PILOCARPINE AND OTHER REAGENTS IN RELATION TO PRECIPITIN IMMUNITY.

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Introduction.

It is generally recognised that, in certain diseases, recovery mainly depends upon the prompt and efficient reaction of the tissues forming antibodies, such as bacteriolysins, antitoxins, and the like, and it is possible that these peculiar biological responses may be influenced by the administration of drugs.

The problem is open to direct experimental investigation. For this purpose it is necessary to select one of those reactions of immunity which will lend itself to easy quantitative estimation. It was suggested by Dr Nuttall that the precipitin reaction would conveniently suit this purpose, and that the action of pilocarpine should be first investigated.

This suggestion was based upon the work of Salomonsen and Madsen[1] in the case of antitoxin production. These authors found a large and rapid increase of antitoxin in the serum of the horse immunised against diphtheria toxin; this increase occurring after the intravenous injection of pilocarpine. They found that this increase in the antitoxic power of the serum occurred at the time when the horse was showing the obvious physiological effects of the drug. They remarked that, when the doses of pilocarpine were repeated the effect upon the antitoxic strength of the serum became less with repeated doses. And they also noted that the increase in antitoxin after pilocarpine could best be elicited when the animal was naturally decreasing its yield of antitoxin.

[1] Cited by Nuttall, *Blood Relationship and Immunity* (Cambridge, 1904).

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The precipitin reaction is suitable for this kind of investigation, the technique being comparatively simple. It is obvious that, should the effect of pilocarpine with precipitins be like that already described with antitoxins, a confirmation of some importance is obtained.

There is a certain ambiguity in the meaning of the term "*the precipitin reaction.*" When discussing pathological processes we speak of the inflammatory reaction of the tissues; and in a similar vague manner this term is used to express the biological processes concerned in the formation of precipitins in the immunised animal. This formation of precipitins is a biological response, and will be termed the "*precipitin response.*" The "precipitin reaction" will then be used in the chemical sense concerning events in the test-tube; thus the "precipitin reaction" is that between serum and antiserum which results in the deposit of a precipitum. The term "precipitum" is technically limited to this particular form of precipitate. Thus the "precipitin reaction" is an observable phenomenon, the "precipitin response" is a biological process of which we know nothing except by inference from observed precipitin reactions.

If a rabbit be taken and subjected to a series of injections of serum, the serum being obtained from an animal of a different species, the blood of this rabbit in process of time acquires a new property. For if the serum of the rabbit so treated be mixed in the test-tube with the serum such as was used for injection, a cloudiness gradually appears in the mixture. As the cloud increases, its uniformity gives place to a granular appearance; the finer particles then become aggregated into small discrete masses, which finally sink to the bottom of the test-tube, leaving the fluid above quite clear.

The serum of the rabbit which has acquired this specific property is called an "antiserum" to the serum with which it reacts. Such an antiserum is regarded as containing a substance called "precipitin" which combines with the "precipitable substance" in the serum, the mutual neutralization being evidenced by the appearance of the cloud of "precipitum."

The course of the biological response in the tissues of the rabbit may be gauged by daily estimations of precipitin in samples of blood taken from the rabbit, the quantity of precipitum formed being estimated volumetrically.

The question arises whether, other conditions remaining fixed, the quantity of observed precipit*um* is proportional to the amount of hypothetical precipit*in*. No systematic observations on this point have yet

been published. It is possible that the law which formulates the relation of toxin to antitoxin will be found to apply in the case of the precipitins also.

So far as the precipitin reaction is concerned, it has been shown that, after the precipitum has become deposited from the mixture, free precipitin and precipitable substance may be found in the supernatant fluid. Hence if a further addition of one or the other be made, a fresh deposit of precipitum may be obtained. Let us suppose that with a definite mixture of serum and precipitating antiserum there results *in the end* a definite quantity of the combination, "precipitin-precipitable substance." Now is the precipitum which we see the same in quantity as the "precipitin-precipitable substance" which results from the reaction? does all the precipitum "come down"? Nuttall has found that, by adding antiserum drop by drop to undiluted serum, the evanescent precipitum observed at the moment the drop is added, redissolves or disappears in the excess of serum present; the precipitum is said to be soluble in excess of serum. Does the "precipitin-precipitable substance" here dissociate into precipitin and precipitable substance; or does the substance remain in combination but mechanically dissolved in excess of serum?

There are experiments which suggest that the precipitin reaction is reversible. It is true that toxins deteriorate on keeping, and become, as we say, converted into toxoid. It is also true that precipitating antisera can by heating be inactivated. In this condition the power of specific union with precipitable substance is retained, while the property of bringing about the clouding is lost. Müller regards the combining antibody in inactivated antiserum as "precipitoid"; in Ehrlich's terminology this would be spoken of as precipitin in which the zymophoric group had been destroyed. If then precipitable substance be mixed with such "precipitoid" a soluble compound is formed, so that, on now adding precipitin, there is no available precipitable substance with which the precipitin may combine. No precipitum is formed; the precipitoid prevents the precipitating action of precipitin when the latter is added later to the mixture. So far we may consider precipitoid to have a strong avidity for precipitable substance, so that precipitable substance no longer exists, and there is nothing for precipitin to combine with. But the following experiment, due to Eisenberg, may be performed. The reaction is that between precipitable substance, precipitoid, precipitin, and their compounds when mixed together. First definite quantities of precipitable substance and precipitoid are

mixed together, and it is found, as before, that on adding a certain amount of precipit*in* no deposit of precipit*um* takes place; the experiment is then repeated in a series, the amount of precipit*in* added being each time increased. It is then found that when a sufficient quantity of precipitin is present a deposit of precipitum appears. Here then the combination of precipitoid with precipitable substance must have been dissociated, the precipitable substance so freed then combined with precipitin, the product becoming evidenced by a deposit of precipitum. Thus the reaction appears to be reversible and largely dependent upon the relative masses of the reagents present.

