THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Siegfried Frederick Herrman for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

W. P. Parsons
Chairman

E. J. Bell.

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June 6th 1918

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Siegfried Frederick Herrmann final oral examination for the degree of Master of Science. We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota
May 29 1919

W. P. Parson
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THE EFFECT OF FOREIGN PROTEIN ON ANTIBODY PRODUCTION

A Thesis submitted to the
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Many clinical reports have been published in recent years concerning the treatment of various acute and chronic infections by the intravenous injection of foreign proteins. This treatment has been notably applied to typhoid fever, the arthritides, and certain skin diseases. It apparently developed as an outgrowth of the injection of vaccines. Vaccine therapy, once the greatest hope of modern therapeutics, had failed to produce the promised results, and, with the exception of prophylactic vaccination against typhoid, variola, rabies, and perhaps acne and allied pyogenic skin diseases, was being more and more generally abandoned. Now there is occurring an apparent revival of allied therapeutic methods in the form of foreign protein injections. The following historical resumé is based upon a recent article by Millen(9).

The beginning of foreign protein therapy dates back to the work of Fraenkel(1) in 1893, who treated fifty-seven cases of typhoid with subcutaneous injections of typhoid vaccine. He reported that most of the cases were favorably modified, and that a few terminated by rapid lysis.

This use of typhoid vaccine during the active, acute stage of the disease was a new departure, which demanded further investigation.

Rumpf(2), using a vaccine of Bacillus pyocyaneus, repeated the work in another group of cases, and observed equally favorable results.

There followed many attempts by different workers to produce the same abortive results by injections, usually intravenous, of a variety of substances. Colon vaccine, chicken serum, whole blood, proteose, albumose, horse serum, etc., all seemed equally efficacious.

Ichikawa(3), using intravenous injections of sensitized vaccine, and Kraus and Mazza(4), using a polyvalent vaccine, were able to produce favorable modifications in all but 40% of their cases. They also observed equally good results in paratyphoid and found that colon vaccine could be substituted for the typhoid vaccine.

More recent work on typhoid has been reported by Lüdke(5) with albumoses; Saxl(6), using a solution of caffeine and camphor; Kibler and McBride(7), using typhoid vaccine; etc.

In this country much work has been done on foreign protein treatment of the arthritides. Notable is the report of Miller and Lusk(8). Miller later summarizes his clinical results and gives an extensive bibliography of work done by other clinicians(9). The results as reported appear very promising, especially in acute arthritis.

Other favorable results have been reported in skin diseases, puerperal sepsis, conjunctivitis, iritis, trachoma, typhus, tuberculosis, etc.

Closely related to the response to foreign protein is the old and familiar observation that a specific disease may sometimes improve with the advent of some intercurrent infection. For example, the symptoms of acute gonorrhea have been observed to subside with the incidence of typhoid fever. Attention has also been called to the increased resistance of the body to various common infections, following any vaccine injection.

It is evident that foreign protein therapy as reviewed above is absolutely empirical and non-specific. Almost any foreign protein seems to be effective, and may be employed more or less successfully, under suitable conditions, in a variety of diseases. The question

arises as to what factor is responsible for the apparent therapeutic benefits observed. If this manner of treatment is to be placed on a scientific basis this factor must be determined. Clinical investigations have thrown no light upon this question. The type of reaction is apparently the same in all cases. As described by Kibler and McBride(7) the reaction of a typhoid patient to the intravenous injection of typhoid vaccine is as follows:

1. Temperature. Within a few minutes to one hour after the injection a chill occurs, lasting twenty to thirty minutes. This is followed by a rapid rise of temperature which later falls abruptly, and then tends to remain remittent in favorable cases.

2. Leucocytes. Usually there is an early leucopenia within four hours, due mainly to a disappearance of polymorphonuclears. Within about twelve hours this is followed by a moderate polymorphonuclear leucocytosis.

3. Blood pressure is usually decreased by 10 to 15 millimeters after the chill.

4. Coagulation time. Only slight and insignificant changes have been observed.

5. Antibodies. No essential difference from the specific response to the injection of typhoid antigen in a normal individual was observed in these typhoid patients.

It is to be noted however that conditions here differ somewhat from those in which an heterologous protein is injected.

The reaction in arthritis cases agrees essentially in the first four particulars with the reaction outlined above. The antibody response will be considered later.

Most clinicians seem agreed in regarding the severe chill as

the most important factor in this reaction. It is evident that a chill alone cannot be responsible for the marked relief from pain and for the remission in the course of an acute arthritis. It is possible, however, that the resulting hyperpyrexia may have an effect in favoring immune reactions and antibody production.

Rolly and Meltzer(10), in a study on the effect of high temperatures, injected a series of rabbits with small doses of typhoid bacilli or cholera vibriones. Some of these rabbits were kept in an oven so that their body temperatures remained between 40° and 42°C. The controls were kept at ordinary room temperature. They observed a higher average production of agglutinins and lysins in the high temperature rabbits than in the controls. They also showed that phagocytosis by human leucocytes in vitro occurs at its optimum between 37° and 40°C.

More recently, Winslow, Miller and Noble(11) investigated the effect of moderately high temperatures on the production of lysins against sheep's red blood corpuscles. They kept their rabbits in an oven at a moderately high temperature, 29° to 32°C. Injections of sheep's cells were given, and the hemolytic titre estimated each week. They found that the rate of lysin production in the heated rabbits was slower than that in the controls.

These experiments may indicate that a moderately high temperature lowers the general vital resistance to a beginning infection. Once an infection is established, however, according to Rolly and Meltzer's results, hyperpyrexia may bring about a greater production of antibodies.

If a specific leucocytosis could be observed, one might suspect that this would be a factor in overcoming the infection, but the de-

gree of leucocytosis seems only to parallel the severity in each case(12). Gay and Claypole reported a specific hyperleucocytosis in typhoid immune rabbits, following injections of typhoid bacilli(13), but McWilliams(14) later showed that both normal and immune rabbits respond to about the same degree. The leucocytosis therefore cannot be regarded as specific.

The slight variations in blood pressure and coagulation time are evidently insignificant.

We pass now to a consideration of the antibody response. Very little laboratory work on this aspect of the question has been done, yet it seems as if the explanation of a therapeutic response, in the case of any infection, to the injection of a substance not specifically bactericidal to the infecting organism, should logically be sought in the liberation of specific antibodies against that organism. Ordinarily when the body overcomes a streptococcic infection, for example, we believe this is accomplished by the production of antibodies against the streptococcus. Why, then, should we not suspect that the therapeutic effect of foreign protein injections in infections, obviously not due to the foreign protein per se, has as its basis the liberation of specific antibodies against the causative organism? In the case of an arthritis we believe that the symptoms persist because the antibodies which should normally be produced are for some reason not liberated.

