PRECIPITIN ANTISERA AND THEIR STANDARDISATION.

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It is usually accepted that in precipitin reactions a definite quantity of precipitin interacts with a definite quantity of homologous proteid, and further that in ordinary circumstances the interacting quantities combine to give rise to a precipitate, the precipitum. From this it follows that, if a known weight of serum containing precipitin be allowed to react with various weights of homologous proteid and if the quantities of proteid be sufficiently varied, certain reactions will be completed and others will be incomplete. The addition of precipitin and of homologous proteid to the superfluid of each reaction will determine the presence of excess of precipitin or of homologous proteid in such fluid. Conversely, if a known weight of the homologous proteid be allowed to interact with various weights of serum containing precipitin, it will be possible to determine in a similar manner those reactions which have been completed and those in which the homologous proteid or the precipitin is in excess. If the first stated proposition be correct, the results of these two series should coincide. A definite weight of serum containing precipitin should interact with a definite weight of proteid to form the precipitum.

We have endeavoured by the application of this method to determine exactly the strength of antisera, so that we might state the weight of homologous proteid which would completely react with a given weight

https://doi.org/10.1017/S0022172400002916 Published online by Cambridge University Press

of antiserum. In carrying out these experiments we have been led to an entirely new conception of the nature of the so-called precipitation of the homologous proteid. This we have discussed elsewhere.

We may still, however, apply this method of standardising antisera, and shortly we find that, while a certain quantity of homologous proteid completely neutralizes and precipitates a given weight of precipitin antiserum, the converse does not hold true. A given weight of proteid is not precipitated by any weight of antiserum. A considerable portion of the proteid remains in solution however small be the quantity originally taken.

Methods. We have carried out most of our experiments with material which has been dried *in vacuo* over calcium chloride at 38° C. The rabbit has been immunised by intraperitoneal injections of solutions of dried material. The antiserum has been dried and tested with its dried homologous proteid. Elsewhere[1] we have shown that this is frequently a more convenient method than that commonly adopted of utilising sterile fluids. In other cases, where it has been more suitable to use a fluid for the homologous proteid, we have set apart and dried a portion so that our results could be based upon weights of dried proteid. Since the solutions have been left for some days for interaction to occur, and since these have to be tested a second and sometimes a third time, all the apparatus and the salt solution with which solutions have been made, have been sterilised. The tests have been carried out in tubes of small bore (4—5 mm.) plugged with sterile cotton-wool. Where a bacterial deposit may have simulated or concealed a slight precipitum, the tubes have been rejected. We have taken note only of definite deposits which have generally settled to leave clear superfluids. The dried antisera, sera, and egg albumens have dissolved in ·75 % sodium chloride and have not required to be filtered. We have standardised the antisera by a modification of Nuttall's method so that other workers might note the relative strength of our antisera. These latter yield precipita between ·01 c.c. and ·1 c.c.

EXPERIMENT I. Reaction between egg albumen and dried egg antiserum. Rabbit immunised by the injection of 6·48 grammes of dried egg-white in 6 doses. Antiserum dried. All the primary tubes made up to 2·6 c.c. with ·75 % sodium chloride. Precipitum from ·1 c.c. antiserum by modified Nuttall's method ·024 c.c. A series of tubes arranged as in Table 1.

[1] *Proc. Linn. Soc.*, N. S. W., Sept. 1905; *Austral. Med. Gaz.*, Jan. 1906.

https://doi.org/10.1017/S0022172400002916 Published online by Cambridge University Press

TABLE 1.

No.	Amount of dried egg-white in grammes	Amount of dried antiserum in grammes	Deposits in 48 hours
1	·0000012	·01	slight
2	·0000024	·01	,,
3	·0000036	·01	,,
4	·0000048	·01	,,
5	·000006	·01	,,
6	·0000072	·01	,,
7	·0000084	·01	,,
8	·0000096	·01	marked
9	·0000108	·01	,,
10	·000012	·01	,,
11	·000024	·01	,,
12	·000036	·01	,,
13	·000048	·01	,,
14	·00006	·01	,,
15	·00012	·01	,,
16	·0006	·01	,,
17	·00012	none	none
18	none	·01	,,

After 48 hours the superfluid was removed and filtered. The removed fluid was divided into three portions, A, B, and C, of ·5 c.c. each. To each tube of series A ·1 c.c. of 1 % egg-white in salt solution (·00012 gramme dried) was added. To each tube of series B ·01 gramme of dried antiserum dissolved in ·1 c.c. saline was added. The tubes of series C formed controls. After 48 hours the readings were taken and these are recorded in Table 2.