There is evidence to show that the same general principles apply with regard to the allied haemolytic phenomena which involve the reactions between red cells, specific immune body, and complement.

Finally the question arises in the case of the precipitin reaction: is the precipitum which we see and whose volume may be measured, itself the combination "precipitin-precipitable substance"? or may it be that this product of the reaction is always invisible to us, but by a secondary action precipitates other substances present in the serum, and so causes the cloud of precipitum? Obermayer and Pick separated the different constituents of egg white and injected them severally into animals in order to produce corresponding antisera. These observers were surprised to find that one substance might in the animal lead to the formation of a precipitin, which did not react with this substance, but with some other constituent of egg white. It may also be remarked that pure crystallised egg albumin was quite incapable of exciting any precipitin response at all.

With regard to the precipitin reaction, it is clear that no definite statement as to the quantitative relation of precipit*um* to hypothetical precipit*in* can be made. There is first the quantitative relation of precipitin to "precipitin-precipitable" substance; and, secondly, the relation of the latter to the precipitum which we observe. The answer to these questions can be supplied by experimental investigation only. Moreover each precipitin reaction observed on injecting different sera may be the resultant of a number of precipitin reactions occurring together.

Methods.

It will be remembered that at the outset the precipitin response was distinguished from the reaction *in vitro*. It is the effect of drugs

upon the course of the precipitin response which we wish to investigate, and it becomes necessary here to define our position clearly.

Just as the temperature chart may be taken to indicate the course of a fever, so a chart may be obtained to indicate the course of the precipitin response in the animal yielding antiserum. Each day a few drops of blood are withdrawn from the animal's vein, and after clotting, the antiserum is obtained. A constant volume of the antiserum is then taken and tested by mixing it with a constant and definite quantity of the serum with which it gives the precipitin reaction. The amount of precipitum formed under these conditions is charted. Thus an arbitrary method of estimating precipitating power is fixed upon, while what is indicated on the chart is the precipitating power of a unit volume of blood from day to day. Putting aside the question of the exact relation of precipitating power of the sample to its precipitin content it is obvious that not only is the precipitating power of the sample dependent on the formation of precipitin by the tissues, but it is also dependent on the dilution or concentration of the blood itself from time to time.

Such precipitin charts serve the present purpose, for the daily readings mark out a curve sufficiently uniform to be taken as a normal, so that sudden and marked variations in the curve following the administration of the drug are clearly seen. It is, of course, essential that the daily precipitin estimations should be performed in an exactly similar manner, so that, whatever may be the complicated sequence of events in the reaction *in vitro*, still if the blood itself should vary uniformly from day to day the precipitin chart should also vary uniformly and map out a curve.

The following experiment shows the necessity of keeping the conditions uniform during the formation of the precipitum in the test-tube. In performing estimations of precipitating power it is usual to add a definite quantity of the antiserum to be tested to a larger quantity of fluid consisting of serum largely diluted with normal saline; here the antiserum naturally sinks to the bottom of the test-tube, so that the contents must be mixed together by inversion. When performing several estimations it is easy to fill in antiserum to a series of test-tubes containing the serum solution, and then afterwards to mix them up one after the other. In this case an initial and variable time has been allowed for events to occur at the zone of contact between antiserum below and serum solution above. It is obvious that in this zone the relation of the masses interacting is very different from that

which comes into existence when they have been shaken up together. When the contents are not shaken up the cloud of precipitum after a time makes its appearance in this region, and it might be supposed that in any case the subsequent mixing up would equalise matters. This, however, does not appear to be the case always, and it becomes necessary as an invariable routine to mix up the reagents thoroughly immediately on adding the one to the other.

Two similar estimations were performed in test-tubes *A* and *B*; like quantities of the same antiserum and serum solutions were used in both. The antiserum was a weak one, the precipitation occurring very slowly. In the case of tube *A* two clear days were allowed to elapse without shaking up the contents, but the other tube, *B*, was mixed at once. *A* was then shaken up, and the tubes allowed to sediment completely. Quantitative measurements of the deposits showed that *A* contained 5·7 c.mm. of precipitum, while *B* contained ·9 c.mm. only. Thus, in the zone of contact, in *A*, a considerable amount of precipitum was formed which on subsequent mixing was not redissolved. The matter was not further pursued, as the purpose was only to find a uniform method of procedure, and in consequence it became a routine practice to mix the contents immediately after adding antiserum to serum solution.

