# Section of Obstetrics and Gynæcology

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## Serum and Vaccine Therapy in Combination with Sulphanilamide or M and B 693

By ALEXANDER FLEMING, F.R.C.S.

IN the past many chemicals have been recommended for intravenous injection or for introduction into the body in other ways with the object of directly killing infecting bacteria. With the exception of the salvarsan group they have been found wanting, although sometimes one and sometimes another has had, for a time, a certain vogue.

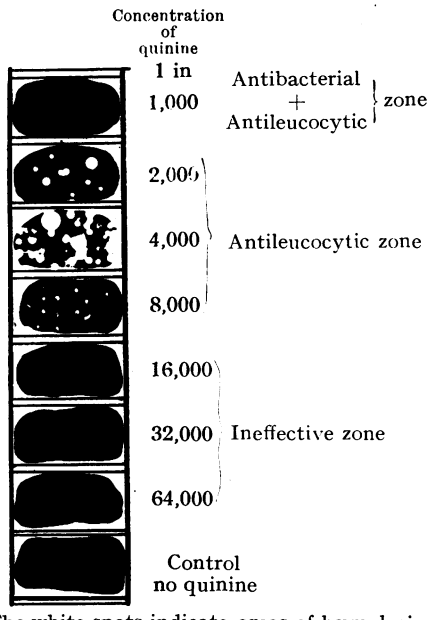
Let us see what happens when the antibacterial action of these chemicals is tested in human blood ; and we may take as an example quinine, which has been recommended as an intravenous injection for the treatment of puerperal septicæmia due to *Streptococcus pyogenes*. If human defibrinated blood infected with a suitable dose of *Streptococcus pyogenes* is mixed with serial dilutions of quinine and incubated in slide cells the resulting growth is as illustrated in fig. 1.

In the blood without quinine there is no growth as the phagocytes of the blood can deal with all of the infecting bacteria. In the weakest concentrations of quinine there is the same result, but as the concentration of quinine increases so hæmolytic colonies of streptococci appear until when there is a concentration of 1 : 4,000 in the blood all the streptococci implanted in the blood grow out. This is due to the fact that chemicals like quinine have a more poisonous action on leucocytes than they have on streptococci. Had it been possible to administer quinine in a dose sufficient to produce in the blood a concentration of 1 : 4,000 the result would have been serious as the blood would have lost all its anti-streptococcal power and have become a first-class culture medium for the streptococcus. The dose recommended was, however, 2 gr., which if introduced into the blood-stream could not give a concentration of more than 1 : 40,000. It will be seen by referring to fig. 1 that this concentration is well off the scale, and would have no effect on the bacteria or on the leucocytes.

When sulphanilamide was introduced I was interested to see whether it had any such antileucocytic action. The test was made in exactly the same way except that the indicator organism was a hæmolytic enterococcus—a microbe which is quite insensitive to sulphanilamide. The result obtained is shown in fig. 2.

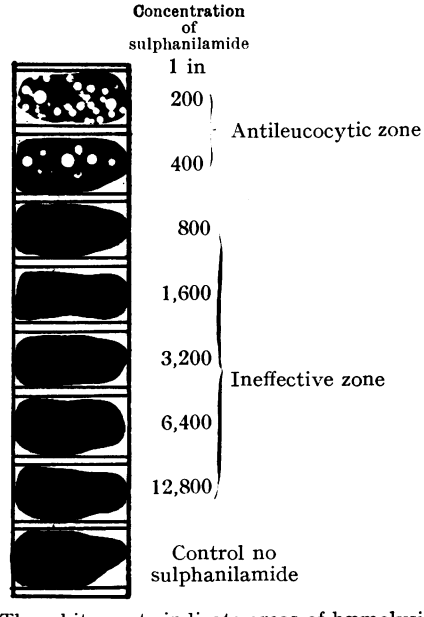
This shows that there is no antileucocytic effect until a concentration of sulphanilamide of 1 : 400 is reached—a concentration far greater than can be attained in the blood therapeutically. More recently similar experiments were made with M & B 693 and it was likewise found that this drug had no antileucocytic effect in any significant concentration.

