STUDIES ON INFLUENZAL MENINGITIS

I. The Problems of Specific Therapy*

By HUGH K. WARD, M.B., and JOYCE WRIGHT, B.M., B.Ch.

(From the Department of Bacteriology and Immunology of the Harvard University Medical School, Boston)

(Received for publication, November 9, 1931)

INTRODUCTION

Whatever the relation of Pfeiffer's *Bacillus influenzae* to epidemic influenza—and that is not under discussion here—there is no doubt that this organism is the cause of influenzal meningitis, a disease that is much more common than is generally realized. The records of The Children's Hospital, Boston, disclose that in the last 5 years there have been 56 cases of meningococcus meningitis and 25 cases of influenzal meningitis in children under 2 years of age. In both diseases the cases have occurred only sporadically during this period.

Influenzal meningitis is a disease of infancy and early childhood and is almost always fatal. Rivers (1), in an analysis from the literature of 220 cases under 2 years of age, gives a mortality of 92 per cent. It is remarkable that no record can be found of any attempt being made to treat these cases with an appropriate antiserum, despite the striking results that have been obtained with antimeningococcus serum. Wollstein (2) in 1911 injected antiserum intraspinally 24 hours after she had infected a *rhesus* monkey, the animal recovering, but no account can be found of the use of this serum in human cases. Rivers (3) has pointed out, and this has been our own experience, that the strains of the influenza bacillus that are found in influenzal meningitis are fairly homogeneous, thus simplifying the problem of specific serum treatment. Under these circumstances we thought it worth while to investigate the possibility of specific therapy. Through the courtesy of the Massachusetts Antitoxin Laboratory, after some months we

* This work was largely financed by the Philip Ellis Stevens, Jr., Memorial Gift.

had available for use the serum of a horse that had been injected with formalized cultures of *B. influenzae* that had been recently isolated from cases of the disease. This antiserum was tried on a number of cases, some 10 to 12 cc. being injected intraspinally either once or twice a day. Although the serum agglutinated the organisms isolated from the cases and was therefore apparently specific, it had no effect whatever on the course of the disease, and influenza bacilli could be cultured from every specimen of cerebrospinal fluid up to the day of death.

The disappointing clinical results with this antiserum and the information that it contained no precipitins[1] prompted us to make a more thorough investigation into the problem instead of merely treating the patients empirically with a specifically agglutinating antiserum. How was this investigation to be carried out? Direct animal experimentation was not promising, because none of the ordinary laboratory animals are susceptible to virulent *B. influenzae* except in large doses and there is very little margin between the lethal dose of living organisms and the lethal dose of dead organisms. This suggests that the toxicity of the culture is of much greater importance than the virulence in overcoming the resistance of the animals and in the beginning at any rate it was the virulence-resistance mechanism rather than the toxicity of the organism that we wished to study.

In the case of the pneumococcus, bactericidal tests either with whole blood or mixtures of leucocytes and serum have given useful information in the problems of pneumonia, several workers, including one of the authors, having employed this technique in recent years. With this experience as a general guide, it was decided to use a similar method in influenzal meningitis, but one modified to meet the special characteristics of the influenza bacillus. It is admitted that, in attempting to find out what goes on in the body in infectious diseases by studying the behaviour of the specific virus in the blood only, one has to be very cautious in drawing inferences. For although the serum and leucocytes may be the most important part of the defense mechanism, they are still only a part. Nevertheless, the technique of the bactericidal blood test is so easy and simple compared to *in vivo* tests

[1] We owe this knowledge to Dr. Margaret Pittman of The Rockefeller Institute who tested the serum and kindly passed on the information to us.

that it facilitates the study of the practical problems of specific therapy, as well as the study of the relationship between defences of the parasite (upon which virulence depends) and the defences of the host (upon which resistance depends). And in diseases in which there is no susceptible animal available, it seemed to us the only way at present to study these problems.

Technique

The technique used in these experiments is essentially one devised originally by Todd, but slightly modified by one of the authors and described in previous publications (4). One determines the maximum number of organisms that a constant amount of the whole defibrinated blood will kill, in the following manner:

Known but varying numbers of organisms are incubated for 24 hours with the constant amount of blood (0.5 cc.) in small, sealed test-tubes placed in a rotating box. The tubes are then broken open and the contents plated out to determine growth or sterility. Growth products of the organisms or antibodies or both can be added to the contents of the tubes before incubation if one wishes to study the effect of these substances on the bactericidal action of the blood. Details of technique can be found in the original communication (4).

