this organism showed no appreciable change in the content of the haemolysin.

TURPENTINE ABSCESS.

The subcutaneous and intramuscular injection of turpentine for the purpose of producing an artificial abscess has been applied in the medical treatment of certain infective conditions. Madsen states that inflammation occurring in an antitoxin-producing animal may be accompanied by a considerable increase in antitoxin formation.

In one experiment the turpentine abscess was associated with a two-fold increase of haemolysin, but in a subsequent test the content remained unaltered except for a depression immediately following the injection.

EFFECT OF REPEATED BLEEDINGS.

A considerable amount of work has been done with regard to the effect of bleeding on antibodies (see O'Brien, 1913; Hartley, 1924). O'Brien has shown how, in the case of a horse injected with sheep's red cells and in a condition of constant haemolytic titre, the withdrawal of 122 litres of blood in fractions over a period of eleven months produced a fall in haemolytic titre to 66 per cent.

only of the original value. Thus the tissues may be stimulated as a result of bleeding to form an increased amount of an antibody so that the content is maintained at the original value or exhibits only a relatively small decrease.

Certain of the investigations on this subject tend to show that the concentration of antitoxins and other antibodies may even be increased by large bleedings. Langer (1921) has claimed that in rabbits immunised with typhoid bacilli repeated 20 c.c. bleedings may produce an increase in the agglutinin concentration. Hartley has found recently that repeated small bleedings at short intervals amounting in 24 hours to 10–12 c.c. may be followed after a few days by an increase in typhoid agglutinin in immunised rabbits.

The question has arisen as to whether the bleedings required for obtaining blood samples might *per se* increase the antibody content. In the control animals, already referred to, no increase has been noted. These included animals bled at varying intervals in parallel with actual test rabbits. Two rabbits were also subjected to repeated bleedings within 24 hours as in Hartley's experiments and then examined later at longer intervals. In one of these there was a decrease in the antibody content persisting on the eighth day of the experiment (Table XVI). In the other animal no alteration occurred.

Table XVI.

Rabbit 484

Day of experiment	Bleedings	Concentration of antibody
1	3 c.c. blood drawn on four occasions during 24 hours; total bleeding—12 c.c.	5 (in first specimen)
2	2 c.c. sample taken	2·5
6	2 „ „ „	2·5
8	2 „ „ „	2·5

Three experiments were carried out in which relatively large quantities of blood were withdrawn on successive days so that a total amount of over 100 c.c. of blood had been removed. In one rabbit (Table XVII), from which 130 c.c. were withdrawn within five days, the antibody content remained absolutely constant. This experiment illustrates how an equilibrium as it were is maintained in spite of extensive bleeding.

Table XVII.

Rabbit 462.

Day of experiment	Bleedings	Concentration of antibody
1	30 c.c.	20
2	25 „	20
3	25 „	20
4	25 „	20
5	25 „	20
8	usual sample	20
12	„	20
19	„	20

In another test similar to the foregoing, after the last large bleeding there was a fall in the amount of antibody, but six days later the original content

was restored. The third experiment (Table XVIII) showed, however, a progressive reduction of the haemolysin followed by a progressive increase until ten days after the bleedings the content was five times greater than the original value. Seven days later the original content was restored. In this case bleeding stimulated an increased production of antibody which temporarily rendered its concentration in the serum greater than the original.

Table XVIII.

Rabbit 446.

Day of experiment	Bleedings	Concentration of antibody
1	40 c.c.	40
2	25 ,,	20
3	23 ,,	10
4	20 ,,	10
5	usual sample	5
8	,,	<5
11	,,	10
13	,,	100
15	,,	200
22	,,	40

DISCUSSION.

These experiments indicate that (apart from the injection of substances which act as heterophile antigen) the natural haemolytic antibody of the rabbit for sheep's red cells may be markedly stimulated in some instances by certain non-specific agents, *e.g.* the intravenous injection of manganous and beryllium chlorides, colloidal manganese, salvarsan and sodium nucleinate, the subcutaneous injection of normal ox serum, and successive large bleedings. Such effects are however extremely variable and depend apparently on the individual animal; certain rabbits react well while others show no response[1]. It is obvious, therefore, if any general deduction can be made from the results as regards such non-specific influences on antibody formation, that these agents are of doubtful value as a practical means of augmenting antibody formation. The inconstancy of the results with the metallic salts contrast with the claims of Walbum and Madsen, but may explain the divergence between their results and the completely negative results recorded by others. Of course it has not been found possible to give the larger doses used by Walbum. In this connection it must be noted that individual animals also show considerable variation in their reponse to heterophile antigens, *e.g.* guinea-pig's kidney. In the writer's own experience one rabbit may exhibit a 100-fold increase in the anti-sheep haemolysin while in others the increase may be less, or even absent altogether. This variation has also been noted in experiments with another heterophile antigen, *B. dysenteriae* (Shiga), referred to above.

[1] Horgan has also recorded a similar variability in the effect of manganous chloride on agglutinin in immunised animals and has suggested that there is some relation between the power of the animal to produce antibodies and their response to manganese (*Brit. Journ. Exper. Path.* 1925, VI. 108). In the writer's experiments there has been no apparent relationship between the degree of normal production of the antibody and the response to non-specific agents.

In none of the experiments with these non-specific agents has the stimulation of the antibody been so marked as that produced by the homologous antigen or even a heterophile antigen. The greatest increase noted was 20-fold, whereas in animals immunised with guinea-pig's kidney or *B. dysenteriae* (Shiga) a 100-fold increase may occur. Injections of normal horse serum produced a 30-fold increase but the tissues and serum of this species contain heterophile antigen.

While it would be difficult to make any definite generalisation from these experiments, they are of some interest as a contribution to the somewhat controversial subject of non-specific immunisation which requires careful analysis both from the standpoint of experimental immunisation and also of therapeutics.

CONCLUSIONS.

1. The natural haemolytic antibody of the rabbit for sheep's blood is remarkably constant in amount in normal healthy rabbits and *may* remain unaltered even after extensive bleedings. A marked increase in the concentration of the antibody has been noted however after successive large bleedings. Small bleedings have no such effect.

2. Apart from immunisation with heterophile antigen, this antibody may be increased markedly in amount in the serum following the injection of certain non-specific substances: metallic salts (manganous chloride and beryllium chloride), colloidal manganese, salvarsan, sodium nucleinate, normal ox serum.

3. This effect is inconstant and variable in degree.

4. The increase produced in such non-specific manner is less than that usually noted after immunisation with the homologous or even a heterophile antigen.

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