In the light of Ehrlich's receptor theory, we recognize three distinct phases in the production of antibodies: (1) the sensitization of the tissue cells; (2) an overproduction of sessile receptors; (3) the liberation ("Abstossung") of these receptors from the cells. A perfect antigen is one which causes all of these phases to be com-

pleted. It seems a plausible hypothesis that in those cases of arthritis, etc., which show a rapid therapeutic response to injections of foreign protein, the etiological organism is acting only as an imperfect antigen. It sensitizes the cells and causes sessile receptor production, but does not give the necessary stimulus to cause these receptors to be thrown off. The foreign protein then supplies this stimulus, the system is flooded with specific antibodies, and a remission of the infection results. Altho a variety of proteins may serve to complete this second phase, the reaction is still absolutely specific, since there must be a specific stimulation of cells, and a final liberation of specific antibodies.

In this connection recall the classic experiments of Bruck(15). According to Ehrlich's theory a molecule of tetanus toxin is composed of a haptophore group, which unites with the cell receptor, and a toxophore group, which is responsible for the poisoning of the cell. This toxophore group can be destroyed by various methods such as heat, light, and long preservation. The toxin has then become a toxoid. The haptophore group can still unite with a sessile cell receptor, or with a specific antibody, but because of the absence of the toxophore group the molecule has become innocuous. Bruck injected rabbits with small repeated doses of such a toxoid. The rabbits were found to produce very little, or no, free antitoxin, the amount being dependent on the amount of effective toxophore groups which still remained in the toxoid. He further showed that rabbits injected with tetanus toxoid, during the first few succeeding hours possessed a greater tolerance for tetanus toxin, but that later they showed an actual hypersensitiveness, being killed by only a fractional portion of the ordinary lethal dose of toxin.

These results Bruck interpreted as follows: The haptophore group of the toxoid at first anchors on to the specific tissue cell receptor. The toxin molecule introduced soon afterward, therefore, cannot reach the cell, since all the receptors are blocked. Later, however, an overproduction of sessile receptors occurs, and a hypersensitiveness to toxin results, since the avenues of approach to the cell are increased many times. The toxoid has acted as an inferior antigen. It has brought about the first and second steps of antibody production, namely (1) sensitization of cell receptors and (2) the overproduction of similar sessile receptors, but it has lacked the necessary stimulus to cause the "Abstossung" of these receptors. Had the rabbits been immunized with small doses of unchanged toxin this third stage would have been completed. Bruck therefore sees in the toxophore group the necessary stimulus for the setting free of the receptors.

Might not a foreign protein, in an analogous way, supply such a stimulus?

It was with this hypothesis in view that we undertook our investigation of the effect of foreign protein on antibody production. The experiments have been conducted entirely with rabbits, because all the factors can thus be much more accurately controlled than in a purely clinical investigation.

Several other investigations have been reported which touch on the immune reactions in foreign protein therapy.

Jobling and Petersen(16) report a "mobilization of ferments", notably protease and lipase, following foreign protein injections. Later(17) they point out an "increase in antiferment", dependent on "highly dispersed, unsaturated lipoids", and conclude that "the main

therapeutic factor is probably an increased dispersion of colloids."

Culver(18) investigated the opsonin and lysin content in the serum of patients with gonorrheal arthritis, after intravenous injections of gonococcic vaccine and proteose, He reports a rise in titre of these antibodies.

Bull(19) reports a rise in the antibodies of typhoid immune rabbits after the injection of typhoid bacilli; but his results could not be confirmed by Teague and McWilliams(20). These latter workers suggest a new theory to explain the therapeutic effect upon cases of typhoid, of intravenous injections of typhoid vaccine. Upon the supposition that blood serum is more bactericidal than lymph, they base the theory that the vaccine causes a greater flow of bactericidal serum into the lymphoid organs, and thus overcomes the local infection. We do not believe that this theory has been substantiated.

Upon the basis of the hypothesis as outlined above, namely that the foreign protein reaction serves as a stimulus to set free specific antibodies, we first investigated opsonins against the streptococcus. This organism is notably a poor antigen. In other words, it is difficult to obtain a high antibody titre against it. An animal sensitized with this organism should therefore be a favorable subject for the liberation of antibodies by foreign protein.

Series I. Two rabbits were injected intravenously at three day intervals with one cubic centimeter of a fresh suspension of killed Streptococcus viridans. The organism was grown on blood agar slants, and a twenty-four hour growth washed off with ten cubic centimeters of physiologic saline solution. This emulsion was heated at 60°C. for one hour to kill the bacteria, and one cubic centi-

meter of this vaccine used as a dose. A fresh vaccine was made each time. The fourth injection consisted of 1/2 c.c. of living streptococci. Five days later the opsonic index was determined, and 2 c.c. of human ascitic fluid injected intravenously.

The method of determining the opsonic index was that of Wright, using the experimenter's own leucocytes and serum as a standard.

Results are charted in Plate I. Both rabbits were found to have a very low opsonic index five days after the fourth injection of streptococci. 2 c.c. of human ascitic fluid were then injected, and a marked rapid rise of opsonins occurred, the total increase being 700% in 48 hours. Apparently the foreign protein acted as an effective stimulus to liberate sessile receptors.

After 18 days the opsonic content of the rabbits' sera was again found to be at a low level. Two further injections of streptococci were then given, as charted. This time an interesting variation in response occurred. The opsonic index of No. 17 rose, while that of No. 311 continued to fall. In the former the streptococci evidently acted as a perfect antigen, causing all three stages of antibody production, while in the latter the final liberation of antibodies did not take place. This supposition was confirmed by the effect of an injection of ascitic fluad. In No. 17 there resulted no further increase in opsonins. The stimulus of the antigen had been sufficient to liberate all antibodies. In No. 311 a rapid liberation of opsonins followed the foreign protein injection. In this case we picture the tissue cells as loaded with an overproduction of specific sessile receptors. The foreign protein reaction then acted as an effective stimulus to cause the cells to throw off these receptors.

Series II. See Plate II. In this series two rabbits were in-

jected with fresh suspensions of meningococci, and human serum was used as foreign protein. Rabbit No. 395 received two injections of serum with a resulting rise after each injection. Apparently the cells were not completely desensitized by the first serum injection. Rabbit No. 268 received no second serum injection and showed a gradual decrease in opsonins.

In order to demonstrate that these antibodies were specific for meningococci, and also as a control check on our technique, the rabbits' sera were also tested each time for opsonins against B. typhosus. The typhoid opsonic index remained low and unchanged thruout.

Series III. See Plate III. Two rabbits were given four intravenous injections of Streptococcus viridans at three day intervals, as in Series I. Six days after the fourth injection the opsonic index was determined. Rabbit No. 31 then received 1 c.c. of human serum intravenously, while rabbit No. 32 was used as a control and received no serum. The former showed a rise of 38% on the third day following, while the opsonic index of the latter remained stationary. The control test for B. typhosus remained unchanged in both cases.