In working with *B. influenzae*, certain minor changes have been made:

1. In experiments involving phagocytosis, human defibrinated whole blood has been used, because rabbit blood is not suitable for this purpose, but in all other experiments rabbit defibrinated whole blood has been employed. Fresh rabbit serum has been tried occasionally, but the results are the same as with the whole blood, and it is more convenient to use the blood. In some experiments where it was desirable to reduce the bactericidal power of the normal blood in order to show the effect of adding antibody, the blood has been diluted with an equal volume of 5 per cent pepsin-digested horse blood broth. There is still enough complement left in this special blood broth mixture to show bactericidal action in the presence of antibody, and this digested blood broth is an excellent medium for the *B. influenzae*. Fildes (5) advocated the use of this broth and described the method of preparation in 1920.

2. The smooth, virulent cultures of *B. influenzae* have been kept in defibrinated rabbit blood for the greater part of the time. As far as we can tell, this method has been successful in maintaining the original virulence of the cultures, a very important consideration in most bacteriological work, and especially in studies of this nature. The organism growing out from the culture of cerebrospinal fluid is inoculated into about 0.5 cc. of fresh, defibrinated rabbit blood in either a sealed or a wool-plugged tube. The tube is incubated for 24 hours and then placed in the ice box. At the end of 10 days a tube of 5 per cent digested blood broth is inoculated from the blood tube. The broth culture is incubated and the process repeated.

The rough, non-virulent cultures of *B. influenzae* have been kept in the 5 per cent digested blood broth in the ice box, transplanting at 10 day intervals.

3. When a culture of the virulent organism is wanted for the bactericidal blood test, an 18 hour culture is made in the 5 per cent digested blood broth from one of the stock blood tubes described above.

4. At the end of the incubation of the blood tubes in the bactericidal test, the tubes are broken open and their contents plated out with a platinum loop on to chocolate agar instead of blood agar.

EXPERIMENTAL

In any test involving the bactericidal action of the blood on a certain species of organism, it is obviously necessary to begin by analysing this action. Generally speaking, the Gram-positive organisms are first sensitized by the antibody in the serum and then phagocyted by the leucocytes, no organisms being killed in the absence of the cells; but with the Gram-negative organisms, the cells play a very minor rôle, the bacteria being sensitized by the antibody and then killed by the complement. Under certain conditions the sensitized organisms are not only killed, but undergo lysis. *B. influenzae* falls into this category, but it was found that the cerebrospinal fluid in cases of influenzal meningitis never contained a trace of complement, but leucocytes were always present, often in great numbers. Under these circumstances it was desirable to find out whether phagocytosis of the bacilli could occur in the presence of antibody and cells, but in the absence of complement.

Table I shows the results of an experiment designed to demonstrate the bactericidal action of the various constituents of normal human blood against a virulent culture of *B. influenzae*. The blood was separated into serum and cells by centrifugation. Part of the serum was heated to 56°C. for 30 minutes. The cells were washed four times with saline before using. Antiserum in various concentrations was added to the cells, because the cells with saline or with heated serum had no bactericidal action at all.

Table I shows:

1. That 1.0 cc. of normal human blood can kill at least 10,000,000 virulent influenza bacilli, and it may be asked how does this very strong bactericidal power of the blood fit in with the known susceptibility of human beings to influenzal meningitis, if such findings are at all typical. As far as we know at present, this result is typical of

adult blood, but not of infant blood, and influenzal meningitis is a disease of infancy. This, however, is a subject for separate investigation.

2. That the action of the unheated normal serum alone is just as strong as that of the whole blood, and that the cells either with saline or heated normal serum have no action at all. In other words, the bactericidal action of normal whole blood against *B. influenzae* is wholly due to the natural antibody-complement mechanism.

3. That the organism does not grow well in heated normal serum alone, but that there can be no bactericidal action, as is shown by the

TABLE I

No. of organisms added	Whole blood	Unheated serum	Heated serum	Washed cells + saline	Washed cells + heated serum	Washed cells + heated serum + concentration of antiserum		
						1/20	1/200	1/2,000
5,000,000	0	0	++	++++	++++	++++	++++	++++
500,000	0	0	++	++++	++++	++++	++++	++++
50,000	0	0	++	++++	++++	++++	+++	++++
5,000	0	0	++	++++	++++	+++	+++	++
500	0	0	+	++++	++++	++	+	+
50	0	0	+	++++	++++	0	+	0
5	0	0	0	++++	++++	0	0	0

Here, and in the following tables, ++++, +++, ++, + = various degrees of growth. 0 sterility.

addition of cells to the heated serum, the cells probably supplying a necessary growth factor.

