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THE CLINICAL SIGNIFICANCE OF THE Rh FACTOR*

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The discovery of the Rh factor† (Landsteiner and Wiener, 1940) and the demonstration of its importance in various fields of medicine (Wiener and Peters, 1940; Levine, Katzin, and Burnham, 1941) have recently aroused the greatest interest. Not only has an explanation now been found for many of the hitherto unexplained haemolytic reactions following certain homologous-group blood transfusions, but also a dramatic advance has been made in the understanding of the haemolytic anaemias of the newborn.

It is well known that human erythrocytes contain, in addition to the A and B agglutinogens of Landsteiner, a variety of antigenic components. These other antigens, of which M and N have hitherto been the best known, are usually ignored by clinicians in the selection of blood donors because the corresponding antibodies occur only very rarely in human sera, and, moreover, these factors, although they act as antigens when injected into certain animals, very rarely stimulate the production of immune antibodies in man. By contrast, the importance of the Rh factor lies in its ability to stimulate the formation of specific immune agglutinins in man.

The presence of the Rh factor in certain human erythrocytes was first discovered by testing samples with anti-rhesus sera, prepared by injecting the blood of rhesus monkeys into rabbits. It was found that 85% of human bloods, irrespective of group, were agglutinated, whereas 15% were not. The former are termed "Rh-positive" and the latter "Rh-negative." Persons whose erythrocytes are Rh-negative are capable, under certain circumstances, of forming an antibody which reacts with the Rh antigen. This may occur after transfusions of Rh-positive blood or, probably more commonly, when a woman (herself Rh-negative) becomes pregnant with a baby whose erythrocytes are Rh-positive.

The first demonstration of the practical importance of the discovery of the Rh factor was made by Wiener and Peters (1940). These authors described 4 cases in which haemolytic reactions occurred after the repeated transfusion of blood which would ordinarily have been considered compatible. They were able to demonstrate that the sera of 3 of these patients contained an atypical agglutinin which gave identical reactions with the anti-rhesus serum. They showed that all 3 of these patients were Rh-negative and that the blood responsible for the haemolytic transfusion reactions was Rh-positive. Ten further similar cases were next reported by Wiener (1941). In many of these cases it was possible to show that the transfusion of Rh-negative blood was by contrast as successful as an ordinary compatible transfusion.

Slightly earlier, Levine and Stetson (1939) had suggested that immunization of the pregnant woman to an antigen contained in her foetus might explain the presence of atypical agglutinins in her serum and thus account for the comparative frequency of intragroup transfusion accidents in pregnant or recently delivered women. Later Levine, Katzin, and Burnham (1941) pointed out that these women in whom atypical agglutinins were found had often given birth to infants affected with erythroblastosis. They suggested that the haemolytic anaemia in the foetus might be due to the passage of the immune agglutinin across the placenta, with subsequent destruction of the foetal erythrocytes. At the same time they stated that the majority of the atypical agglutinins had been found to give reactions identical with those of anti-Rh sera.

The role of iso-immunization in the pathogenesis of erythroblastosis foetalis was more fully considered by Levine, Burnham, Katzin, and Vogel (1941). These workers showed that whereas only 15% of the random population were Rh-negative, of 153 mothers whose infants were affected with erythroblastosis foetalis 141 (92%) were Rh-negative. Of 76 infants and 89 husbands in this group who were tested, all were found to be Rh-positive. In 70 cases the mother's serum was examined within two months of delivery; Rh antibodies were demonstrated in 33 of these. From this examination it was concluded that iso-immunization of an Rh-negative mother to the Rh antigen contained in her (Rh-positive) foetus, with subsequent passage of the immune anti-Rh agglutinin back across the placenta, was the cause of the erythroblastosis in the great majority of cases. In the small remaining group, in which the mother was Rh-positive, it was considered that other blood group differences were responsible. Attention was drawn to the fact that 8 out of the 141 mothers had had severe transfusion reactions although homologous-group blood had been used (but no attention had been paid to the Rh grouping of the donor). In the same paper it was claimed that the affected infant maintains higher levels of haemoglobin and R.B.C. counts after transfusion of Rh-negative blood than after transfusion of Rh-positive blood.