During the next forty days four injections of a dextrose broth culture of streptococcus were given. Six days after the last injection the opsonic index was again determined. It was found lowered in both rabbits. Rabbit No. 32 then received 1 c.c. of human serum. There followed a rise of nearly 300%. Rabbit No. 31 showed a spontaneous rise, but to a much less degree.

A check opsonic index determination for meningococci showed no variation.

It was now thot that if an increase in opsonins following foreign protein injection occurred, it should also be possible to demon-

strate a simultaneous rise in the agglutinin titre for streptococci.

The agglutinin titre was determined by the microscopic method as follows:

The rabbits were bled from the ear veins, the blood allowed to clot, and the serum separated by means of rapid centrifuging. Varying dilutions of this fresh serum were then made in 0.9% salt solution. Hanging drop preparations were then made by placing upon a coverslip, with a small platinum loop, one drop each of diluted serum and of a 15 hour dextrose broth culture of streptococcus. The coverslips were then inverted on vaselined hollow ground slides, and left at room temperature. Readings were then taken under the microscope at the end of three hours. Positive agglutination could be observed in the lower dilutions, consisting of definite clumping of the streptococcal chains. The highest degree of dilution in which a positive test occurred was recorded as the agglutinin titre. Results are reported in the lower half of Plate III.

The first tests were performed simultaneously with the last opsonic determinations. In response to the first serum injection No. 32 showed a rise in agglutinin titre of 250%. No. 31, which served as a control at this time, also showed a slight rise of 50%, and continued to rise gradually. The agglutinin titre of No. 32 began to fall after the third day. All the sessile receptors apparently had been freed by the foreign protein injection, while in No. 31, without the stimulus of foreign protein they were liberated to a less degree and more slowly.

In order to resensitize the tissue cells 2 c.c. of broth culture were now injected. Seven days later the agglutinin titre had fallen very low in both rabbits. This time No. 31 received 1 c.c.

of human serum, while No. 32 served as control. A rapid rise of agglutinin titre occurred in the former which then remained stationary. In the control rabbit a more gradual rise occurred. Thus the specific response to the foreign protein injection was again observed.

Series IV. See Plate VII. It now became necessary to investigate whether or not foreign protein injections would serve to bring about a mobilization of antibodies against an organism which was known to be a good antigen, that is, one which when injected into an animal, would readily cause the production of a high titre of free antibodies. The typhoid bacillus is known to be such a perfect antigen, when injected into rabbits. Indeed, it is not unusual to obtain an agglutinin titre against typhoid as high as 1-500,000 dilution.

Two rabbits were injected repeatedly with emulsions of typhoid bacilli, until they showed an agglutinin titre of 1-50, upon testing by the macroscopic method. 1 c.c. of guinea pig serum was then injected and the agglutinin titre again determined in 48 and 72 hours. No rise in titre was apparent.

Further injections of typhoid bacilli were then given until the agglutinin titre reached 1-100. Then 1/2 c.c. of human serum was injected. Again no increase in agglutinins could be observed during the next 72 hours. A second injection of human serum did not change the titre.

In order to check these negative results two more rabbits were immunized with typhoid. After two injections of bacilli the agglutinin titre was found to be zero, by the microscopic hanging drop method, as described under Series III. 1/2 c.c. of human serum was then injected, and the antibody titre again determined on the third

day following. Again no agglutinins were found. Evidently the rabbits had not yet been sufficiently sensitized against the bacilli.

Immunization with typhoid bacilli was then continued until the microscopic method revealed an agglutinin titre of 1-3000. 2 c.c. of human ascitic fluid were then injected intravenously. No rise in agglutinin content resulted.

The sera of these rabbits were also tested for complement fixing bodies, with the idea in mind that the foreign protein reaction might possibly bring about a liberation of such antibodies, altho it apparently had no effect on the agglutinin content of the rabbits' sera.

As an antigen in the complement fixation test an old suspension of typhoid bacilli in normal saline, to which 0.3% tricresol had been added, was used. The test was carried out with the usual technique of a complement fixation test, using this typhoid antigen, washed sheep's red blood cells, fresh guinea pig complement, and rabbit amboceptor. The serum of our typhoid immune rabbits was inactivated by heating at 56°C. for 1/2 hour, then diluted, and the complement fixing power of a given quantity tested.

Results:- The ability of the inactivated serum to fix complement was not changed in the slightest degree by foreign protein injections.

We must conclude that foreign protein injections have no effect upon the antibody production in typhoid immune rabbits. The typhoid bacillus is apparently a perfect antigen in rabbits, and no foreign protein is needed to stimulate the liberation of antibodies against it.

How then can we reconcile this with the apparent therapeutic benefits observed in some human patients? Perhaps the improvements

reported have been only the result of a clinical over-enthusiasm. On the other hand may it not be possible that some patients do not react completely to the antigenic stimulation of the typhoid bacillus? In such cases the foreign protein might well serve as a stimulus causing the sensitized tissue cells to throww off their sessile receptors. This sudden flooding of the system with antibodies might then be responsible for the remission in the disease. This hypothesis seems to be supported by the succeeding series.

Series V. Charted in Plates IV, V, and VI. Thus far we have recorded observations on opsonins and agglutinins. It seemed desirable also to investigate the response of a third type of antibodies, lysins, to foreign protein injections. Since it is difficult to make accurate determinations of the lysin content of a serum for a specific bacterial cell, we decided to use as our antigen washed sheep's red blood corpuscles. A hemolytic titre can be very accurately obtained while a bacteriolytic titre, obtained by the method of extinction and plating, is modified by too many possible errors of technique.

It is well known that rabbits will readily produce lysins against sheep's corpuscles. Common use is made of this fact in producing amboceptor for the hemplytic system of the Wassermann reaction. We may say therefore that sheep's cells act as a good antigen when injected into rabbits. This would allow us to predict, on the basis of the preceding series, that foreign protein injections into rabbits sensitized against sheep's cells would have little effect on the liberation of specific lysins for these cells. It has however been frequently observed in this laboratory that a great variation exists in the readiness with which different rabbits will produce

amboceptor when injected with sheep's cells. Rubinstein(21) recently studied the hemolytic titre of rabbits after three injections of sheep's red cells. The titre was determined before each injection, and seven days after the third. He found that out of eleven rabbits only eight showed a definite marked rise in lysins after each injection. In one of the remaining three the titre remained the same, and in the other two there was even a decrease in lysin content after successive injections. In such rabbits as the latter three the sheep's cells evidently acted only as an inferior antigen, and in such cases we would expect, on the basis of our previous work, to be able to liberate antibodies by means of foreign protein injections.