4. That the addition of antiserum to the cells and heated serum results in a very slight bactericidal action presumably by phagocytosis.

Kolmer (6) pointed out several years ago that complement was absent from the cerebrospinal fluid of cases of influenzal meningitis, and this finding has been confirmed in the present investigation. It is interesting in this connection that Dr. L. D. Fothergill of The Children's Hospital, Boston, has shown the presence of complement in the cerebrospinal fluid of all cases of meningococcus meningitis that he has tested. The method of carrying out the test for complement in the two diseases was identical.

The results of the above experiment and the proved absence of complement in the cerebrospinal fluid in this disease showed that a modification of the treatment was essential, since although leucocytes are present in the cerebrospinal fluid, nothing but a very feeble bactericidal action could be brought about by the injection of antiserum alone. The obvious change that was indicated was the introduction of complement along with the antiserum, as indeed had been advocated by Kolmer (6). This method of treatment has now been tried on 8 cases.

In the first 5 cases (which are reported in another paper) some improvement took place temporarily, although all of the 5 patients died eventually. In the next 2 cases, the disease was very far advanced on admission to hospital, and not even temporary improvement was noted. The most encouraging feature of the first 5 cases was the fact that the cerebrospinal fluid was sterilized at one time or another during the disease, in 1 case for 5 days, in a second case for 10 days and in a third case for 14 days. Clinical improvement coincided with the temporary sterilization of the cerebrospinal fluid. Autopsy findings in these cases showed that abscesses were definitely walled off from the general subarachnoid space, and presumably the walls of the abscess protected the organisms from the action of the antiserum and complement. The eighth case, reported to us by Dr. A. Kuttner of the Johns Hopkins Hospital, was a child aged 2½ years, admitted 3 days after the onset of influenzal meningitis. The child was treated daily with the influenzal antiserum and human complement. The cerebrospinal fluid was found to be sterile 24 hours after the first treatment, it remained sterile and the child recovered.

It is clear from these cases that it is possible to sterilize the main subarachnoid space, provided the treatment is commenced reasonably early in the disease, but it is not clear how the formation of abscesses is to be prevented or how they are to be treated once they have formed, because their site at the base of the brain taxes the technique of the most highly skilled brain surgeon. To prevent the formation of abscesses in this region, Dr. F. D. Ingraham and Dr. L. D. Fothergill are attempting to find a better method of introducing the antiserum and complement than by simple lumbar or ventricular injection, and provided the cases are diagnosed early enough—this is perhaps the greatest of the difficulties one has to contend against—they have not lost hope of eventually being able to overcome the obstacle of abscess formation.

Meanwhile, it is our part to make the mixture of antiserum and

complement as efficient as possible, since it is logical to assume that the more rapidly the meninges are sterilized, the less likely is the formation of abscesses.

In the case of antimeningococcus serum, in which no animal test is available, it is customary to rely on agglutination as a test of the efficiency of the antiserum. Accordingly, when our original supply of antiinfluenza serum agglutinated a certain meningeal strain of the influenza bacillus up to a dilution of 1/1000, we assumed that it would be effective as a bactericidal serum. After some months, however, we began to have some doubts, especially when Dr. Margaret Pittman tested it and found that it contained no precipitins. It was at this time that we began to investigate the whole problem more thoroughly,

TABLE II

No. of organisms in tube	Concentration of Antiserum A					Concentration of Antiserum B					Control, no antiserum
	1/25	1/250	1/2,500	1/25,000	1/250,000	1/25	1/250	1/2,500	1/25,000	1/250,000	
200,000	+	+	+	+	+	+	+	+	+	+	+
20,000	+	+	+	+	+	+	0	0	+	+	+
2,000	0	+	+	+	+	+	0	0	+	+	+
200	0	+	+	+	+	+	0	0	0	+	+
20	0	0	0	0	0	+	0	0	0	0	0

and obviously one of the first things was to test the bactericidal action of the antiserum.

In setting up the experiment, the results of which are shown in Table II, we compared the bactericidal action of the original antiserum (Antiserum A) agglutinating *B. influenzae* in a dilution of 1/1000, with serum taken some months later from the same horse (Antiserum B). The horse was being regularly injected with formalized cultures throughout all this period. Antiserum B agglutinated the same meningeal strain of *B. influenzae* up to a dilution of 1/8000.