The heredity of the Rh factor was considered by Landsteiner and Wiener (1941). They suggested that two allelomorphic genes—Rh and rh—were concerned, Rh being dominant. When the husband's phenotype is Rh-positive and that of the wife Rh-negative the phenotype of the baby will depend upon whether the husband's genotype is RhRh or Rhrh. If the former, the infant's genotype will always be Rhrh and the phenotype therefore Rh-positive; if the latter, the phenotype will only be Rh-positive in 50% of the siblings. These considerations help to explain the different incidence of erythroblastosis in different families.

From this brief consideration of the circumstances in which the Rh factor has been shown to play an important part, it is clear that tests for Rh antigens and antibodies have considerable application in clinical medicine. This paper seeks to confirm many of the observations reported above and to present some new findings.

Sources of Test Serum

Test serum may be obtained from two sources. Either an animal, preferably the guinea-pig (Landsteiner and Wiener, 1941), may be immunized by being given a course of injections of blood from rhesus monkeys, or serum may be obtained

* A report to the Medical Research Council from the S.W. London Blood Supply Depot.

† Throughout, the terms "Rh factor" and "Rh antigen" are used interchangeably, and the corresponding antibody is referred to as the Rh antibody, Rh agglutinin, or anti-Rh agglutinin.

from a human individual who has become immunized to the Rh antigen. In the first case the serum will always have to be absorbed, since it will contain other antibodies acting upon human erythrocytes. Furthermore, only certain animals produce satisfactory antibodies, and even from the good reactors only a small amount of serum will be obtained. The advantages of animal sera are simply that they can be produced at will and that their reactions are more uniform than those of human sera. By contrast, the advantages of using immune human sera for testing are that with good fortune a comparatively large amount of serum may be obtained with very little trouble. Unabsorbed human immune sera containing anti-Rh agglutinins can only be used for testing bloods of the same group or of group O, however, because, of course, they may also contain anti-A and anti-B agglutinins. These agglutinins either can be removed by absorption with appropriate human erythrocytes or, according to Wiener (1942), may be neutralized by the addition of saliva from individuals of groups A and B who are known to be "secretors."

In the present work a start was made by finding a serum, from a woman who had recently given birth to a baby with icterus gravis, which agglutinated the majority of bloods of its own group. The reactions were better at 37° C. than at 5° C. This serum was found to give reactions identical with those of a second similar serum that was obtained shortly afterwards, and the incidence of positive reactions produced by these sera suggested strongly that the agglutinins concerned were anti-Rh. Shortly afterwards, by the courtesy of the Rockefeller Institute, a sample of anti-Rh serum was sent to the Medical Research Council, and so it was possible to confirm that these human sera contained anti-Rh agglutinins. Through the kindness of Dr. Morgan of the Lister Institute we also had at our disposal some other samples of animal anti-rhesus serum obtained by the injection of guinea-pigs.

During the course of the work some 49 sera were found which contained demonstrable Rh antibodies. In accordance with the findings of other workers (Wiener and Peters; Landsteiner and Wiener; Levine, Burnham, Katzin, and Vogel) it was noted that the sera did not give entirely parallel reactions. The majority were observed to react with the same cells as those with which the animal sera reacted. A few sera, however, gave a higher percentage of negative reactions with a random selection of human bloods. Also, in occasional cases bloods were negative with one serum and positive with another. If human sera are used for testing, as they were in most cases in the present investigation, it certainly does not seem advisable to use less than 3 anti-Rh sera for testing (see also Davidsohn and Toharsky, 1942). At least 3, and often more, different sera were therefore used as a routine.

Test Cells

In agreement with the findings of Landsteiner and Wiener great differences were observed between the sensitivity of the cells of different Rh-positive individuals towards a given anti-Rh serum. Whereas with a given serum the cells of one individual may be completely agglutinated, those of another may show only a weak reaction. We have found that, although these differences are constant with any one serum, they differ from one serum to another, so that cells which react well with one serum may react poorly with another, while other cells may react poorly with the first serum but well with the second.

In examining a given serum suspected of containing anti-Rh agglutinins it is clearly advisable to test it against several different known Rh-positive bloods. Furthermore, in titrating the serum against Rh-positive cells it is essential to use the same cells for every estimation if accurate information is to be obtained about the changes in titre of the serum.

Technique of the Tests

Historical

In the reports of the first cases in which anti-Rh agglutinins were detected in human sera it was stated that the reactions were more pronounced at low temperatures and that no noticeable reaction at all occurred at 37° C. (Wiener and Peters). Accordingly, it was recommended that the mixture of cells and serum should be chilled. It was also suggested that the mixtures should be centrifuged before being examined microscopically for evidence of agglutination.