The technique of obtaining the hemolytic titre in our rabbits was as follows:- A rabbit was bled from the ear vein into a dry Wassermann tube. The blood was allowed to coagulate and was then centrifuged. The serum was decanted into a fresh tube and heated in a water bath at 56°C. for one half hour, in order to inactivate it.

Fresh complement was obtained by exsanguinating a guinea pig into a sterile centrifuge tube; allowing the blood to clot; centrifuging and pipetting off the serum. This fresh serum was then diluted ten times with 0.9% sodium chloride solution, and used as complement in the subsequent titration.

Fresh sheep's red blood cells were obtained by bleeding a sheep aseptically from the jugular vein. The blood was stirred until defibrinated and then filtered thru cotton. The filtrate was centrifuged rapidly to throw down the corpuscles and the supernatant serum pipetted off. The corpuscles were then washed repeatedly with 0.9% saline and recentrifuged. A 2% suspension of these washed cells in 0.9% saline was then made and used in the subsequent titration.

For the hemolytic titration six Wassermann tubes were set up, each containing 1.5 c.c. of 0.9% saline solution. The inactivated rabbit's serum was diluted 1-10 in saline and to each tube were added respectively .02, .03, .04, .06, .08, .10 c.c. of this diluted serum. To each tube were then added .10 c.c. of complement (1-10) and 0.5 c.c. of red cell suspension. The rack was then placed in the incubator at 37°C. for one hour. At the end of this time the readings were taken, and the tube containing the least amount of rabbit's serum, and in which complete hemolysis had occurred, was recorded as the lysin titre of that serum. If hemolysis occurred in all six tubes the inactivated serum was diluted more highly, and the titration repeated until a definite end point was obtained.

Results of our experiments are recorded in Plates IV, V, and VI. The ordinate axis in these charts represents the least amount, in cubic centimeters, of undiluted inactivated rabbit's serum which would cause complete hemolysis in the titration as outlined above. Suppose hemolysis was complete in the fifth and sixth tubes of a titration with serum diluted 1-20. The fifth tube contains .08 c.c. of diluted serum. .08 c.c. of serum diluted 1-20 represents .004 c.c. of undiluted serum, and would be recorded so on the chart. The abscissa represents days, as in preceding charts.

A total of twenty rabbits was used in this series. To avoid undue repetition only nine have been charted. The rabbits were injected intravenously with two initial doses of sheep's corpuscles suspended in saline solution. As a rule the lysin content was then measured, 10 to 12 days after the last red cell injection, it having been determined that the lysin titre normally reaches its height by the end of this period. After foreign protein injections, either human

serum or typhoid vaccine, the lysin content was again determined, usually in 72 hours, in order to give ample time for any resulting change in titre to take place.

Plate IV represents a record of the hemolytic titre of the rabbit apparently most favorable for this investigation. Sheep's cells evidently acted as an inferior antigen in this case. The lysin content eleven days after the second red cell injection was very low, and remained so. 1/4 c.c. of human serum injected on the seventeenth day after the last red cell injection did not influence the low lysin content. Believing that this was due to the fact that the tissue cells were no longer properly sensitized, we then gave another injection of sheep's cells. Eleven days after this the titre was still at the same low level. An injection of foreign protein (typhoid vaccine) was then given, and an immediate marked liberation of lysins occurred. These antibodies disappeared rapidly. A second injection of typhoid vaccine in seven days had no effect on raising the lysin titre, but the curve continued to fall rapidly. A third injection of red cells was then given and this time acted as a perfect antigen. The lysin content rose to its height in ten days and remained high for several days. After it began to decrease another injection of red cells was given, but this time an injection of ascitic fluid was given only four days later, in order to determine whether an early stimulation by foreign protein would serve to liberate antibodies. A very pronounced mobilization of lysins followed.

This alternating introduction of red cells and foreign protein was continued as recorded on the chart. Every time the mere injection of antigen (red cells) failed to produce lysins in the rabbit's serum a mobilization of these antibodies could be effected by foreign protein.

When, on the other hand, there was a normal response to the antigen, the foreign protein was followed by no further liberation of lysins (see the reaction on the 119th day). After the last two successive injections of red cells apparently all the receptors were not spontaneously liberated because there was a slight response to foreign protein ten days later.

Plate V. Rabbits Nos. 387, 362, and 6 on Plate V are of this same type. They failed to respond to injections of red cells by a proper production of antibodies, but when then injected with foreign protein a marked liberation of lysins occurred. Note especially the pronounced rise in No. 6.

Nos. 30 and 39 were rabbits which responded very well to the antigenic stimulation of sheep's cells. No. 39 was used as a control while No. 30 received human serum on the eleventh day following the last injection of antigen. No marked difference can be noted in the reactions of the two rabbits. This was observed also in a number of other cases. When the titre is already high, foreign protein will not bring about a further rise in antibody content.

Plate VI. Rabbit No. 51 represents a negative result which can not be readily explained. The typhoid vaccine injected on the 29th day should have been followed by a rise in antibodies, according to our other observations, since the red cells 11 days earlier had not produced a liberation of lysins. In other respects, however, this curve is in accord with the general results.

Nos. 61 and 66 are charted only to show that normally the lysins begin to disappear, under the conditions of our experiments, after the tenth day. No. 61 also repeats the observation that, when a proper liberation of antibodies follows the injection of red cells, in

other words, when the red cells act as a perfect antigen, no rise in titre can be expected to result from foreign protein injections after the tenth day. Earlier injections, that is, before the tenth day, are followed by a rise in titre, but since this rise must also be expected to occur normally without foreign protein, not much stress can be laid on results obtained at this time. No distinction can be made in these early injections between a liberation of antibodies due solely to the effect of the antigen, and a liberation as the result of a foreign protein reaction. We therefore have not charted results obtained in this way.

SUMMARY.

1. In rabbits sensitized with streptococci a definite liberation of specific opsonins and agglutinins follows the injection of foreign protein.

2. A similar rise in specific opsonins also occurs in rabbits sensitized with meningococci.

3. Foreign protein injections have no effect on antibodies in typhoid immune rabbits.

4. In suitable rabbits, which do not readily produce lysins against sheep's red blood corpuscles, the injection of foreign protein within ten days after the injection of antigen, is followed by a marked liberation of specific lysins.

5. A variety of foreign proteins can be used. Human serum, typhoid vaccine, human ascitic fluid, and guinea pig serum proved equally efficacious.

CONCLUSION.

The intravenous injection of foreign protein serves as a stimulus for the liberation of specific antibodies, in those animals in

which the previously injected antigen is unable to cause such a liberation. This insufficiency may lie either in the antigen or in the rabbit.