Normal rabbit blood diluted with equal parts of 5 per cent digested blood broth was used as the source of complement. (Each tube contains 0.5 cc. of this rabbit blood broth mixture, a known amount of antiserum and a known number of organisms. The tubes were sealed, incubated, then broken open and plated out. The details of this technique have already been referred to.

Table II shows that the original Antiserum A, although it had an agglutination titre of 1/1000, had a bactericidal action in a concentration of 1/25 only (compare with control); whereas Antiserum B, with an agglutination titre of 1/8000, had a

slight bactericidal action in a concentration of 1/25,000. In other words, a serum which was but eight times stronger in its agglutinating action was approximately a thousand times stronger in its bactericidal action. This discrepancy is analysed in Paper II.

We need hardly say that from this time onwards Antiserum B was used in the treatment of the cases. Actually, it was used for the first time on the first of the 8 cases previously mentioned.

It will be noticed in Table II that the strong Antiserum B shows the Neisser-Wechsberg phenomenon very plainly, in that the strong concentration of this antiserum has no bactericidal action at all, in contrast to the marked action of the weak concentrations. The reasons for this phenomenon are still in dispute, but is has been known for a long time that for optimal action, complement and antibody have to be present in certain definite proportions to one another. These experiments are always carried out *in vitro*, and we do not know for certain whether this law holds good *in vivo* as well, but if and until there is definite evidence to the contrary, we must assume that it *is* true *in vivo*. And here in the treatment of influenzal meningitis, when we attempt to introduce a bactericidal mixture of complement and antibody into the subarachnoid space, we come face to face with the practical application of the law. In other words, how much antiserum and how much complement are we going to inject, so that the mixture shall have the optimal effect? The answer to this question is not a matter of working out a simple problem in arithmetic, as may appear at first sight. The dilution factor, the diffusion factor and the absorption factor in the cerebrospinal fluid are all uncertain. Further, we have evidence that the antiserum forms a specific precipitation with the cerebrospinal fluid, and we would suspect that the antiserum would be thereby weakened, not to speak of the probability that complement would be adsorbed by this specific precipitate. We have indeed noted in one case, where 12.0 cc. of fresh, human serum (active as complement in a final concentration of 1/150 *in vitro*) was injected by the lumbar route, not a trace of complement could be detected in the cerebrospinal fluid 2 hours later. It must be admitted that so far we have not had the courage to advise a change in the proportion of antiserum to complement, and 15 cc. of antiserum and 6 to 8 cc. of fresh, human serum have been injected twice a day throughout the

disease. Most of the cases responded favourably at first, and one has to be very sure of a theory to change a treatment under these conditions. However, the above proportion is probably far from the optimal, and in the light of the ultimate fate of most of these cases, some modification in the proportion of antiserum appears to be indicated.

In order to gain as much information as possible on this point, we have recently carried out some experiments designed to simulate the conditions in the cerebrospinal fluid.

The experiments have shown that the stronger the concentration of complement, the better the sterilizing effect, so that in the experiment shown in Table III we

TABLE III

No. of organisms in tube	Fresh human serum + concentration of antiserum					Fresh guinea pig serum + concentration of antiserum				
	1/15	1/150	1/1,500	1/15,000	0	1/15	1/150	1/1,500	1/15,000	0
7,000,000	++	+++	+++	+++	+++	+	0	+++	+++	+++
700,000	+++	+	+++	+++	+++	+	0	+++	+++	+++
70,000	++	+	++	+++	+++	+	0	+++	+++	+++
7,000	++	0	+	++	+++	++	0	+	0	+++
700	++	0	0	++	+++	+	0	+	0	+++
70	+	0	0	++	+++	++	0	0	0	+++
7	+	+	0	++	+++	+	0	0	0	+++

have worked with a complement concentration of 1/7.5 in saline. Saline was used as the diluent instead of the special broth, because although the organisms do not grow out as well, and the results are more irregular, saline approximates much more closely to cerebrospinal fluid. A complement concentration of 1/7.5 was used, because that is approximately the final concentration one might expect on injecting 10.0 cc. of fresh serum into the cerebrospinal fluid of a young child. Finally, the effect of fresh guinea pig serum was compared to that of fresh human serum, because of the known high complement content of the former serum.