By contrast, sera described by Levine, Katzin, and Burnham (1941) were found to contain Rh agglutinins more active at 37° C. than at low temperatures. In a paper published a little later Wiener (1941) also recorded the finding of sera containing Rh agglutinins more active at 37° C. He stated that, despite the use of the centrifuge technique, no Rh agglutinins were found in 6 cases, in all of which there was a strong suspicion of their presence. At the end of the paper, however, Wiener refers to the fact that the tubes containing the mixtures of cells and serum from one of the cases showed a peculiar pattern of the sediment. In an article published soon afterwards (Landsteiner and Wiener, 1941) particular attention was drawn to this sediment pattern as a sign of a positive reaction. It was pointed out that after a mixture of cells and serum has been allowed to stand for some time the sediment at the bottom of the tube presents different appearances, according to whether or not agglutination has occurred. "Negatively reacting bloods [then] show a circular deposit with a smooth edge, while positive bloods have a wrinkled sediment with a serrated border or show a granular deposit."

It was pointed out that sometimes, in spite of a distinctly positive sediment picture, when the suspensions are examined microscopically the clumping may be quite weak. In our experience, rough treatment, such as sharp tapping of the tubes before the withdrawal of a sample for microscopical examination, can completely break up weak agglutination.

Using the cold centrifuge technique originally advocated by Wiener and Peters, we had several failures at first in attempting to demonstrate Rh antibodies. Later, by treating the mixtures more delicately and by working at a temperature of 37° C. instead of 5° C., it was possible to show that some of the sera examined did in fact contain Rh antibodies. Wiener (1942) has stated that the majority of Rh antibodies are more active at 37° C. than at 0° C., although in his opinion many of them are not much affected by alterations in temperature.

Procedure Recommended

(*a*) *Testing Unknown Cells.*—The following technique is very similar to that recommended by Landsteiner and Wiener (1941), although the importance of carrying out the tests at 37° C. when using human immune sera was first emphasized by Levine (1941).

A suspension of the cells to be tested is prepared by adding one small drop (0.02 c.cm.) of blood to 1 c.cm. of saline; the final concentration should be approximately 1 to 2% in terms of blood sediment. One volume of this suspension is placed with one volume of the test serum in a small tube, 7 mm. in diameter. As mentioned above, it is desirable to test cells with not less than 3 different sera. At the same time known Rh-positive and Rh-negative cells should be put up with the same sera to act as controls. The tubes should be left for at least one hour, and preferably for two hours, before being examined. For reasons discussed above, the tubes should be kept at 37° C. whenever possible, although with animal sera the reactions are just as satisfactory at room temperature (Wiener, 1942).

After the tubes have stood for one to two hours the sediment which has formed is examined, preferably with the help of a hand lens. The difference between typical negatives and positives has already been described, but it must be added that only the examination of a large series of sediments can make one familiar with all the possible appearances. When the sediments have been examined and the reactions have been scored, the sediment in each tube is gently mixed with its supernatant serum by means of a Pasteur pipette, and a little of the mixture is then withdrawn and spread on a slide, and examined microscopically. Usually, definite clumping is seen in blood taken from the tubes giving macroscopic positives. Occasionally, however, only very small agglutinates are seen despite a characteristically positive sediment, and, as we have said, harsh treatment of the mixtures in such cases sometimes completely abolishes the agglutination. Nevertheless we prefer to rely upon seeing agglutinates, macroscopically or microscopically, as a sign of a positive reaction, and simply to use the sediment pattern as a strong suggestion of the reaction to be expected. The danger of false negatives is of course reduced by the use of 3 different sera.

When the reactions are weak it may be very difficult to decide from the microscopical appearance whether the reaction is true agglutination or pseudo-agglutination (rouleaux-formation).

This is a further reason for the use of several different sera and for carrying out control tests with known Rh-positive and Rh-negative cells. Whenever sufficiently potent sera can be obtained it is desirable to add a volume of saline to the volume of serum and of cells in order to diminish rouleaux-formation.