A very different picture is obtained when the antibacterial power of sulphanilamide or M & B 693 is tested on a sensitive microbe such as the *Streptococcus pyogenes*. If such a test is made in exactly the same way as is illustrated in fig. 1 except that sulphanilamide or M & B 693 is used instead of quinine a result is obtained which is shown in fig. 3.



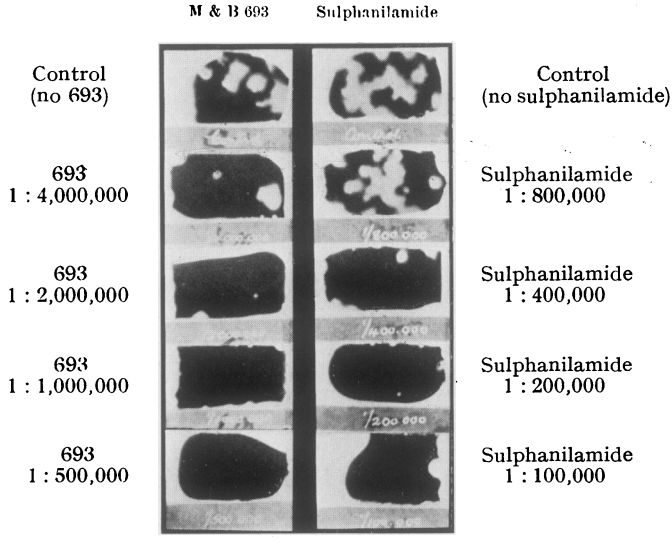

The white spots indicate areas of hæmolysis due to the growth of *Streptococcus pyogenes*.

FIG. 1.—Effect of quinine on the growth of *Streptococcus pyogenes* in human blood.




The white spots indicate areas of hæmolysis due to the growth of hæmolytic enterococcus.

FIG. 2.—Effect of sulphanilamide on a hæmolytic enterococcus in human blood.




The light patches represent areas of hæmolysis caused by growth of the streptococci.
M & B 693 appears to be ten times stronger than sulphanilamide on a typical Group A hæmolytic streptococcus.

FIG. 3.—Comparison of the antistreptococcal power of sulphanilamide and 693.

Here it can be seen that in human blood some antibacterial action is manifest in concentrations of the drugs far weaker than can be obtained by their oral administration. It can also be seen that on *Streptococcus pyogenes* M & B 693 is something like ten times as potent as sulphanilamide. (Complete inhibition of growth with 1 : 1,000,000 of M & B 693 and 1 : 100,000 of sulphanilamide.)

Leaving aside the question of toxicity of such drugs a simple test of this nature gives very valuable information ; a result such as is shown in fig. 3 gives a very strong indication that therapeutic success would follow the administration of these drugs.

A criticism might be offered that it is easy to say this now that sulphanilamide and 693 have proved themselves therapeutically successful, but I first published this method of testing antibacterial chemicals in 1924 and it has appeared since in various publications including the *Proceedings* of this Society.

The exact mode of action of drugs like sulphanilamide or M & B 693 is still unsettled, but it is generally accepted that the action is on the infecting bacteria and not on the host ; and that it is bacteriostatic rather than bactericidal. The chief result of the drug on the bacteria is some interference with their growth, and this is well brought out in two experiments which I have already published (1938).

*Demonstration of the Bacteriostatic Action of M & B 693 on Pneumococci*

Human blood was deprived of its leucocytes by passing it through a tight cotton-wool filter. Such deleucocyted blood has no antibacterial action on pneumococci. This blood was infected with a suitable dilution of a pneumococcus culture and was mixed in equal volumes with serial dilutions of M & B 693 in normal saline. The mixtures were incubated in slide cells for twenty-four hours, when the resultant growth was noted. The results obtained are shown in Table I.