BIBLIOGRAPHY

1. Fraenkel. Ueber Spezifische Behandlung des Abdominal Typhus. Deutsche med. Wochenschr., 1893, vol. 19, p. 985.

2. Rumpf. Die Behandlung des Typhus Abdominalis mit Abzetötenten Kulturen des B. Pyocyaneus. Deutsch. med. Wochenschr., 1893, vol. 19, p. 987.

3. Ichikawa. Abortivbehandlung von typhösen Krankheiten. Ztschr. f. Immunitätsforsch. u. exper. Therap., 1914, Orig., vol. 23, p. 32

4. Kraus and Mozza. Zur Frage der Vakzinetherapie des Typhus Abdominalis. Deutsch. med. Wochnschr., 1914, vol. 40, p. 1556.

5. Luedke. Behandlung des Typhus abdominalis mit intravenösen Injektionen von Albumosen. München. med. Wochenschr., 1915, vol. 62, p. 321.

6. Saxl, P. Ueber die Einwirkung pyrogener Substanzen und Fieber. Wiener med. Wochnschr., 1916, vol. 66, p. 115.

7. Kibler and McBride. Intravenous injection of typhoid vaccines. Jour. Inf. Dis., 1917, vol. 21, p. 13.

8. Miller and Lusk. Use of foreign protein in the treatment of arthritis. Jour. A. M. A., 1916, vol. 66, p. 1756; and Jour. A. M. A., 1916, vol. 67, p. 2010.

9. Miller. The non-specific character of vaccine therapy. Jour. A. M. A., 1917, vol. 69, p. 765.

10. Rolly and Meltzer. Experimentelle Untersuchungen über die Bedeutung der Hyperthermie. Deutsch. Arch. f. klin. Med., 1908, vol. 94, p. 335.

11. Winslow, Miller and Noble. The effect of moderately high atmospheric temperature upon formation of hemolysins. Proc. Soc. Exper. Biol. & Med., 1916, vol. 13, p. 764.

12. Cross. Foreign protein injections in the treatment of acute infections. Journal-Lancet, 1917, vol. 37, p. 764.

13. Gay and Glaypole. Specific hyperleucocytosis. Arch. Int. Med., 1914, vol. 14, p. 662.

14. McWilliams. Is the hyperleucocytosis following the injection of typhoid bacilli into immunized rabbits specific? Jour. Immuno., 1916, vol. 1, p. 159.

15. Bruck. Experimentelle Beiträge zur Theorie der Immunität. Zeitschr. f. Hyg. u. Inf., 1904, vol. 46, p. 176.

Also - Beiträge zur Kenntniss der Antitoxinbildung. ibid, vol. 48, p. 113.

16. Jobling and Petersen. Bacteriotherapy in typhoid fever. Jour. A. M. A., 1915, vol. 65, p. 515.

17. Jobling and Petersen. Non-specific factors in the treatment of disease. Jour. A. M. A., 1916, vol. 66, p. 1753.

18. Culver. Antibodies in gonorrheal arthritis after the intravenous injection of specific and non-specific protein. Jour. Lab. & Clin. Med., 1917, vol. 3, p. 11.

19. Bull. The influence of typhoid bacilli on the antibodies of normal and immune rabbits. Jour. Exp. Med., 1916, vol. 23, p. 419.

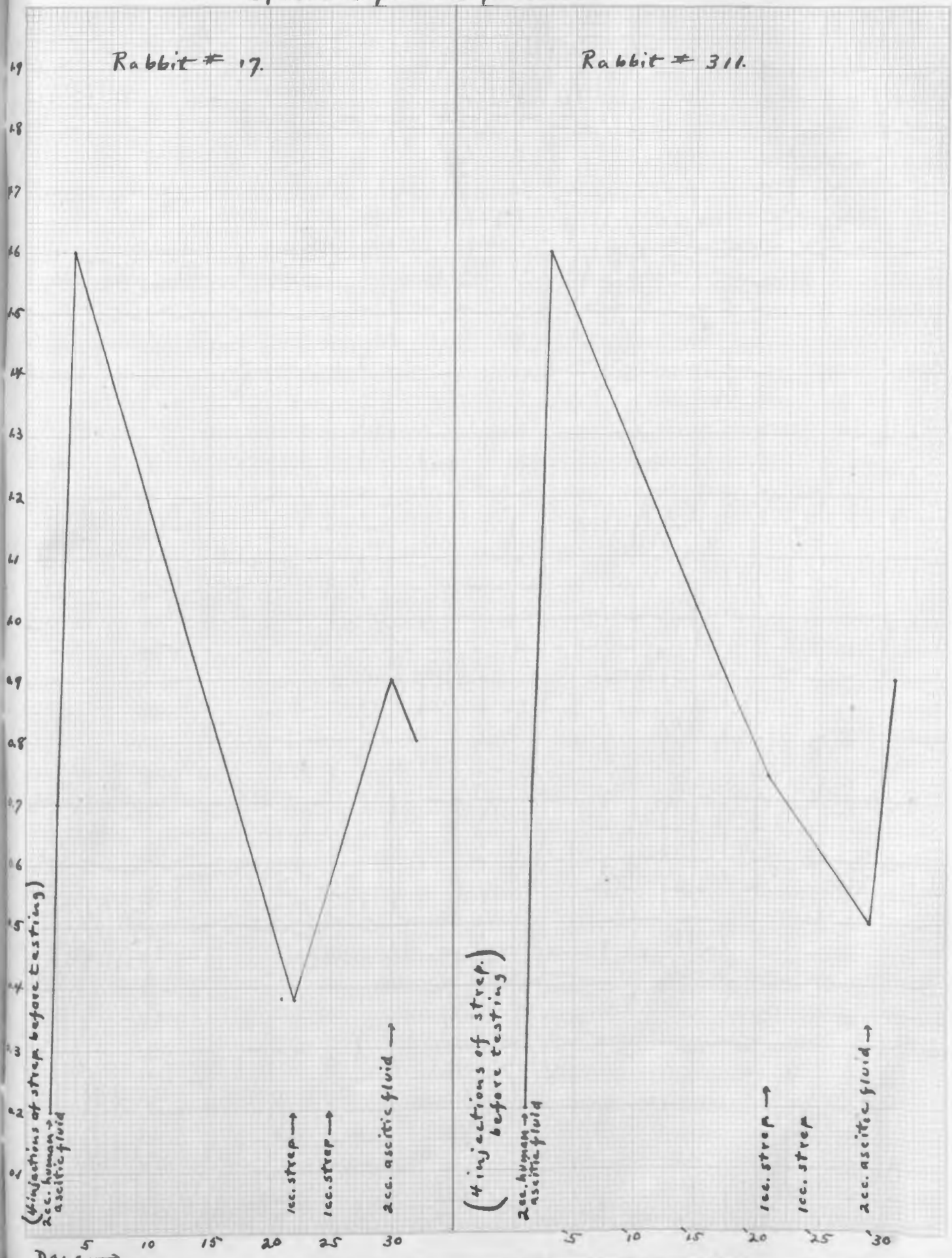
20. Teague and McWilliams. Experiments with a possible bearing upon the treatment of typhoid fever with typhoid vaccine administered intravenously. Jour. Immun., 1917, vol. 2, p. 185.