(*b*) *Testing Unknown Sera.*—Sera suspected of containing Rh antibodies should be tested against at least 10 different group O bloods collected at random and also with not less than 2 known Rh-positive and 2 known Rh-negative bloods, using the technique described above. If the reactions are positive with the known Rh-positive bloods and negative with the known negatives, and if the serum reacts with the majority of the unknown group O bloods, it is practically certain that the atypical agglutinin is anti-Rh. It is advisable to test the serum with the cells of the individual from whom it came, to exclude the presence of abnormally powerful auto-agglutinins. If the latter are present they may be partially removed by leaving the serum in contact with its own clot at refrigerator temperature and then removing the serum while it is still cold.

(*c*) *Direct Matching Test.*—In any case in which there is a possibility that the recipient's serum contains Rh agglutinins it is most desirable to perform the direct matching test in such a way that reactions due to the presence of these agglutinins will be detected. As emphasized by Wiener (1942), Rh reactions are usually not detectable by an open slide method, and it is therefore necessary to set up the reactions in tubes as described above. We have tested the most potent anti-Rh sera that we have encountered against known Rh-positive cells, using the ordinary slide technique, and failed to detect any evidence of agglutination. It is therefore necessary to prepare a suspension of the donor's blood and mix it with the recipient's serum in a small tube as described above. Wherever possible the tube should be kept in an incubator for 1 to 2 hours; when an incubator is not available the tubes should be left to stand at room temperature for a similar period. Although it appears from Wiener's observations that occasional anti-Rh sera are more active at refrigerator temperature, it is our impression that such sera will prove to be very uncommon, and since testing at low temperatures introduces considerable additional difficulties it is suggested that in the present state of knowledge it is unnecessary to carry out a test at this temperature as well.

At the end of 1 to 2 hours the sediment at the bottom of the tube is examined, and the impression gained is confirmed by gently withdrawing a portion of the sediment and examining it microscopically. The least evidence of agglutination must be taken as a definite contraindication to the use of the particular donor.

When it is desirable to complete the test as rapidly as possible the tube containing the mixture of cells and serum may be centrifuged slowly at the end of 30 minutes (500 r.p.m. for one minute) (Levine, 1941). The tube is then gently shaken and the contents examined. We do not, however, consider this technique to be as reliable as that already described.

It may be emphasized that Rh agglutinins occurring in human sera are immune bodies and that therefore their titre varies considerably, according to the time which has elapsed since their stimulation by the Rh antigen. Following the transfusion of Rh-positive blood, for instance, there may be a negative phase in which the titre is low and it may be impossible to demonstrate Rh antibodies at all. During the next few days the strength of the antibody increases, and reaches a peak some 7 to 21 days after the transfusion. Thereafter the titre falls slowly.

Little evidence has so far been collected about the period of gestation at which Rh antibodies occur in cases where their formation is stimulated. We have, however, noted that the titre is low soon after delivery and that it rises to a maximum between 7 and 21 days later. The antibody may have practically disappeared in a few months or may still be detectable 5 years later, as in one of our cases.

Results

Random Samples of the Population Examined

A random sample of blood donors (all white; groups A + O) was tested with several human anti-Rh sera. All the sera used for these tests were considered to give similar reactions to the guinea-pig sera, and presumably correspond to the standard type described by Wiener (1942) or to the "No. 1 in our series" of Davidsohn and Toharsky (1942).

Of 1,610 cases, 1,371 (85.15%) were found to be Rh-positive and 239 (14.85%) Rh-negative. These figures correspond closely with those recently quoted by Wiener (1942) (of 777 persons examined, 14.4% were found to be Rh-negative).

Mothers of Infants affected with Erythroblastosis

Diamond, Blackfan, and Baty (1932) first suggested that universal oedema of the foetus, icterus gravis neonatorum, and anaemia of the newborn were all manifestations of the same underlying disease process and could be conveniently grouped together and known as "erythroblastosis foetalis."

Before mentioning the findings in a group of these cases, attention is drawn to the difficulty that is sometimes encountered in diagnosing erythroblastosis in the infant. Clinicians have stated that no line can be drawn between mild "icterus gravis" and physiological jaundice of the newborn (Hawksley and Lightwood, 1934). Certainly, if the passage of immune incompatible agglutinins across the placenta is accepted as the main causative factor in this disease, all degrees of severity will be expected.