TABLE I.—INFLUENCE OF M AND B 693 ON PNEUMOCOCCUS IN DELEUCOCYTED BLOOD.

| Concentration of M and B 693 | Number of colonies developing from an implant of 100 cocci |
|---|---|
| 0 (control) | 106 |
| 1 : 500,000 | 114 |
| 1 : 250,000 | 118 small |
| 1 : 128,000 | 108 smaller |
| 1 : 64,000 | 102 minute |
| 1 : 32,000 | 90 minute |
| 1 : 16,000 | 107 minute |
| 1 : 8,000 | 94 minute |

This shows that the drug in a concentration of 1 : 8,000, which is about the maximum which can be obtained in the body therapeutically, has no bactericidal action on pneumococci, but there is a marked inhibition of growth as shown by the very small size of the colonies in all concentrations greater than 1 : 250,000.

With a more sensitive microbe such as the *Streptococcus pyogenes* inhibition of growth may be complete, and even after prolonged incubation no colonies appear but the cocci may still survive as is shown in the next experiment.

Serum from a patient who was taking M & B 693 in large doses was infected with a sensitive *Streptococcus pyogenes* so that 25 c.mm. of the serum contained 115 cocci. This infected serum was incubated in 25 c.mm. volumes in capillary tubes, and at intervals the whole of the contents of one tube were planted into glucose broth to ascertain whether the streptococci had been killed. The result obtained was as follows :—

Planted in glucose broth after { 18 hours / 24 hours / 48 hours } growth

It was estimated chemically that this sample of serum contained M & B 693 in a concentration of 1 : 10,000, but it is clear that 25 c.mm. could not kill 115 streptococci in two days.

As these drugs do not actually kill the infecting bacteria something else must do it to account for the striking success which has followed their use in experimental infection of animals and in medical practice. That something is the natural defensive mechanism of the body which, while it might be powerless against a rapidly growing streptococcal or pneumococcal infection, can cope with the infection when the growth of the bacteria is interfered with by the drug.

Even with the most sensitive organisms these drugs do not effect a cure in every case, and I am bringing forward evidence to show that the results obtained by increasing the power of the defensive mechanism, in other words, by increasing the immunity, are, in combination with these drugs, far superior to immunotherapy or chemotherapy alone. The immunity can be increased specifically by vaccine and serums and non-specificially by a great variety of measures.

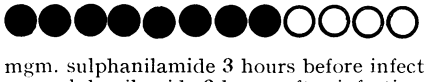
*Passive Immunity in Association with Chemotherapy*

Several workers have shown that specific antiserums enhance the apparent effect of sulphanilamide treatment.

De and Basu (1938), working with staphylococcus, used a combination of an antitoxic and an antibacterial serum, and showed that mice infected with staphylococci could be saved by the administration of these serums combined with sulphanilamide treatment although neither the serum nor the sulphanilamide treatment saved the animals.

Control mice—untreated.

50 units staph. antitoxin
0·5 c.c. staph. antibacterial serum
½ hour before infection



3·5 mgm. sulphanilamide 3 hours before infection.
4 mgm. sulphanilamide 3 hours after infection.

Serum as above + sulphanilamide as above.

(Black circle = mouse died. White circle = mouse survived.)

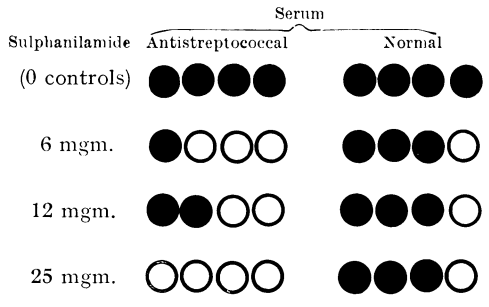
FIG. 4.—Combined action of antistaphylococcal serum and sulphanilamide. Adapted from De and Basu.

Fig. 4, which is adapted from the figures given by De and Basu, shows graphically the results which they obtained. All the controls died, all the animals having the combined treatment lived, and only a few survived that had serum or chemotherapy alone.