Not only in order to obtain a uniform curve on the precipitin chart must this method of estimation be a uniform routine, but the animal giving the response must be kept under uniform conditions also. It was found that the precipitating power of the sample of blood was affected by the state of digestion of the animal at the time when the sample of blood was taken. This of course might be an ultimate effect of assimilation upon the metabolism concerned in producing antibody; on the other hand it might be due to dilution or concentration of the total quantity of blood so affecting the power of the antiserum. To avoid this source of variation the sample was drawn in the morning before feeding; and the rabbits were kept in cages bedded with saw-dust, and fed at a definite time upon turnips.

The method of measuring precipitum which was used in this work, as well as the method employed for estimating the specific gravity of the blood, have been described elsewhere[1]. It is only necessary here to describe the method of preparation of the animal, and then to indicate briefly the routine of the precipitin estimation.

[1] Nuttall and Inchley (1904), this *Journal*, IV. No. 2; Inchley (1904), *Journ. of Physiol.* XXXI.

A rabbit was taken and subjected to a series of injections of hippopotamus serum; this serum had been previously heated to 55° C., thereby doing away with the normal haemolytic effects when injected. The animal received 1 c.c. of the serum into the ear vein, two clear days being allowed to elapse between each injection.

During the course of the ensuing precipitin response a few drops of blood were obtained daily from the ear; the sample was received into sterile Petri dishes, the serum separated, and its precipitating power estimated in the manner to be described.

When dealing with drugs which affected the distribution of leucocytes, a leucocyte count was made in the ordinary way, but no differential count was attempted.

Concentration of the blood.

The concentration of the blood was determined, when necessary, by estimating at the same time the specific gravity of the sample. On other occasions the depth of colour only was recorded by means of Oliver's haemoglobinometer, this test being likewise used to determine fluctuations in concentration. It is evident that concentration or dilution of the blood as a whole would cause an alteration in the result obtained with the precipitin test.

Thus, should any alteration in the precipitating power of the sample be observed, it becomes necessary to determine whether altered concentration is sufficient to account for the change. If neither this nor alteration in the number of leucocytes in the sample will explain the result, then presumably it is due to some deep-seated effect upon metabolism.

The precipitin estimation.

A standard dilution of hippopotamus serum was made, 1 part of serum being added to 20 parts of sterile normal salt solution. In each estimation a definite quantity of this was used as a reagent. ·5 c.c. of this solution was taken, and to it was added ·1 c.c. of the antiserum to be tested.

When an animal is treated with a foreign blood this can be shown to persist in the animal's circulation for a variable period by tests made with an antiserum appropriate to the foreign blood injected. Thus, if hippopotamus serum is injected into a rabbit, the presence of hippo-

potamus serum in the rabbit's circulation can be demonstrated for some time by the use of anti-hippopotamus serum. In carrying out such tests I diluted the antiserum 1:5 in saline before adding to it the serum to be tested.

The routine of each estimation occupied six days, consequently the state of the animal giving the precipitin response was not known till about a week later.

The order of procedure was as follows:

1st day—15 drops of blood were collected as a sample, and the antiserum allowed to separate from it.

2nd day—the precipitin reaction performed, ·5 c.c. of hippopotamus serum dilution was transferred into a small test-tube, and to this was added ·1 c.c. of the antiserum, and the contents then immediately mixed together.

3rd day—the precipitum which had now sedimented was transferred to the capillary tube.

4th day—in the capillary tube the column of precipitum was broken up by means of a horse-hair introduced from above and twirled about.

6th day—the height of the column of precipitum was measured and recorded.

It was found possible to avoid bacterial growth by ordinary precautions. When not in use the capillary tubes were washed through with tap water, the interior being easily cleaned with a pipe brush the bristles of which had been cut short for the purpose. The tubes were then kept immersed in chloroform water. In the precipitin charts which follow there is recorded either the height in millimetres of the column of precipitum, in which case a definite set of capillary tubes of uniform calibre was specially used, or the volume of the precipitum in cubic millimetres, this being calculated from the calibration of the tube. In the later curves the former method was employed.

The following drugs were employed: *Pilocarpine* was given in doses of 2–4 mgs. dissolved in saline solution and injected into the ear vein or hypodermically. *Turpentine*, a 1 % emulsion in saline was prepared, and injected subcutaneously in doses of 1 c.c. *Cinnamate of Soda*, 1 c.c. of a 4 % solution in saline was injected under the skin. *Quinine*, 1 c.c. of a 4 % solution of the chloride given subcutaneously. *Nuclein*, a 5 % solution of nucleinic acid from yeast, the dose injected being 1 c.c. of the solution. *β tetra-hydro-naphthylamine*, 5 c.c. of ·5 % solution in saline injected subcutaneously.

(1) *Action of pilocarpine.*

The effect of subcutaneous injections of pilocarpine upon the course of the precipitin response is shown in the accompanying chart (fig. 1).

In this experiment the rabbit received eight primary injections of hippopotamus serum; the chart shows the precipitating power of the serum above, while below is shown the disappearance of precipitable substance from the blood. The experiment covered a period of 76 days. The gradual onset of the response and increased facility of dealing with the foreign serum is observable. Towards the height of the response on the 28th day a subcutaneous injection of 2 mgs. of pilocarpine nitrate was made, and a corresponding increase in the precipitating power of the serum is shown on the chart. Similar but more marked effects are to be seen later, when the response was diminishing. It will be noted that the 8th injection of hippopotamus serum was given on the 22nd day, and that the decrease in precipitating power followed after the 31st day. In each case the sample of blood was taken half-an-hour after the injection of pilocarpine, so that of course the maximum attained is not necessarily the reading which is marked as maximum upon the chart. On the 54th and 55th days the injection of 2 mgs. of pilocarpine was followed by a distinct increase of precipitating power. On the 58th day three such doses were injected at intervals of half-an-hour and the sample of blood was taken half-an-hour after the last injection; in this instance practically no increase was observable.