21. Rubinstein. Reaction de fixation preparation du serum hemolitique. Comptes Rendus Soc. de Biol., Paris, 1917, vol. 80, p. 908.

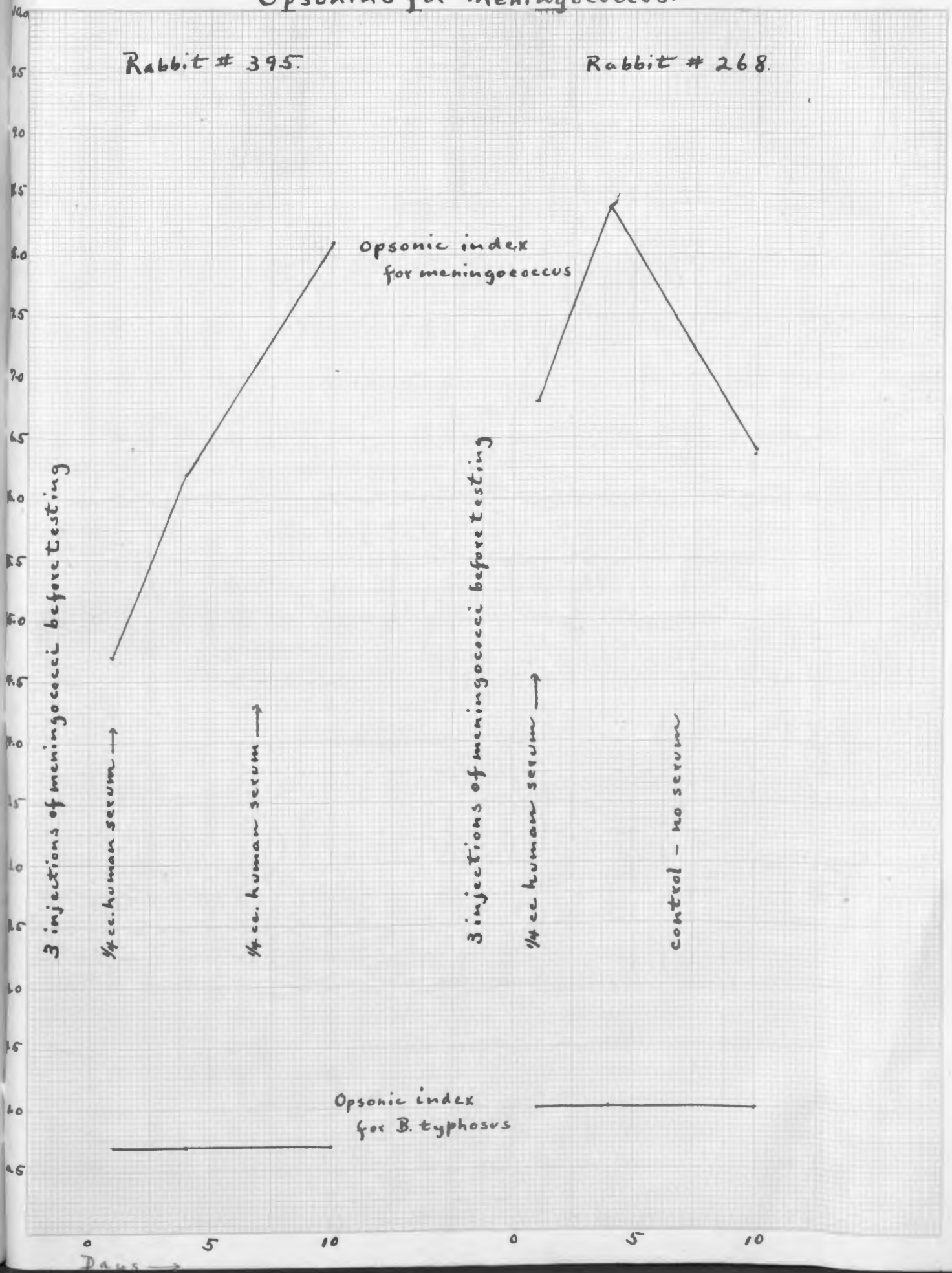
Series I

Plate I

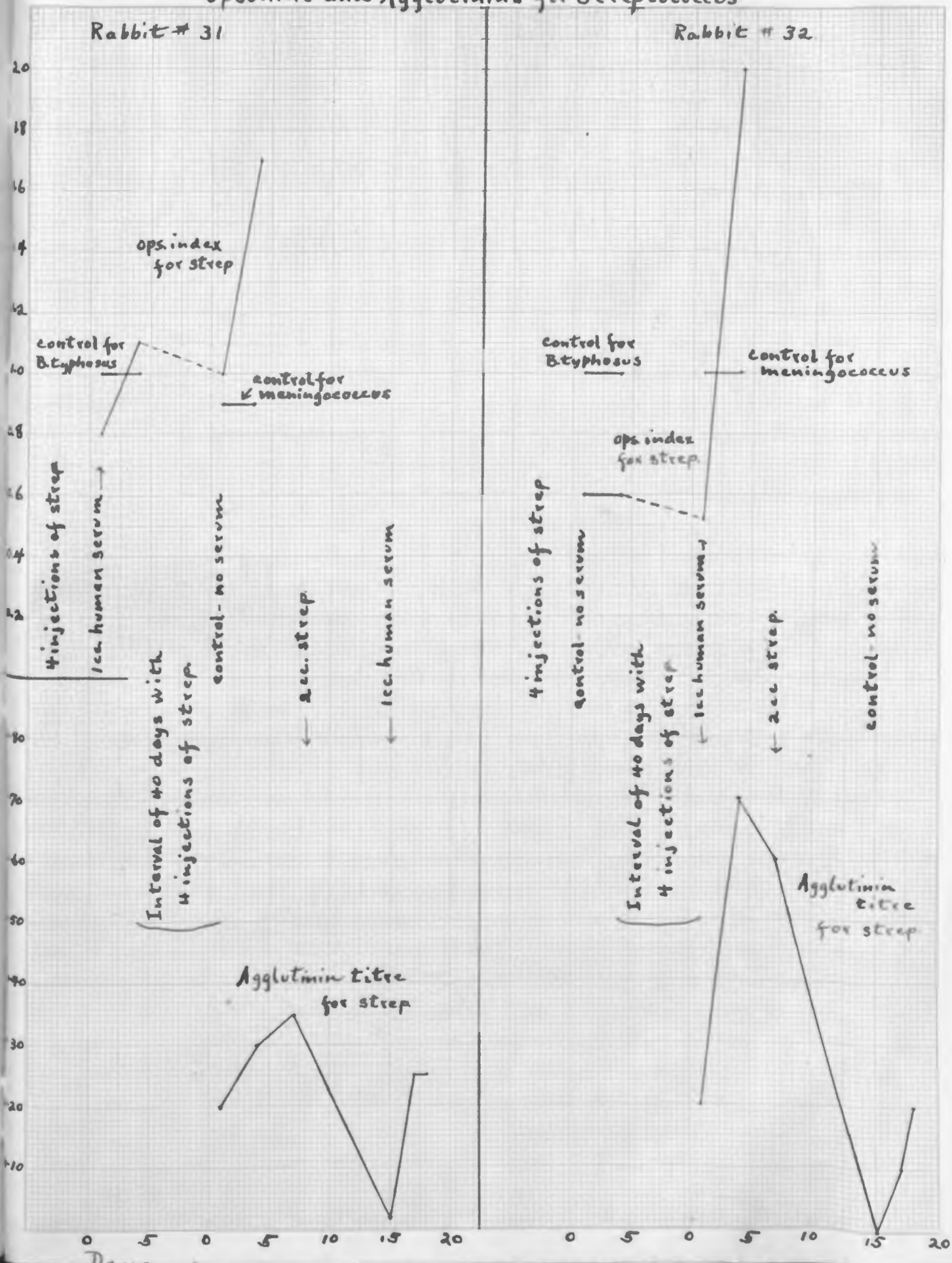
Opsonins for Streptococcus.