Of greater interest possibly are the results published by Loewenthal (1939) on the synergic action of antistreptococcal serum and sulphanilamide in experimental streptococcal infections in mice.

Loewenthal chose a dose of serum which he knew would not protect the mice against his experimental infection, and likewise a dose of sulphanilamide too small

to be completely effective. In one series of experiments he gave the serum eighteen hours before the animals were infected and then immediately after infection the mice received orally a single dose of sulphanilamide. I have set forth graphically in fig. 5




(Black circle = mouse died. White circle = mouse survived.)

FIG. 5.—Combined serum and sulphanilamide treatment.
Serum given 18 hours before infection.
Sulphanilamide given immediately after infection.
(Adapted from Loewenthal.)

the results which he obtained, which show clearly that even with a very small dose of sulphanilamide a greater number of the serum-treated animals survived ; and with a dose of 25 mgm. all the mice recovered although in the control series only one out of four survived.

In a second series the mice were infected with streptococci and nothing was done for eighteen hours, by which time the mice were very ill.

Then the mice were divided into four batches, which were treated as follows :—

(1) 0·5 c.c. of normal rabbit serum.

(2) 0·5 c.c. of antistreptococcal serum (rabbit).

(3) 0·5 c.c. of normal rabbit serum + 20 mgm. of sulphanilamide.

(4) 0·5 c.c. of antistreptococcal serum + 20 mgm. of sulphanilamide.

His results I have adapted into fig. 6, which shows at a glance that the only treatment which was effective was the combination of antistreptococcal serum and sulphanilamide.

There is as yet no published work on the combination of serum therapy and M & B 693, but I have shown *in vitro* that the addition of antipneumococcal serum greatly enhances the antipneumococcal power of a mixture of human blood and M & B 693.

Normal human defibrinated blood was infected with pneumococcus Type 1, and 25 c.mm. of this was mixed with 25 c.mm. of dilutions of M & B 693 and 10 c.mm. of Type 1 antipneumococcal serum or saline. These mixtures were placed in slide cells in the incubator and after twenty-four hours the number of colonies was enumerated.

TABLE II.—INFLUENCE OF M & B 693 ON PNEUMOCOCCI (TYPE 1) IN HUMAN BLOOD WITH AND WITHOUT IMMUNE SERUM.

| 25 c.mm. | + | 25 c.mm. | + | 10 c.mm. | No. of colonies |
|---|---|---|---|---|---|
| Blood | | Saline | | Saline | ∞ |
| Blood | | Saline | | Immune serum | 360 |
| Blood | | M & B 693 1 : 16,000 | | Saline | ∞ |
| Blood | | M & B 693 1 : 32,000 | | Immune serum | 0 |
| Deleucocyted blood | | M & B 693 1 : 16,000 | | Immune serum | ∞ |

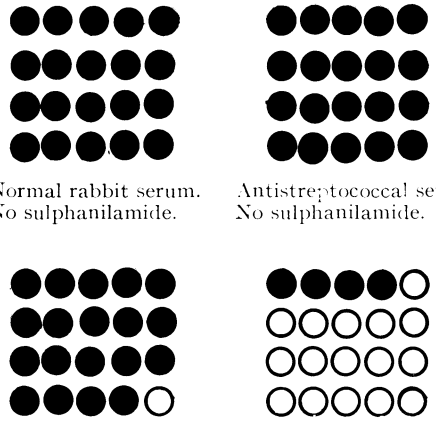



FIG. 6.—Combined serum and sulphanilamide treatment of mice infected 18 hours previously with a hæmolytic streptococcus.
(Adapted from Loewenthal.)