The same chart also shows towards the end (beginning 61st day) the effect of further injections of hippopotamus serum; this however will be considered later.

It is to be remarked that the pilocarpine does not appear to have any permanent effect upon the curve. Within an hour or two at most the blood has returned to its condition prior to the experiment. Consequently, when the slow disappearance of the response as a whole is considered, such a rapid oscillation of the curve after pilocarpine is opposed to the view that this drug had stimulated the cells to secrete an additional quantity of precipitin into the blood, for it would seem reasonable to expect that the maximum would be maintained for some time at least.

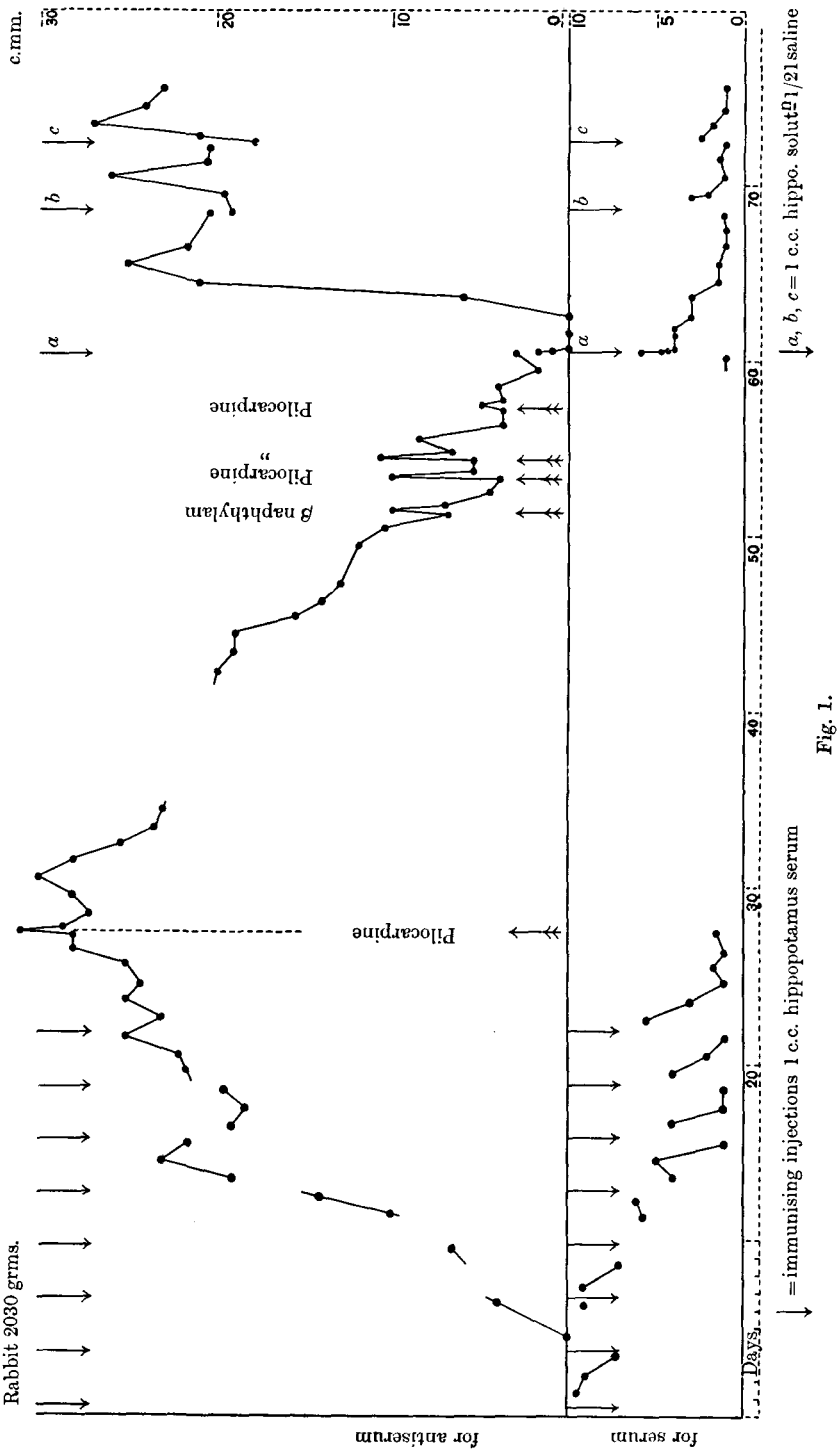

Fig. 1.

Concentration of blood.

On the supposition that these effects might be simply due to concentration of the blood, the haemoglobin percentage was observed as each sample of blood was taken. This variation acted as an index of concentration. The close correspondence between the haemoglobin curve and that of the precipitating power is shown in the following figure (fig. 2).