TABLE 2.

No.	Deposits in Series A (plus egg-white)	Deposits in Series B (plus antiserum)	Deposits in Series C (Control)
1	marked	slight	none
2	,,	,,	,,
3	,,	,,	,,
4	,,	less marked	,,
5	,,	,,	,,
6	less marked	,,	,,
7	,,	,,	,,
8	,,	,,	,,
9	,,	marked	,,
10	slight	,,	,,
11	trace	,,	,,
12	,,	,,	,,
13	none	well marked	,,
14	,,	,,	,,
15	,,	,,	,,
16	,,	,,	,,
17	,,	,,	,,
18	marked	none	,,

A well-marked deposit is roughly equivalent to 2 mm. in other series; a marked deposit to 1 mm.; a less marked deposit to ·5 mm.; and a slight deposit to ·3 mm.

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This experiment shows that in reactions 1 to 12 a quantity of precipitin has not been neutralised by the albumen. In reactions 13 to 16 the precipitin has been neutralised. It also shows that in every tube in which albumen is present the further addition of antiserum caused a precipitate: this will be discussed later.

On examination of the quantities it will be seen that a quantity of albumen between ·000036 gramme and ·000048 gramme completely neutralised the precipitin in ·01 gramme of antiserum. The deposits given in Table 2, series A, show that the amount of precipitin decreased from tube 1 to tube 12, and that very little was present in tubes 11 and 12. It should be noted that not more than one-fifth of the original precipitin can be present in the tubes of series A and one-fifth of the original albumen in the tubes of series B recorded in Table 2.

EXPERIMENT II. Reaction between horse serum and dried horse antiserum. Rabbit immunised by the injection of 4·45 grammes of dried horse serum in 8 doses. Antiserum dried. All tubes made up to 2·6 c.c. with ·75 % sodium chloride. Fresh horse serum diluted with salt solution used for homologous proteid. Each cubic centimetre of serum contained ·097 gramme of dried material. The precipitin from the antiserum by modified Nuttall's method was ·024 c.c. A series of tubes as in Table 3 was arranged. Tubes of about 5 mm. diameter with a column of about 15 cm. of fluid were used.

TABLE 3.

No.	Amount of dried serum in grammes	Amount of dried antiserum in grammes	Precipita in 48 hours in mm.
1	none	·01	none
2	·0000000097	·01	trace
3	·000000097	·01	·5
4	·00000097	·01	·5
5	·0000097	·01	2
6	·0000194	·01	2
7	·0000291	·01	3
8	·0000388	·01	3
9	·0000485	·01	2
10	·000097	·01	1·5
11	·000485	·01	2
12	·002328	·01	1·5
13	·02328	·01	1·5
14	·000097	none	none

After 48 hours the superfluid was removed and ·5 c.c. placed in each of three tubes, A, B, and C. To each tube of series A ·1 c.c. of 1 % horse serum in salt solution (·000097 gramme dried) was added. To each tube of series B ·01 gramme dried horse antiserum dissolved in ·1 c.c. saline was added. Series C formed controls. After 48 hours the readings were taken and are recorded in Table 4. Tubes of 4 mm. and columns of about 4·5 cm. fluid were used.

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TABLE 4.

No.	Deposits in A series (plus serum) in mm.	Deposits in B series (plus serum) in mm.	Deposits in C series (Control)
1	·5	none	none
2	·5	·3	,,
3	·5	·3	,,
4	·5	·3	,,
5	·3	·3	trace
6	·3	·3	minute trace
7	·3	·3	,,
8	·3	·5	,,
9	·3	·5	,,
10	trace	1	,,
11	minute trace	1·5	,,
12	,,	2	,,
13	,,	2·5	,,
14	none	1	none

In Experiment II reactions 2 to 10 have been completed, since precipitin is still present. In reactions 11 to 13 the precipitin has been completely neutralised. On examination of the quantities that interact it will be noted that ·000097 did not quite neutralise the precipitin in ·01 gramme antiserum. The control tubes show that the reactions in the primary tubes 6 to 13, Table 3, have not been completed in 48 hours.

Tertiary reactions were carried out with the superfluid removed from certain tubes after the interactions recorded in Table 4 were completed. These are summarised in the following table, in which the first column gives the number and letter of the tube in the corresponding secondary reaction in Table 4, the second column gives the kind and weight of the addiment, and the third column the reading in 48 hours.