Each tube in this experiment then contains 0.5 cc. of a 1/7.5 dilution of fresh human serum (or fresh guinea pig serum) in saline, together with a known amount of antiserum and a known number of organisms. The tubes were sealed, incubated, broken open and plated out as before.

Despite the minor irregularities, Table III shows the Neisser-Wechsberg phenomenon again quite clearly, and indicates that with this

concentration of complement, a concentration of approximately 1/150 of this antiserum gives the optimum sterilizing effect. On the assumption that the volume of the cerebrospinal fluid of a child is about 75.0 cc., it would suggest the injection of only 0.5 cc. of the antiserum instead of the 10.0 cc. or more that is ordinarily injected. The experiment also shows that fresh guinea pig serum is more effective than fresh human serum. Actually, this specimen of guinea pig serum was four times more active than the human serum, when tested on sensitized red cells. This is consistent with our previous statement (based on other experiments) that the higher the concentration of complement, the greater the sterilizing effect.

It is fully realised that the inferences to be drawn from this experiment rest on no very secure foundation, because the experiment itself is based on the assumption that the conditions *in vitro* approximate the conditions *in vivo*, and we are very far from sure of this. Nevertheless, one can hardly ignore the test-tube evidence that a large amount of fresh guinea pig serum with a relatively small amount of antiserum is the optimal bactericidal mixture to inject in these cases.

DISCUSSION

The results achieved so far with the specific serum treatment of influenzal meningitis compare unfavorably with the results of similar treatment in meningococcus meningitis. Too little is known of both diseases to permit of any definite reason being given for this. At the same time, the disease processes differ in at least two ways:

1. Complement is present in meningococcus meningitis, absent in influenzal meningitis.

2. Abscesses appear to be walled off quite early in influenzal meningitis.

Although the cerebrospinal fluid was sterilized temporarily in most of these cases, the sterilization took some days to effect, and it is probably significant that in the one case in which the sterilization was permanent, the cerebrospinal fluid was found free of organisms 24 hours after the first treatment, just as in successfully treated meningococcus meningitis.

The antiserum in the presence of complement has a strong bactericidal action *in vitro*, but the observed rapid disappearance of the in-

jected complement from the cerebrospinal fluid leaves some doubt as to the efficiency of this method of supplying the missing complement. Unfortunately, in the one case in which the fluid was sterilized immediately and the patient recovered, we have no information as to the presence or absence of complement in the fluid before treatment with antiserum and complement was commenced.

Early diagnosis in this disease is no doubt a very important factor indeed, and was perhaps the main reason why one case was treated successfully. But the cases are often in an advanced stage when admitted to hospital and the fact that even these cases sometimes show a temporary improvement under treatment gives a little hope that if the surgical and serological techniques can be improved, some of them may be cured. "Diagnosed too late" perhaps comes to the tongue too readily, if treatment is unsuccessful.

CONCLUSIONS

1. An acute purulent meningitis due to the invasion of the meninges by Pfeiffer's influenza bacillus is not a very uncommon disease in infants and young children. It has a very high mortality.

2. Complement is entirely absent in the cerebrospinal fluid of these cases, and bactericidal experiments suggest that the injection of a specific antiserum will have but slight lethal effect on the organisms unless complement is injected at the same time.

3. Treatment with a mixture of specific antiserum and complement led in some cases to a definite clinical improvement, coincident with sterilization and clearing of the cerebrospinal fluid. But after some days, the patients relapsed and died. Autopsy showed localized abscesses in the vicinity of the base of the brain, the lesions being definitely walled off from the general subarachnoid space. In one case, the patient recovered.

4. Since the walls of the abscesses apparently present an insuperable mechanical obstacle to the action of the antiserum and complement, the possibility of preventing the formation of abscesses is discussed. Earlier diagnosis and more rapid sterilization are the most obvious measures. Bactericidal experiments indicate that the proportion of antiserum to complement may be an important factor in bringing about a more rapid elimination of the bacilli.

REFERENCES

1. Rivers, T. M., *Am. J. Dis. Child.*, 1922, **24**, 102.
2. Wollstein, M., *J. Exp. Med.*, 1911, **14**, 73.
3. Rivers, T. M., and Kohn, L. A., *J. Exp. Med.*, 1921, **34**, 477.
4. Ward, H. K., *J. Exp. Med.*, 1930, **51**, 675, 685.
5. Fildes, P., *Brit. J. Exp. Path.*, 1920, **1**, 129.
6. Kolmer, J. A., *Proc. Assn. Research Nerv. and Ment. Dis.*, 1924, **4**, 71.