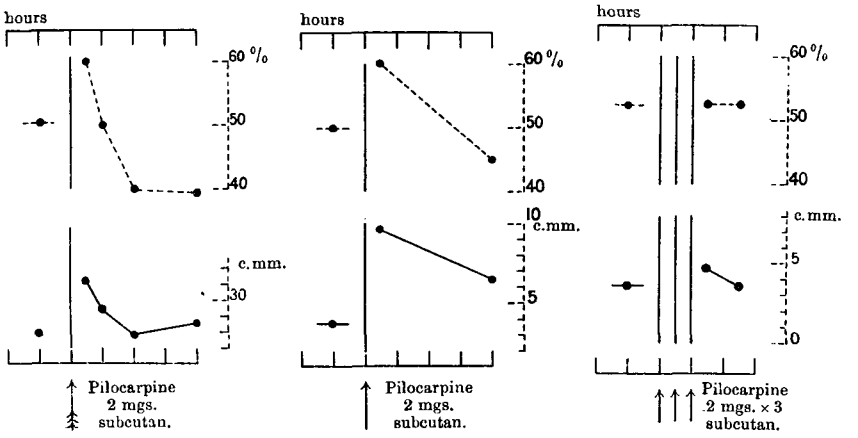

Fig. 2.

In these figures the precipitum is shown below, while above is represented the corresponding variation in the haemoglobin percentage. They show in greater detail the experiments indicated in the general chart (fig. 1) on the days 28, 54, and 58.

As the haemoglobinometer is calibrated not for rabbit but for human blood, it was necessary to employ some other method of estimating the amount of concentration. The experiment was consequently repeated, the specific gravity of the blood being observed. The haemoglobin estimations show that a diminution in the volume of the plasma had occurred. A simple increase in specific gravity alone might possibly be attributed to the addition of heavier bodies to the plasma.

In the following chart (fig. 3) the effect of an intravenous injection of 4 mgs. of pilocarpine is shown.

This curve was obtained from the same rabbit as before, after an interval of three weeks, the animal being again treated with hippopotamus serum. Fifteen minutes after the injection of pilocarpine the precipitating power had risen from 19·3 to 30·6 mm., and there was

a corresponding alteration in the specific gravity of the blood from 1049 to 1055.

Three days later the same dose was administered again, this time subcutaneously. The sample of blood was taken half-an-hour after this, and a very slight effect on the precipitating power was observed; as before pointed out, the negative result may be simply due to the sample

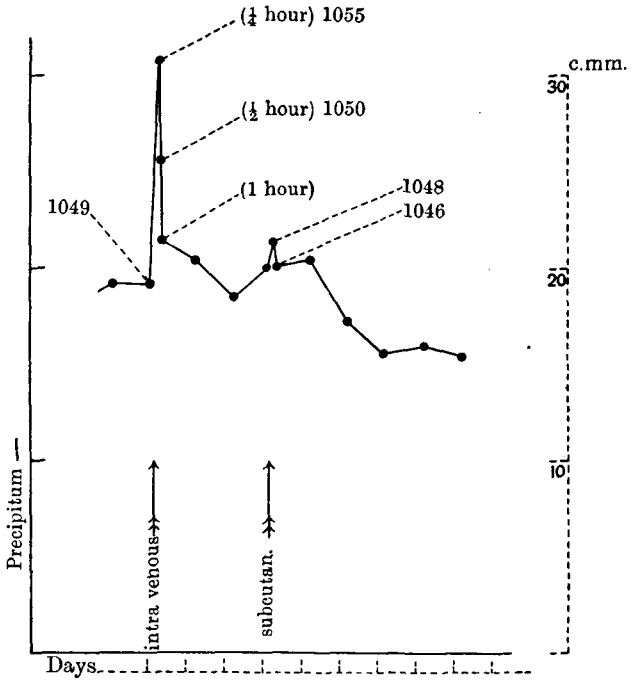

Fig. 3.

not being taken at the right time. But it is to be noticed here that with a slight alteration in precipitating power there is a corresponding slight alteration in the specific gravity of the blood.

In the first case then there was an increase of 50 % in precipitating power, while the density of the blood was altered from 1049 to 1055. This alteration in density is equivalent to the abstraction of about 12 % of water[1]. If the fluid removed be of greater density than water it is

[1] Let x be the number of c.c. of water added to 100 c.c. of blood with sp. gr. 1055, so that the mixture $(100+x)$ c.c. has now a sp. gr. of 1049;

$$\text{then } (100 \times 1055) + (x \times 1000) = (100 + x)\,1049;$$
$$\therefore\ x = 12.$$