According to Diamond, Blackfan, and Baty, the features which enable jaundice associated with erythroblastosis to be differentiated from "physiological jaundice" are its earlier appearance and greater duration, and the associated features of an excess of erythroblasts in the peripheral blood at birth and their persistence for many days or weeks after delivery, together with anaemia, splenomegaly, and hepatomegaly. However, even when these diagnostic criteria are considered there may still be doubt as to the diagnosis because great variations in all these features are encountered. In making observations upon the serological findings in the mothers of infants with erythroblastosis it has been thought preferable to keep to classical cases of the disease, and this we have done. Nevertheless, attention will be drawn to a group of jaundiced babies who possibly exhibit a mild form of the condition.

We tested 48 mothers whose babies had definite erythroblastosis; 46 of these were found to be Rh-negative, and in 44 cases Rh antibodies were present. In all 48 cases the infant was Rh-positive. In the 4 cases in which no Rh antibody was found in the mother's serum, other group differences were evident—e.g., the mother belonged to group O and the foetus to group A. The difference was always such that the mother's serum contained an agglutinin incompatible with the infant's erythrocytes. The possible relevance of this finding is discussed below. In 9 cases in which the mother's serum contained Rh antibodies the infant's serum was examined for the presence of these antibodies. In 8 cases in which the infant was 3 to 10 days old at the time of examination no antibodies were detected. In the ninth case, however, in which the sera of identical twins were examined within 24 hours of birth, definite Rh agglutinins were found in both. This is believed to be the first recorded instance of this finding.

The Rh agglutinins in the mother's serum were titrated at room temperature and at 37° C. in every case, using a standard technique (Taylor and Ikin, 1939), but the mixtures were handled more delicately than usual. In most instances the agglutinins were titrated at intervals after delivery, and these results will be reported separately. It may be useful, however, to mention that out of 41 cases examined within a month after delivery very high titres (over 512) were found in 3, moderate titres were found in 19, and very low titres (under 8) were found in 19 cases. These figures refer to the maximum titre recorded in each individual case; in the majority of instances the figures probably approach the maximum titre reached, but they do not necessarily do so in all cases.

Cases of "Physiological" Jaundice

As mentioned, this does not seem to be a sharply defined group. For the present purpose any baby developing jaundice has been included provided that there were none of the classical features of erythroblastosis described above and provided that there was reasonable evidence that syphilis, sepsis, or congenital obliteration of the bile ducts was not responsible for the jaundice. Tests were made in 24 mothers; 3 were Rh-negative. In these cases the baby was found to be

Rh-positive and the mother's serum contained an Rh antibody. Moreover, in one of these cases the mother had previously given birth to an infant affected with icterus gravis neonatorum. This finding suggests that certain cases usually diagnosed as physiological jaundice would be more properly regarded as mild cases of erythroblastosis. The supposition that the aetiology of some cases of mild jaundice of the newborn is connected with the passage of incompatible agglutinins across the placenta receives further support from the findings in the other mothers and infants investigated. In these the incidence of cases in which the mother's serum was incompatible with the infant's erythrocytes (due to the presence of anti-A or anti-B agglutinins) was considerably higher than the expected incidence in a random sample of mothers and infants.

(The concluding part, with a list of references, will appear in next week's issue)

TREATMENT OF SHOCK BY DIRECT ACTION ON THE VEGETATIVE NERVOUS CENTRES*

BY

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The Mechanism of Traumatic Shock

Efforts to produce shock experimentally play an important part in the elucidation of the mechanism of traumatic shock. The criteria of shock conditions in all these experiments were: (1) sudden and persistent fall in blood pressure, with a rapid and feeble pulse, (2) shallow breathing, (3) the absence of reaction on irritation, (4) lowering of the temperature. All this points to a disturbance of the vegetative nervous system, particularly the sympathetic, which in itself leads to a diminution of volume of the circulating blood, due to the accumulation of blood in the blood depots and diminution in the liquid part of the blood from plasmorrhoea. This disturbance of the vegetative nervous system is also a cause of chemical changes which characterize the course of shock (appearance of acidosis, diminution of glucose, etc.). This work also showed that with the weakening of the sympathetic nervous system there is a stimulation of the parasympathetic system which shows itself by a number of inhibitive effects.

Experiments made by us and our colleagues (Rosin, Chvoles) have proved that sensitivity, especially to pain, plays an important part in the development of shock. They showed that irritation of the skin by an induction current for 2 to 6 hours causes shock, whereas with preliminary anaesthetizing of the skin with novocain much longer electrical stimulation is without effect. Given the same conditions, shock is most easily produced by irritation of the skin on the inner surface of the thigh, the peritoneum, and the periosteum, whereas irritation of the dura even for as long as 8 to 12 hours does not cause shock. The rhythm of irritation is important, shock usually appearing much more quickly when the rhythm is quicker.