TABLE 5.

Number and letter of corresponding reaction in Table 4	Weight in gramme and kind of addiment dissolved in ·1 c.c.	Reading of precipitum in 48 hours in mm.
1 A	·0002 dried horse serum	none
3 A	·0001 ,, ,,	,,
3 B	·0001 ,, ,,	1·5
4 A	·0005 ,, ,,	none
4 B	·0005 ,, ,,	1·5
5 A	·01 dried horse antiserum	1
9 A	·01 ,, ,,	1
11 A	·01 ,, ,,	1
11 B	·0001 dried horse serum	·7

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The results of the tertiary interaction given in Table 5 are interesting and require some discussion.

In No. 1 A, ·002 gramme of dried antiserum had interacted with ·0001 gramme of dried serum. The addition of more serum produced no precipitum.

In Nos. 3 A and 4 A, ·002 gramme of dried antiserum already acted on by a minute trace of proteid had interacted with ·0001 gramme of dried serum. The addition of more serum produced no precipitum.

In Nos. 3 B and 4 B, ·01 gramme of dried antiserum had already interacted with the superfluid of primary tubes 3 and 4, Table 3. In this superfluid there was much precipitin (cf. secondary interactions 3 A and 4 A) and a small quantity of the homologous proteid (cf. secondary interactions 3 B and 4 B). The addition in 3 B of ·0001 and in 4 B of ·0005 gramme of the proteid produced equal precipita of considerable size. There would be much precipitin in 3 B and in 4 B consisting of that in ·002 gramme antiserum in primary superfluids plus that in ·01 gramme of antiserum added for secondary interaction less that precipitated in primaries 3 and 4 and in secondaries 3 B and 4 B respectively. We would draw attention to the equal size of these precipita.

In Nos. 5 A, 9 A and 11 A, ·0001 gramme of serum had been added to detect precipitin in the primary superfluids 5, 9 and 11. Precipitin was shown to be present in quantity in 5 A, just present in 9 A, and absent in 11 A. The further addition of ·01 gramme of antiserum produced equal precipita with all the superfluids.

In No. 11 B, ·01 gramme of antiserum had reacted with ·000097 gramme of serum (one-fifth of that in primary tube 11, Table 3) which had already been once acted on by precipitin. The addition of more serum to the superfluid shows that the precipitin has not all been neutralised.

Before discussing further the results of these experiments certain points require criticism.

The time necessary for interaction. Three factors seem of importance in determining the period within which a reaction is completed, (1) the temperature, (2) the dilution of the precipitin, (3) the concentration of the serum in which the interaction takes place.

On the effect of variation of temperature we have made no observations. Our experiments have been conducted at a temperature ranging between 17° and 22° C. and averaging about 18° C.

Dilution of the precipitin retards the interaction, and this factor is correlated with the amount of homologous proteid in solution. The precipitin dilution remaining constant, an interaction in which much homologous proteid is present proceeds more rapidly than one in which the homologous proteid is less, even though the amount of proteid in the latter case is sufficient ultimately to neutralise all the precipitin. These effects are shown in the following Experiment (III).

EXPERIMENT III. Two corresponding series of tubes, A and B, were prepared, each tube containing ·0001 gramme dried horse serum in ·05 c.c. salt solution, and ·01 gramme dried horse antiserum in ·2 c.c. salt solution. Before the antiserum solution was introduced, the serum solution was further diluted by the addition of ·25 c.c., ·75 c.c., 2·25 c.c., 4·75 c.c., and 9·75 c.c. salt solution, so that, when the antiserum solution was finally added, the tubes in each series contained altogether ·5 c.c., 1 c.c., 2·5 c.c., 5 c.c. and 10 c.c. salt solution in which the interacting substances were dissolved. In series A the deposits were read in 24 hours, and the clear superfluids transferred to fresh tubes, in which the concentration of the homologous proteid was so adjusted that each ·5 c.c. of solution contained ·0001 gramme dried horse serum. The deposits in these secondary interactions were read in 48 hours. In series B the primary deposits were read in 24 hours and again in 48 hours, after which time the clear superfluids were removed, the concentration of homologous proteid raised as in series A, and the secondary deposits read in 48 hours, as shown in the following Table (6). The dilution of the precipitin antiserum was practically unaltered by raising the concentration of the homologous proteid.

TABLE 6.