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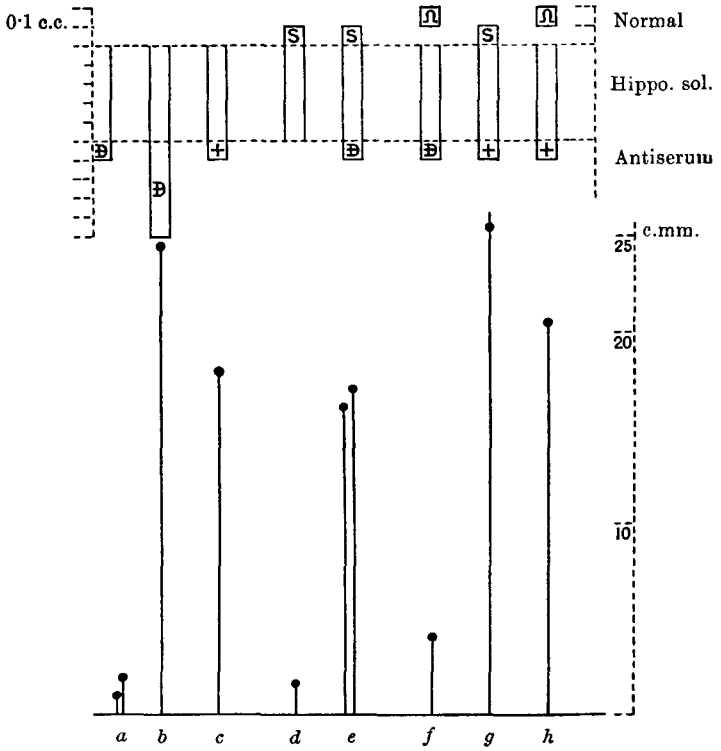
obvious that a larger volume percentage would have to be abstracted in this case. If then the concentration of the blood be only 12 %, how is the increase of 50 % in precipitating power to be accounted for?

The following figure shows diagrammatically the effect produced upon the precipitating power by varying the amount of antiserum in the mixture.



Ω = normal rabbit serum.
S = serum of rabbit 60th day, fig. 1.
+ = stock antiserum.
Ð = stock antiserum dilut. $\frac{1}{5}$ normal saline sol.

Fig. 4.

Here the mixture of serum and antiserum is represented above, while the amount of precipitum which resulted is shown below.

(*c*) indicates that 18 c.mm. of precipitum were obtained when 0·1 c.c. of *stock antiserum* was added to 0·5 c.c. of *hippopotamus* serum dilution.

In (*d*) the same proportions were employed, only 0·1 c.c. of *a very*

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weak antiserum was used instead of the stock antiserum. Here only 1·5 c.mm. of precipitum were obtained.

In (*g*) a further experiment was performed, in which equal parts (0·1 c.c. of each) of both strong and weak antisera were present. It is observed that 26 c.mm. of precipitum now appeared.

Thus with a constant quantity of the precipitable serum solution, a powerful antiserum gave 18 c.mm., a weak one only 1·5 c.mm., while the two together gave as much as 26 c.mm. So that a very slight increment in precipitating substances apparently led to an increase of 50 % in the precipitum deposited in this case.

It may be observed that the stock antiserum here used gave 18 c.mm. of precipitum, while the serum of the rabbit in which the action of pilocarpine was tried gave 19 c.mm. So that the two antisera were of approximately equal strength.

Next the stock antiserum was diluted with normal saline, a dilution of one in five being obtained.

In (*a*) and (*b*) the same quantity (0·5 c.c.) of precipitable serum dilution was again employed, but in the first case 0·1 c.c. of dilute antiserum was added, while in the second case 0·5 c.c. was taken. It will be seen that five times the amount of antiserum present roughly results in about 25 times the amount of precipitum deposited.

If this be taken as roughly indicating the relationship with these solutions, it follows that a 50 % increase in the amount of precipitum deposited would be accounted for by a 10 % increase in precipitating substances present. And this corresponds with the 12 % concentration in the blood which was observed after pilocarpine administration in the last experiment. In other words, the mere concentration of the blood after pilocarpine appears to be amply sufficient to account for the increased quantity of precipitin deposited.

We have therefore reason to believe that the increased precipitating power in the serum which follows pilocarpine is a result of the concentration of the blood which it brings about. Such a view is also in accordance with the known pharmacological action of pilocarpine.

The drug has a specific stimulating effect upon the nerve terminations in glands and unstriped muscle. It leads to increased peristalsis and diarrhoea, and causes shrinkage of the spleen and organs supplied with involuntary muscle fibres. In this connection it may be mentioned that electrical stimulation of the spleen in leukaemia has been found to bring about an immediate increase in the leucocytes in the blood. That pilocarpine leads to a leucocytosis in the normal animal is a state-

ment in which there is not entire agreement. Ewing was unable to obtain this effect in rabbits. Its action upon the terminations of secretory nerves induces a copious general secretion throughout the body, and in this way a considerable loss of fluid occurs through the skin, lungs, salivary glands, etc.

So far as is known, the only glandular tissues which are induced to secrete by the administration of pilocarpine are those having a nervous control, yet a nervous mechanism for the production of antibodies would seem at any rate a most unlikely one.

(2) *Action of nuclein and of fowl sera.*

The following chart records the precipitin response during which the animal received injections of yeast nuclein, and also of fowl serum, that is to say, of a serum different from that which was originally employed to excite the precipitin response.