Opsonins for Meningococcus.



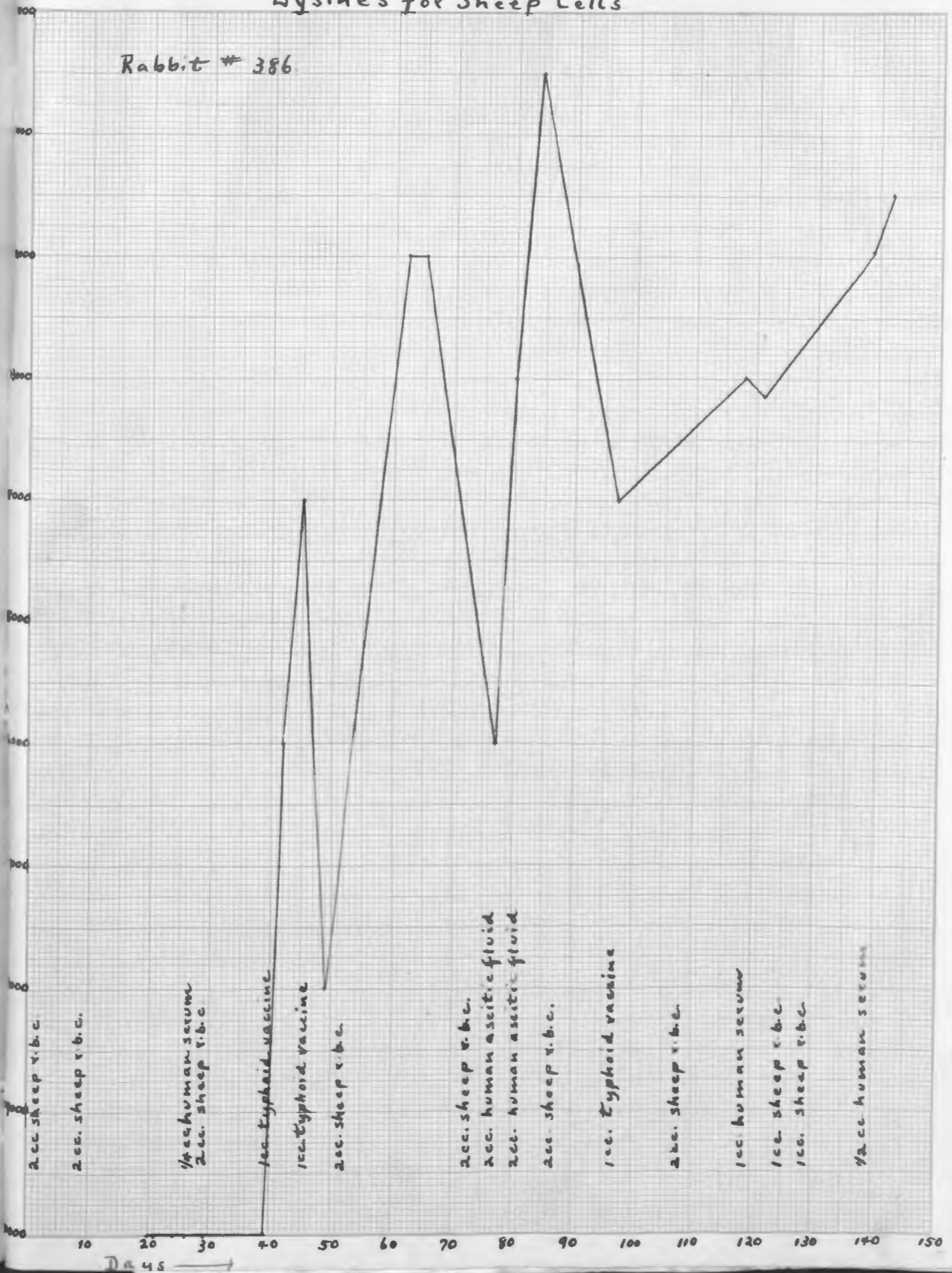
Opsonins and Agglutinins for Streptococcus