No.	Total amount of fluid in c.c.	Primary deposits in 24 hours (A and B series) in mm.	Primary deposits in 48 hours (B series) in mm.	Secondary deposits after increase of concentration (A and B series) in mm.
1 A	·5	2	—	·3
1 B	·5	2	2	none
2 A	1	1·5	—	·5
2 B	1	1·5	2	none
3 A	2·5	1	—	1·5
3 B	2·5	1	1·5	minute trace
4 A	5	·5	—	2
4 B	5	·5	1	trace
5 A	10	trace	—	slight
5 B	10	trace	slight	trace

The tubes in this experiment were of different sizes. The diameter of nos. 1 and 2 was 4 mm., of nos. 3 and 4 about 5 mm., and of no. 5 about 8 mm.

These results show that the reaction was not completed in any case in 24 hours, but was practically completed in tubes 1, 2 and 3 in 48 hours. In nos. 4 and 5 the reaction was not completed in 48 hours,

but was accelerated by raising the concentration of the homologous proteid.

The retarding effect of diluting the antiserum is further supported by experiments with varying amounts of antiserum. When the amount of antiserum employed is so small that the dilution becomes 1 in 500 or more, the precipita increase in control tubes containing clear superfluids removed after a lapse of 48 hours, and the increase may continue for 96 hours.

Following a statement by Nuttall, we at first thought that concentration of serum affected the amount of the precipitum by the solution of precipitum in the serum. Further investigation showed that, if sufficient time be given, the full precipitum for the amount of antiserum employed was usually obtained. That the precipitum separates slowly is shown by filtering the superfluid, or removing it, if clear, from the precipitate and noting whether a further deposit occurs. When horse-serum and fluid horse-antiserum are mixed, a precipitum continues to separate after the 48th hour of the interaction.

Moreover, in Experiment II, when the primary precipita in 24 hours recorded in the tubes of highest serum concentration are compared with those noted in 48 hours the retardation of the interaction is again illustrated, as shown in the appended Table (7).

TABLE 7.

Nos. in Exp. II	Approximate concentration of fluid serum	Precipita in 24 hours in mm.	Precipita in 48 hours in mm.
12	1 in 100	·3	1·5
13	1 in 10	·5	1·5

In two instances (with another hen-egg-antiserum, and with dasyure antiserum) we carried out two full series of parallel observations each similar to Experiments I and II. In each instance one series of primary interactions occurred in a volume of fluid made up to 2·6 c.c. while another series took place in a total volume of ·6 c.c. The superfluids in both series were submitted to the action of fresh homologous proteid and of fresh antiserum, as described in Experiments I and II. The result was that, both with the egg antiserum and with the dasyure antiserum, the data afforded by the one series showed no essential difference from the data supplied by the other, particularly in the secondary interactions, the only noticeable difference in the primary interactions being that the deposits were more easily read in the smaller than in the larger tubes.

The amount of precipitum. For constant quantities of precipitin antiserum interacting with varying amounts of homologous proteid, the amount of precipitum remains constant as soon as sufficient homologous proteid is present to neutralise all the precipitin. This is shown by the following Experiment (IV), the details of which are given in Table 8.

TABLE 8.

No.	Amount of dried horse serum in grammes	Amount of dried horse antiserum in grammes	Amount of precipitum in mm. in 48 hours
1	·05	·01	1
2	·005	·01	1·5
3	·0005	·01	1·5
4	·00005	·01	1·5
5	·000005	·01	·5
6	·0000005	·01	·3
7	·00000005	·01	·3
8	none	·01	none

The interactions took place in tubes of 4 mm. diameter, and each tube contained 1 c.c. salt solution, in separate moieties of which the dried serum and the dried antiserum were dissolved.

On testing with horse serum the superfluid of these reactions, a slight excess of precipitin was found in tube 4, and a full excess in tubes 5, 6, and 7 when the deposits are compared with that yielded by the control tube 8. No residual precipitin was detected in the first three tubes. That the amount of precipitum will become constant when the precipitin is fully neutralised is consistent with its origin mainly from the antiserum and not from the homologous proteid, as we have shown elsewhere[1].

The amount of precipitum is directly proportional to the amount of precipitin (antiserum) interacting with constant quantities of the homologous proteid, even though the homologous proteid is insufficient to neutralise all the precipitin. This is illustrated by the following Experiment (V), Table 9.

TABLE 9.