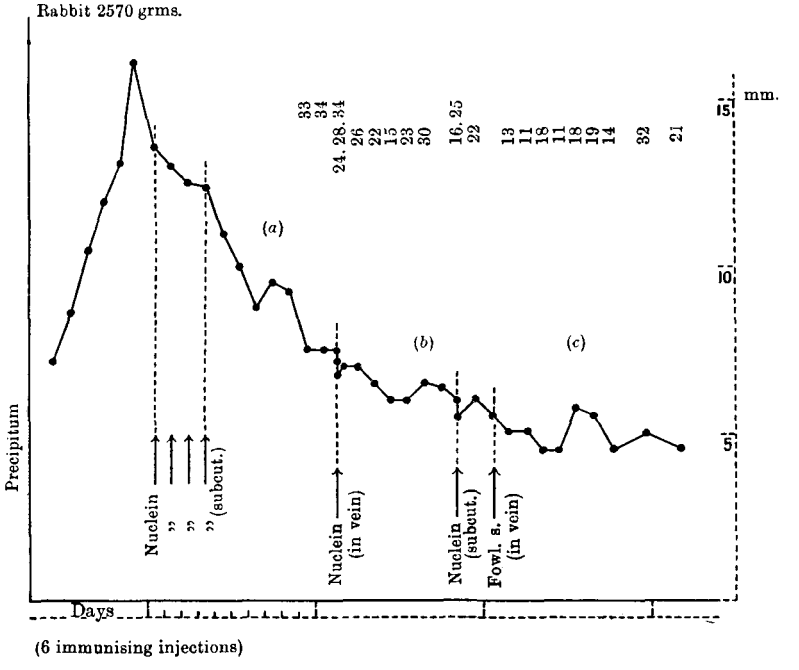

Fig. 5.

It is to be seen that after the injection of fowl serum the curve at first continues its descent apparently unaffected by the treatment; on the 5th and 6th days however a slight rise is recorded at (*c*). This same

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serum tested with fowl serum for the presence of antifowl gave no precipitum, showing absence of specific antibody to fowl.

If now a reference be made to fig. 1 it will be found that a further injection of exciting serum (*a* in fig. 1) led to a second precipitin response which reached its maximum also on the 5th day after the injection. And a similar effect is also shown in fig. 6.

As a series of injections of fowl serum would lead to an antifowl response, the interesting question arises whether the injection of fowl serum in the experiment recorded led to a slight stimulation of the mechanism concerned in the anti-hippopotamus response. To produce a modification in the response other than by its specific exciting serum, the most likely reagent would presumably be a serum of another animal, unless an entirely different sequence of events underlies each precipitin response.

Five days after injections of yeast nuclein still smaller fluctuations in the curve were observed (*a*) and (*b*). These however are so slight as to be within the region of experimental error.

It is manifest that no appreciable effect is produced upon the curve by these agents. That the biological mechanism for the production of antibodies is affected only by specific substances, is in accordance with what is known of glandular mechanisms in general. Drugs which markedly affect secretion usually do so by their action upon the endings of nerves supplying the gland: and conversely we are beginning to see that glands, like the pancreas, which are not coordinated for rapid responses to altered environment, seem to respond to definite physiological substances and to them only.

(3) *Effect of further injections of exciting serum and of various drugs.*

The effect produced by further injections of exciting serum is shown in the later part of the first chart, fig. 1, *a*, *b*, *c*: 1 c.c. of a 1 : 21 hippopotamus serum dilution was injected at a time when the primary precipitin response had almost disappeared.

Here the dose was 1/20 of that used in the primary exciting injections. There is a rapid ($\frac{1}{2}$ hr., 1 hr., 3 hrs. samples) disappearance of precipitin and a corresponding diminution in free precipitable substance in the blood after receiving the injection; on succeeding days there is then a more gradual disappearance of precipitable substance, while precipitin begins to appear on the third day and rapidly rises to a maximum on the fifth day after the injection. It

may be observed that although the dose was very small a high maximum was attained.

At *b* and *c* similar injections were repeated. The sample taken one hour after injection *b*, showed a slight decrease in precipitum, the diminution after injection *c* is that shown by a sample taken $\frac{1}{2}$ hour after the injection. An interesting point to be observed is that the time between the injection and the resulting maximum gets shorter with each injection. Thus after the second injection *b* the maximum is attained on the second day, after the third injection it is reached on the day following the injection. Apparently the previous precipitin response leaves the animal with a mechanism peculiarly susceptible to the exciting serum.

A rabbit was next taken which had given the precipitin response five months previously, 1 c.c. of a 1 : 21 hippopotamus serum dilution was injected intravenously; examinations of the blood showed that no response occurred.

Next two rabbits of approximately equal weight were both treated in a similar manner with six immunising doses, each of 1 c.c. of undiluted serum. The one rabbit *A* had not before been treated with serum; in the other *B* the precipitin response had been induced five months before.

Fig. 5 is the chart obtained from the previously normal animal *A*, while fig. 6 is that of the other one, *B*. It is seen that a much greater response occurred in the one which had previously been treated.

Apparently after the primary response has subsided, there remains for some time a potential mechanism sensitive to the particular stimulus which had been employed, and to which the response owed its origin. Possibly the active formation of antibody during the course of the primary response has given a bias to the cell metabolism, leaving a kind of cell-memory so far as that stimulus is concerned, which in its turn endures for a certain length of time.

In figs. 1 and 6 are recorded certain observations upon the disappearance of foreign serum from the blood. It was found that, after an injection had been made, there followed a gradual disappearance of precipitable substance from the blood. In neither case, however, was the disappearance observed to be complete; towards the end of the response a trace was still obtained.