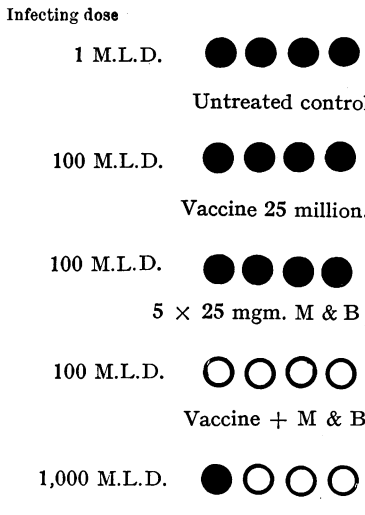
It is clear from this that the combination of immune serum and M & B 693 was the only one effective in preventing the growth of pneumococci in human blood, and there is little doubt that the same combination will be most effective in the treatment of pneumonia in man. You will see, however, that when the leucocytes are removed from the blood the combination of M & B 693 and immune serum is without effect.

*Vaccine Therapy in Association with Chemotherapy*

We have seen that passive immunity by means of serum has a striking effect when induced in association with treatment by sulphanilamide or M & B 693. In passive immunity ready formed antibodies are introduced into the infected animal, but there is no reason why the animal should not make its own antibodies in response to a vaccine, and it is probable that, if there is time, the concentration of antibodies in the blood of the infected animal immunized with a vaccine would be much greater than it would be if a therapeutic dose of serum was administered, for this dose is of necessity of small bulk compared with the amount of blood in the body and would therefore be greatly diluted. Maclean, Rogers and myself, therefore, made experiments to ascertain whether by means of a single dose of vaccine sufficient immunity could be developed in an animal to make any difference to the results obtained when the animals were infected and then treated with M & B 693.

Mice received a single dose of 25 million pneumococcus vaccine, and then after six days they were infected with 100 minimum lethal doses of a virulent pneumococcus culture. (From previous experiments it was known that with that dose treatment with five daily doses of 25 mgm. of M & B 693 failed to save any of the mice.) Then some of the mice were treated with five daily doses of M & B 693 and some were not, and at the same time control unvaccinated mice were infected and of these some were treated in the same way with M & B 693 and some were not.

The results we obtained are shown in fig. 7, and it is clear from this diagram that whereas the vaccine or the M & B 693 failed to save any of the mice infected with 100 minimum lethal doses of pneumococci the mice which had been injected with the vaccine had developed sufficient immunity that when they were infected with 100 minimum lethal doses and treated with M & B 693 they all recovered, and even




(Black circle = mouse died. White circle = mouse survived.)

FIG. 7.—Synergic action of pneumococcal vaccine and M & B 693.
(Adapted from Maclean, Rogers, and Fleming.)

when 1,000 minimum lethal doses of pneumococci were given five out of six mice recovered. From this experiment there is no question as to the synergic action of vaccine therapy and M & B 693 treatment.

To confirm this a second experiment was done in the same way except that the dose of vaccine was 100 million instead of 25 million. The results obtained are shown in fig. 8, and it will be seen that they are essentially the same as in the last experiment.

Further confirmatory tests were done in rabbits, and here the infection of pneumococcus was made intradermally instead of intraperitoneally, as in the case of the mice. The rabbits received a single dose of vaccine six days before they were infected. The control rabbit and the one which received the vaccine, but no M & B 693, died

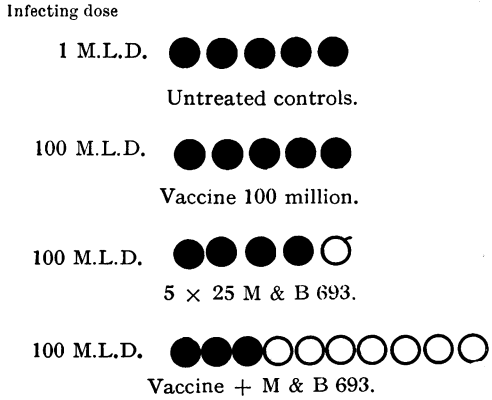



FIG. 8.—Synergic action of pneumococcal vaccine and M & B 693.
(Adapted from Maclean, Rogers, and Fleming.)

with a pneumococcal septicæmia. The unvaccinated rabbit treated with M & B 693 recovered, but had a very serious illness for four days, during which time pneumococci could be recovered from the blood. The vaccinated rabbit treated with M & B 693 also recovered, but had only a trifling illness, and blood cultures taken at intervals over four days remained sterile. The temperature charts of these last two rabbits are shown in fig. 9, and these charts illustrate very well the difference which a single dose of vaccine made in the severity of the illness in animals infected with the same number of the same microbes and both treated in exactly the same manner with M & B 693.