No.	Weight of dried egg-white in grammes	Weight of dried antiserum in grammes	Total amount of fluid in c.c.	Deposits after 48 hours in mm.
1	·0001	·01	·6	2
2	·0001	·0075	·6	1·5
3	·0001	·005	·6	1
4	·0001	·0025	·6	·5
5	·0001	·001	·6	trace

[1] *Proc. Royal Soc.* 1906.

Non-precipitability of homologous proteid. The repeated interaction of homologous proteid and antiserum does not lead to the exhaustion or precipitation of the former. The further addition of antiserum produces a precipitate commensurate to the increase of antiserum. On this account we have not utilised to any great extent experiments in which varying weights of antiserum are added to a constant weight of homologous proteid, for the purpose of determining the combining weights of precipitin and proteid. Such experiments, however, show well the relationship of the precipitum to the quantity of antiserum, as in Experiment V, Table 9, where it is shown that the amount of precipitum varies directly with the weight of antiserum.

EXPERIMENT VI. A known quantity of dried homologous proteid is dissolved in salt solution, and a given weight of antiserum similarly dissolved is added. After 48 hours the precipitum is read and the superfluid is removed and filtered. To the filtrate is added the same amount of antiserum. This process is repeated until the original liquid, of which a little is lost each time, is reduced to a bulk too small for filtration, or until bacterial infection occurs. The results are shown in Table 10.

TABLE 10.

No.	Weight of albumen in grammes	Weight of antiserum in grammes added each 48 hours	Amount of fluid present each 48 hours *in c.c.*	Reading of precipitum in 48 hours *in mm.*
1	·0006	·01	5	·5+
2	—	·01	4	·5+
3	—	·01	3	1
4	—	·01	2·5	2·5
5	—	·01	1·5	2·5
6	—	·01	·5	2

Experiments to determine whether it be possible to neutralise and precipitate the homologous proteid have been conducted as follows.

After interaction 5 in Table 10 5 c.c. of the 1 c.c. of fluid remainder has been added to ·1 c.c. of a solution containing ·0001 gramme of dried egg-white. The resulting precipitum in 48 hours was ·5 mm. The amount of homologous proteid is therefore nearly sufficient to completely precipitate and neutralise all the added antiserum, yet it still (interaction 6) gives a large precipitate with antiserum.

In the next Experiment (VII), Table 11, the amount of proteid is much less. The procedure is the same as that above indicated.

This experiment shows that even with minute traces of proteid exhaustion has not occurred. It also shows, what we have frequently noted, an increase in the precipitum when many interactions have occurred in the same fluid.

TABLE 11.

No.	Weight of dried egg-white in grammes	Weight of dried antiserum in grammes added each 48 hours	Quantity of fluid at each interaction in c.c.	Reading of precipita in 48 hours in mm.
1	·00000001	·01	5	·3
2	—	·01	4	·3
3	—	·01	3	·3
4	—	·01	2	·3
5	—	·01	1·25	·5
6	—	·01	·75	1

We have carried out five similar experiments with other weights of proteid lying between those utilised in Experiments VI and VII, Tables 10 and 11. The results have been consistent throughout. We have always obtained a precipitum, and also found that, when the quantity of homologous proteid has been small (·0000... gramme), the addition of ·0001 gramme of the proteid at the last interaction has produced a very great precipitum. Much precipitin, therefore, exists with the trace of proteid which produces a precipitum on the addition of fresh precipitin.

A striking example in which there is no appreciable diminution of homologous proteid is given in discussing the tertiary reactions of Experiment II. Here with 11 B ·000097 gramme of proteid (equivalent to that in primary tube 10, Table 3), already once acted upon by precipitin has interacted with ·01 gramme of antiserum. The precipitin is 1·5 mm. similar to that in primary tube 10, Table 3. The bulk of the superfluid of this secondary interaction is tested with proteid for excess of precipitin. The precipitum is about ·7 mm. On comparison with the similar experiment with primary tube 10, Table 3, recorded in No. 10 A, Table 4, a trace only is obtained. But only one-fifth of the precipitin remaining over after primary interaction 10 is utilised for the secondary reaction, while the greater part of the superfluid of secondary tube 11 B was tested for precipitin. Hence there is no diminution in the capacity of ·000097 gramme of proteid twice acted on to precipitate and neutralise precipitin in antiserum.

The Standardisation of Antisera.