The significance of the preceding conditions for the development of shock in man has been proved by clinical observations. Excessive tiredness, prolonged insomnia, insufficient nourishment, fear, exposure, loss of blood, and, not least, painful irritations, lead, after transient overstimulation of the sympathetic nervous system, to its exhaustion and thus facilitate shock. The results of these experiments show that shock is caused, apart from loss of blood, by a whole complex of circumstances connected with a definite condition of the nervous centres. Only when all these conditions are present does shock develop. In clinical studies of shock, attention has been mainly directed to the functional state of the peripheral organs, such as the cardiovascular and respiratory organs. A more detailed analysis, however, has shown that all these cardinal changes, including the changes in metabolism and in the physical and chemical state of the blood and the tissues, are not actually specfic to shock. In fact, similar changes, such as a fall in blood pressure, diminished nervous reactions, etc., can be caused by a depression of the sympathetic nervous system such as occurs also in other pathological states. All these changes form a syndrome which characterizes shock and shock-like conditions and can be caused by action on the vegetative centres; this points to the important part played by the central nervous system in the production of shock. It therefore follows that in order to be able to understand the mechanism of shock and to apply useful therapy and prophylaxis one must concentrate on the functional conditions of the nervous centres, on their metabolism, and, in conjunction with this, on the character of the cerebrospinal fluid—i.e., their immediate nourishing medium.

* Translated by Dr. H. W. Swann.

Function of Cerebrospinal Fluid

The importance of the cerebrospinal fluid to the functioning of the nervous centres has been shown by observations in laboratories and clinics for many years. These observations have proved that the cerebrospinal fluid (taken in its broad sense as the fluid which fills not only the ventricles of the brain and the subarachnoid space, but the whole of the perivascular and pericellular spaces of the brain tissue) is the immediate nourishing medium of the central nervous system. The chemical composition, as well as the physical, chemical, and biological character, of the cerebrospinal fluid mainly depends on, and is determined by, a special mechanism—the so-called haemato-encephalic barrier. The anatomical substratum of this barrier is, apart from the vascular plexus, the capillaries and precapillaries of the brain, in the first place their endothelium. The haemato-encephalic barrier has a great selective capacity, on account of which not all substances which normally circulate in the blood, or get there accidentally, pass from the blood stream into the c.s.f. There takes place "a certain choice" as the result of which a relative constancy of the composition of the c.s.f. is maintained, and there is a certain independence of possible changes in the consistency and quality of the blood.

It has been shown that not only the introduction of abnormal substances from the blood into the c.s.f., but even a simple disturbance of interrelations between the normally existing substances in the c.s.f., such as between certain electrolytes, causes a more or less severe reaction in the nervous centres which sometimes leads to the death of the animal. In certain cases we were able to notice that definite changes in the c.s.f. are accompanied by some features which resemble the complex of symptoms characteristic of shock and similar states. This particularly applies to the ions of Ca and K. It was shown that change in the concentration of these ions in the c.s.f., leading to a change in the coefficient K/Ca, has a very strong influence on the condition of the central nervous system, particularly on the vegetative centres. For example, the introduction of minimum doses of Ca salts directly into the c.s.f., especially into the ventricles, brings about in the animal a condition of severe depression, whereas the introduction of minimum doses of K salts produces pronounced excitement. This suggests that the direct cause of the change in the functional condition of the cerebrospinal system may be a corresponding change in the composition of the c.s.f. Comparing the occurrence in shock of changes in the different physiological systems, particularly in the cardiovascular and respiratory systems, with the action which causes a stimulation of the vegetative nervous system, we conclude that the sympathetic and parasympathetic nervous centres play the most important part in the production of shock. The cause of changes in the vegetative nervous centres may be strong irritation coming from the periphery. The intensity of effect brought about by such irritations depends on the preceding condition of the vegetative centres (their greater or lesser excitability and reactivity), which in itself is closely connected with the chemical composition of the c.s.f.

The possibility of varying the reaction of the sympathetic and parasympathetic centres by introducing into the cerebrospinal canal certain substances such as K salts and Ca salts points to the influence of the electrolytic composition of the c.s.f. on the condition and activity of the vegetative nervous