There is a certain amount of clinical confirmation of these results which we have obtained in animals. Cokkinnis and McElligott (1938), in a large series of observations

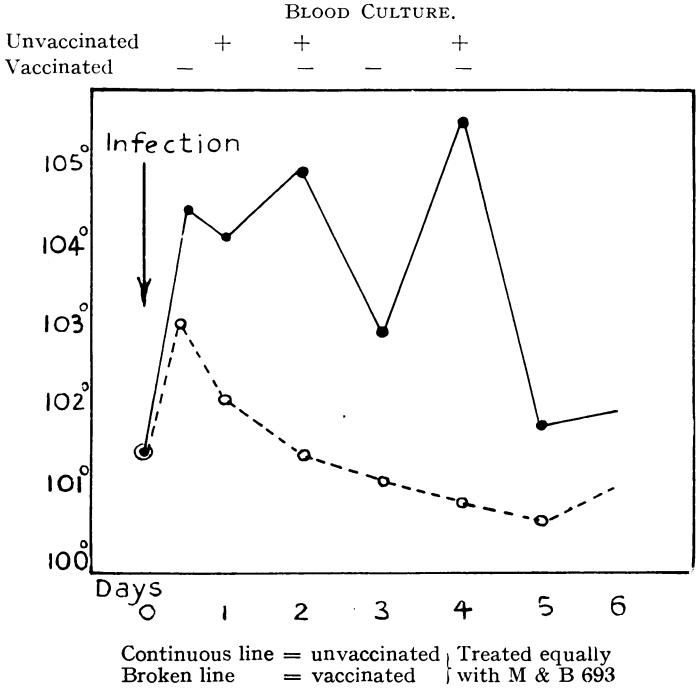



Fig. 9.—Temperature charts of vaccinated and unvaccinated rabbits treated with M & B 693.

(Adapted from Maclean, Rogers, and Fleming.)

on patients suffering from gonococcal infections, have found that better results were obtained when sulphanilamide treatment was withheld for eight to ten days after the onset of the infection. By doing this they obtained a more rapid " cure " and a freedom from relapses. Their explanation of this is " that it takes that much time for the body to acquire enough immunity to dispose of the organisms arrested by sulphanilamide ". They give an interesting table which I have condensed, showing the results obtained in patients treated during the first week of the disease. These patients consisted of over 100 who had never had the disease before, and about half that number who had suffered previously, and in most of whom the complement-fixation test was positive, indicating some degree of increased immunity to the gonococcus. You will see from Table III that much better results were obtained in the second group.

TABLE III.—ANALYSIS OF CASES TREATED IN THE FIRST WEEK OF GONORRHŒA. (ADAPTED FROM COKKINNIS AND MCELLIGOTT.)

| | No. of cases | Good result in one course | Relapsed |
|---|---|---|---|
| First attack .. .. | 103 | 39 (38%) | 61(59%) |
| Not first attack .. | 51 | 31 (61%) | 17(33%) |

*First group complement-fixation test negative in all.*
*Second group complement-fixation test positive in* 42.

Lyons and Mangiaracine (1938) have put forward evidence that the state of immunity of the patient plays a large part in the success or failure of sulphanilamide treatment. They divide acute hæmolytic streptococcal infections into three classes:—

(1) The patients have antibacterial antibody to their organisms at the time treatment is started.

(2) No antibacterial antibody is present and there is a negative blood culture with beginning localization of their infection by the process of inflammatory fixation.

(3) There is a bacteriæmia with no antibacterial antibody.

In this last class they state that sulphanilamide therapy has failed to sterilize the blood-stream.