Series V

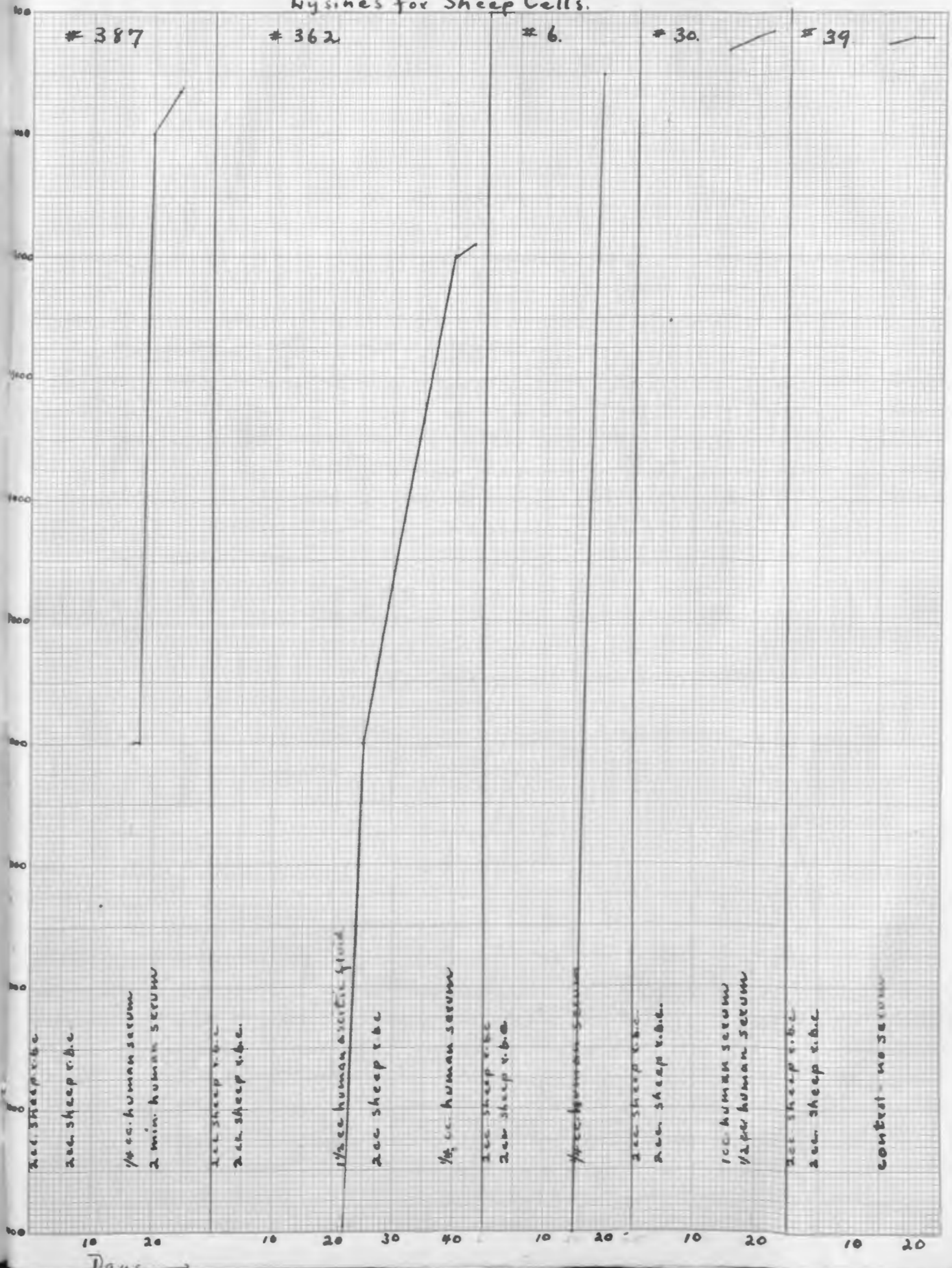
Plate IV

Lysines for Sheep Cells



Series V

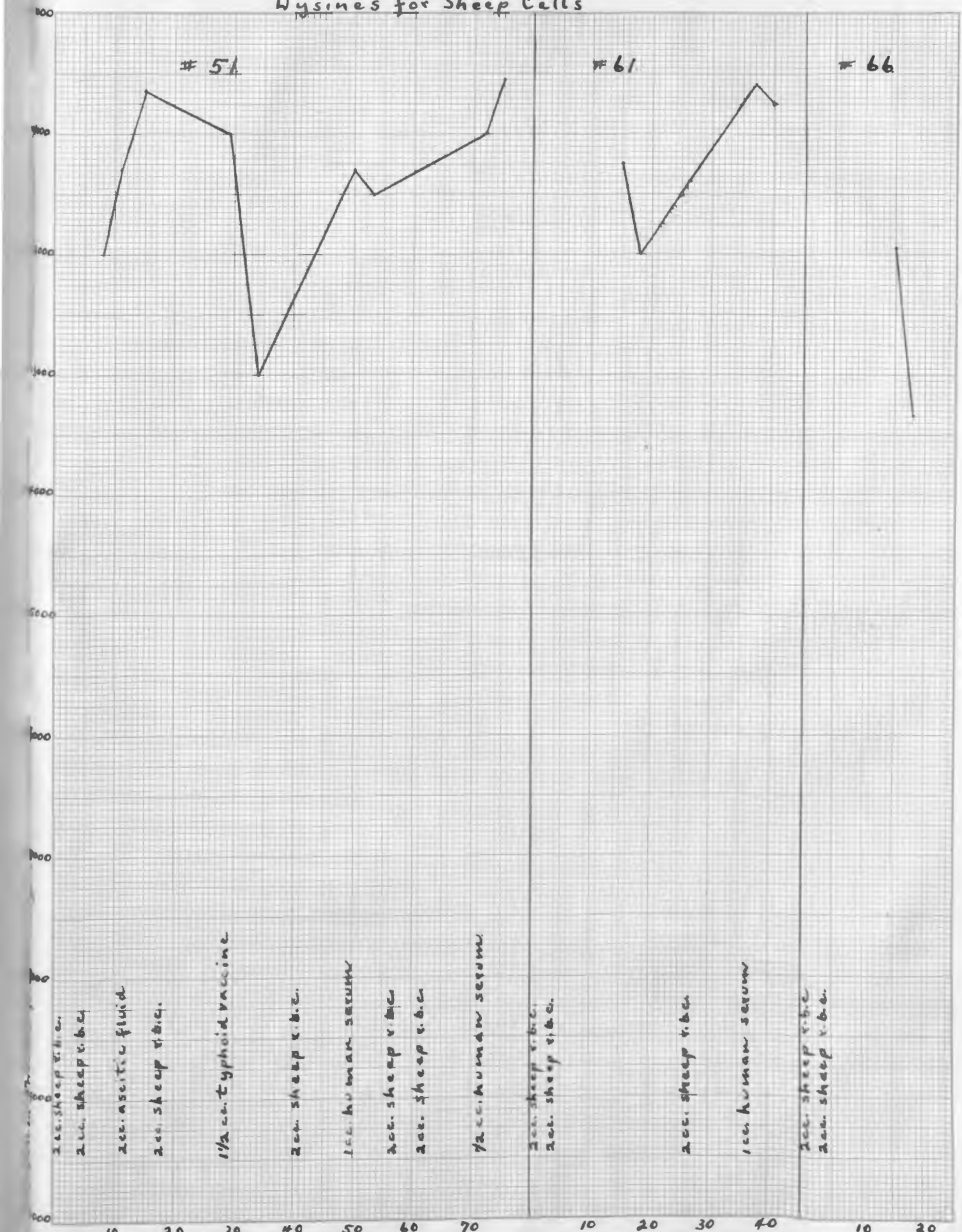
Plate V.



Series V

Plate VI.

Lysines for Sheep Cells



Series IV

Plate VII

Agglutinins for Typhoid Bacilli.