We may now consider the standardisation of antisera. If the precipitum be mainly derived, as we have argued elsewhere, from the antiserum, the estimation of the precipitable content in the antiserum is to be attempted. Nuttall has devised a method by which it is

possible to measure precipita by mixing ·5 c.c. of a 1 in 100 solution of serum with ·1 c.c. of antiserum. This allows about ·0005 gramme of dried serum to interact with about ·01 gramme of dried antiserum. Such a quantity is generally but not always sufficient to neutralise the precipitin in ·1 c.c. of antiserum. We have therefore increased the amount of homologous proteid to ·002 gramme interacting with ·01 gramme of antiserum. We have frequently doubled the amount of antiserum, since the larger precipita give very concordant results when several estimations are made, and the reading of the precipitate is made with less error. Our results with those antisera we have carefully investigated are given in Table 12.

TABLE 12.

Antiserum	Amount of dried proteid in grammes	Amount of dried antiserum in grammes	Precipitum read to constant volume in c.c.	Precipitum for ·01 gramme dried antiserum in c.c.
Hen egg I	·004	·02	·048	·024
Hen egg II	·004	·02	·044	·021
Ostrich egg	·004	·02	·2	·1
Hen egg III	·004	·02	·02	·01
Horse serum I	·004	·02	·048	·024
Horse serum II	·004	·02	·016	·008
Dasyure serum	·004	·02	·005	·0025

Most of the antisera have been tested several times with varying amounts of albumen and in every case the maximal precipitum is given when ·02 gramme of dried antiserum interacts with ·004 gramme of dried proteid.

These antisera have also been examined to determine the quantity of homologous proteid which will precipitate and neutralise the precipitin in a given weight of antiserum. These investigations, of which Experiments I and II are types, have been carried out with usually a greater range of variation in the proteid than that shown in the type experiments. Three of the antisera have been thus tested twice, one four times, and one five times. In these test experiments we have used from 15 to 18 primary tubes, with secondary and sometimes tertiary tubes made as in Experiments I and II to ensure as great accuracy as possible. In this way fourteen observations, each involving about 70 readings, have been conducted. The results may be expressed in the following Table 13.

TABLE 13.

Antiserum	Weight in grammes of proteid always insufficient to neutralise ·01 gramme antiserum	Weight in grammes of proteid usually sufficient to neutralise ·01 gramme antiserum	Weight in grammes of proteid always sufficient to neutralise ·01 gramme antiserum
Hen egg I	·000012	·000024	·000048
Hen egg II	·00001	·00002	·00003
Ostrich egg	·0005	—	·002
Hen egg III	·00001	·000025	·00006
Horse serum I	·00005	·0001	·0005
Horse serum II	·0005	—	·002
Dasyure	·0001	—	·0005

On comparing Tables 12 and 13 it will be noted that there is no correspondence between the precipitable content of the antiserum and the amount of proteid that is necessary to neutralise all the precipitin.

In the light of these results it seems to us that the evidence we have adduced negatives the view that definite amounts of precipitin and homologous proteid unite to form the precipitum. The view that chemical equivalents of these substances combine or react in these interactions to yield the precipitum is also not supported by these results.

Thus the precipitable contents of hen egg antiserum I and of horse antiserum I are identical, yet five times as much proteid is required to neutralise the precipitin in the horse antiserum as is required in the egg antiserum.

Two different factors have therefore to be ascertained in regard to precipitin antisera, (1) the amount of precipitable substance in the antiserum, and (2) the amount of homologous proteid necessary to neutralise and precipitate this precipitable substance.

With the same antiserum we have found that the weight of homologous proteid varies directly with the precipitable content in the antiserum. This has been shown by the increased amount of homologous proteid required to neutralise and precipitate the precipitin in larger amounts of antiserum than ·01 gramme. With different antisera this seems to hold also, but comparison is difficult, as an antiserum with a low precipitable content may require a considerable amount of homologous proteid to neutralise and precipitate it.

We may explain the variation in precipitability in different antisera of the same precipitable content by assuming the number of haptophorous groups, to which the side chains of the homologous proteid may be attached, to be smaller or greater while the number of molecules is the same. This view is supported by the behaviour of antisera with

non-homologous proteids. Here the haptophorous group can only be brought into relation with some few side chains of the non-homologous proteid. As a result, much greater quantities of non-homologous proteid are required to precipitate all the precipitin in the antiserum, and sometimes this cannot be done at all.

In conclusion we should like to convey our thanks to Professor Anderson Stuart, in whose laboratory most of our work has been done, and to express our indebtedness to Dr G. H. F. Nuttall, whose book has been of great service in giving an account of the results of other workers whose original communications we have been unable to consult.