More recently Ordman (1939) has made some interesting observations on the results which he, in conjunction with Agranat and Dreosti, have obtained in the treatment of pneumonia with M & B 693 in S. Africa. Certain sections of the native mining population are inoculated prophylactically with pneumococcus vaccine, and Ordman has compared the results in such communities with other similar uninoculated communities. The Randfontein Estates furnished a group wholly inoculated while the patients entering the Non-European Hospital in Johannesburg were not inoculated. Ordman has compared the duration of pyrexia in these two groups, each of which was divided approximately equally into a control group and a group treated with M & B 693. His comparisons are shown in Table IV, from which it is

TABLE IV.—COMPARISON OF DURATION OF PYREXIA IN PROPHYLACTICALLY INOCULATED AND UNINOCULATED AFRICAN NATIVES SUFFERING FROM PNEUMONIA.

| | Percentage of cases showing a normal temperature within 4 days of admission to hospital | |
|---|---|---|
| | Control (No. 693) | Treated with 693 |
| Inoculated .. .. | 12·6 | 92·9 |
| Not inoculated .. .. | 38·5 | 60·0 |

Agranat, Dreosti, and Ordman.

clear that the duration of pyrexia in the patients who had been prophylactically inoculated with pneumococcal vaccine was, in combination with 693 treatment, much less than in the group which had not been so inoculated.

This clinical evidence is not, of course, conclusive, but it is very significant when taken in conjunction with the experimental evidence which I have given above.

I will conclude by summing up the new chemotherapy by sulphanilamide and M & B 693. These drugs act on certain sensitive bacteria in some way so that their reproduction is retarded or abolished. When this has happened the natural defensive mechanism of the body completes the task by destroying the bacteria. The result, therefore, which will be obtained in any particular case will depend on two things: firstly the sensitiveness of the infecting organism to the drug, and secondly the state of immunity of the patient. I have attempted in Table V to set forth shortly the expectation of a good result in various conditions of immunity and sensitivity of the organism.

TABLE V.

| State of immunity | Sensitivity of infecting organism to drug | Expectation of good result |
|---|---|---|
| High | High | Certain |
| High | Low | Probable |
| High | Nil | Uncertain |
| *Medium* | *High* | *Probable* |
| *Medium* | *Low* | *Unlikely* |
| *Medium* | *Nil* | *Poor* |
| Low | High | Uncertain |
| Low | Low | Very unlikely |
| Low | Nil | Nil |

The ordinary individual may, as regards the common pyogenic organisms, be regarded as having a medium degree of immunity which can be increased actively or passively.

In an acute case one cannot tell whether the infecting organism is one which is very sensitive to these drugs so that, if the patient is to have the best chance, every endeavour should be made at the same time that the drug is administered to increase the immunity by vaccines or serums or by non-specific measures.

Serums are only obtainable for certain infections, but vaccines can be obtained, or can readily be prepared, for practically every acute bacterial infection.

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Professor F. J. BROWNE said he had always understood that a patient who was suffering from an acute infection such as puerperal septicæmia was being sufficiently vaccinated by the infecting organism and therefore did not require any further vaccine.

## The Effects of Injection of Pituitary Extract Immediately after Delivery

By BERNARD L. WILLIAMS, F.R.C.S.Eng.

AT a recent examination discussion arose between the examiners as to whether untoward occurrences resulted from the injection of pituitary extract immediately following the birth of the child, and also as to whether there was any tangible advantage to be gained by this procedure. This experiment was therefore undertaken at the suggestion of Mr. James Wyatt, who was one of the examiners concerned.

The *objects* were as follows :—

(1) To note the incidence of any complication, such as contraction ring, that would in any way be attributable to the use of pituitary extract.

(2) As regards hæmorrhage, to note :—

(*a*) Incidence of post-partum hæmorrhage. (Post-partum hæmorrhage was said to occur when the total loss exceeded 20 oz.)

(*b*) Whether the average loss was affected by the injection of pituitary extract.

(3) To note whether the third stage was shortened.