In view of the work on the nature of the reaction already quoted, it is possible that the antibody in the blood does not completely neutralize the exciting substance; in other words, the process of antibody formation

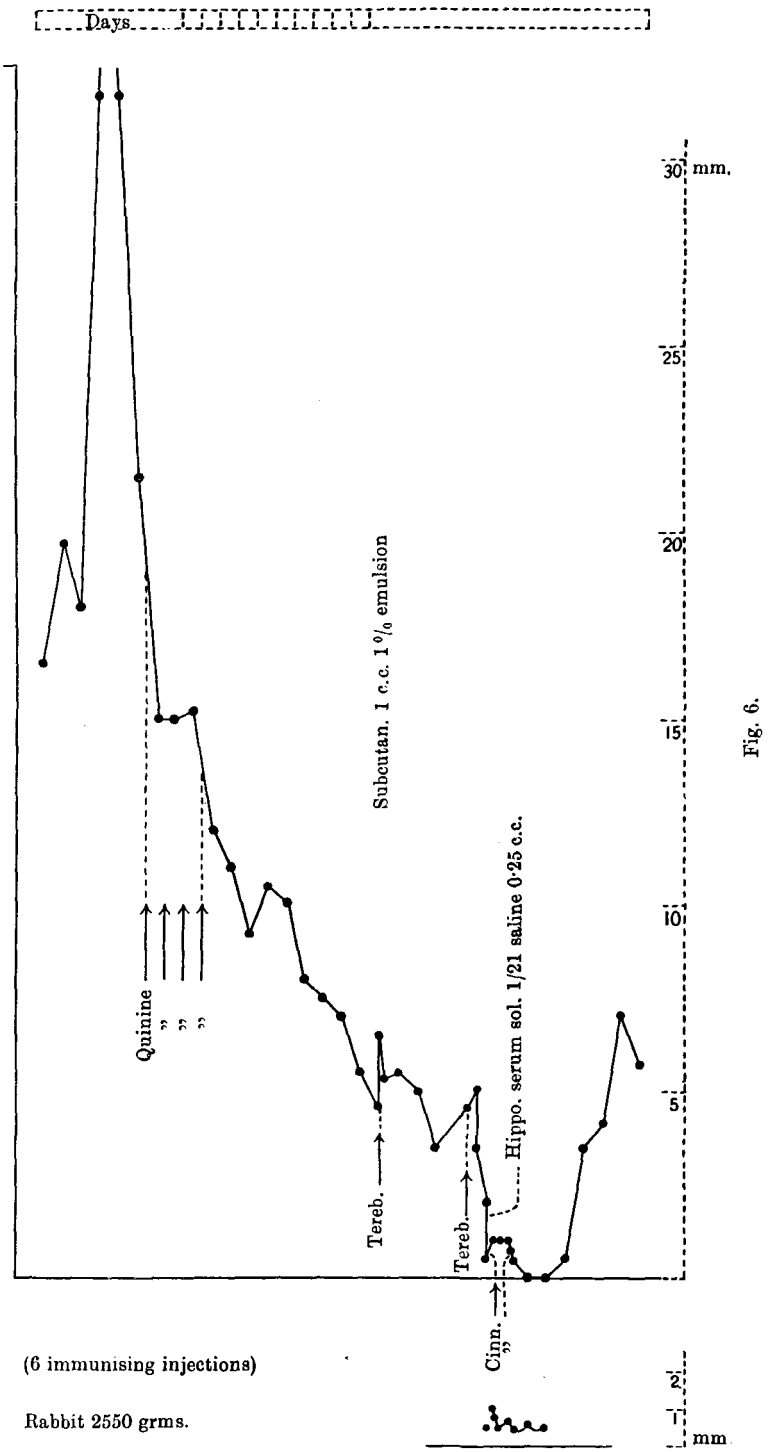

Fig. 6.

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goes on till a residue remains which is tolerated by the tissues, or until the stimulus becomes so small as to be insignificant. The question is an interesting one, only to be solved by further investigation.

Fig. 6 also shows slight irregularities in the course of the curve, which may perhaps be caused by the drugs injected. Thus with quinine injections the disappearance of precipitating power appeared to be less rapid, and 5 days after the last injection a slight increase is observed. Subcutaneous injections of oil of *turpentine* emulsion led to a small and temporary rise in precipitating power, like that observed with pilocarpine. Towards the end of the curve, a small injection of the primary exciting serum was again administered, after an interval of a few days a distinct rise occurred similar to that described in fig. 1. Four hours after this injection a mere trace only of precipitum was obtained. *Cinnamate of Soda* (1 c.c. 4 %) was then administered subcutaneously; the slight alteration in the readings observed is however again too small to be considered.

Conclusions.

As a result of these researches, the general conclusion arrived at is that the processes concerned in the elaboration of specific antibodies are not appreciably affected by these drugs. Where a temporary increase follows the injection of the drug it is probably explainable in other ways. Thus in the case of pilocarpine the concentration of the blood, presumably the result of the general glandular activity, is sufficient to account for the increase in the precipitating power observed.

In conclusion I have to acknowledge my deep indebtedness to Dr Nuttall, F.R.S., throughout; and I have also to thank Dr Dixon of the Pharmacological Department for his advice in the selection of the drugs.