A RAPID TEST FOR THE PRESENCE OF INCREASED COLD AGGLUTININS

BY

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Increased cold agglutinins are most commonly found in patients with virus pneumonia, but the usual method of estimating them, by titration, is time-consuming, and delay in obtaining the result limits the clinical value of the test. Since, however, cold haemagglutination is sometimes seen to occur almost immediately in the barrel of the syringe or along the side of the tube or pipette into which blood is drawn, it was thought possible that a test might be devised which would indicate rapidly whether or not cold agglutinins were present in excess. The purpose of this paper is to show that the rapid chilling of a simple mixture of whole blood and citrate provides a clinically reliable indication of the presence of increased cold agglutinins.

As long ago as 1903 Landsteiner made a study of the naturally occurring cold haemagglutinating antibodies which he had discovered to be present in various animals. He separated the serum of defibrinated blood from the red cells at 38° C. and added a small quantity of a suspension of autologous red cells in serum or saline to it on a slide, which was then placed in the refrigerator. He noted that, provided the proportion of plasma to red cell suspension was 10:1 or greater, the red cells formed large clumps, readily visible to the naked eye. When, however, the proportion of plasma to red-cell suspension was 5:1 or 5:2 no agglutination occurred.

My preliminary experiments were carried out with serum, plasma, and red blood cells, both undiluted and diluted in sodium chloride (0.9%) or sodium citrate (3.8%). These were mixed in different proportions and rapidly chilled. Undiluted specimens of serum or plasma from various children not suffering from virus pneumonia often produced agglutination of autologous cells provided that the suspensions were not too dense. When strong suspensions of red cells were used, however, agglutination regularly failed to appear. The serum or plasma from patients with virus pneumonia, in striking contrast, produced marked agglutination even of concentrated red-cell suspensions. Moreover, the stronger the concentration of red cells the more rapidly did they agglutinate and the larger were the clumps formed.

Dilutions of whole blood were then made in saline and citrate, and the effect of rapid chilling was observed. Sodium citrate was preferred to saline, since it prevented clotting and was incidentally found to enhance agglutination.

The most satisfactory and clear-cut results were obtained with a mixture of whole blood and citrate in equal amounts. Such a mixture constitutes approximately a 20% suspension of red cells in a 1:3 dilution of plasma. Under these circumstances the amount of cold antibody present in the blood of healthy persons produced no agglutination on chilling, whereas the red cells in the blood of patients with virus pneumonia rapidly formed coarse clumps. In whole blood, since in a given sample the ratio of red cells to antibody is constant however it may subsequently be diluted, it might be expected that the relative amounts of blood and citrate could be varied considerably without affecting the result. This proved to be the case within wide limits, but a gross excess of citrate to blood produced a relatively finer pattern of agglutination; a similar excess of blood to citrate resulted in a sticky mixture difficult to read.

Dilutions were initially made on slides which were placed in the refrigerator for varying periods. Mixtures, however, are easier to handle in test-tubes, and tests showed that a small volume (0.4 ml.) of fluid in a Wassermann tube surrounded by ice crystals could be brought to a temperature of 0–4°C. within 15 seconds.

The Test

The following procedure has finally been adopted as a rapid test for the presence of increased cold agglutinins.

Approximately 0.2 ml. of blood, obtained from a finger or ear prick, is allowed to run into approximately 0.2 ml. of 3.8% trisodium citrate in a thin-walled glass Wassermann tube (60 by 7 mm.). The corked tube is rapidly chilled by rubbing it on the cold tray of a refrigerator so as to cover the outside of the tube with ice crystals and is left for about 15 seconds on its side in the tray. The tube is then removed, holding it at the corked end to avoid warming, and is slowly rotated so that the blood–citrate mixture runs gently over the chilled glass surface, to which ice crystals still adhere. The presence of coarse floccular agglutination is recorded as "positive." No agglutination, or the presence of a fine granularity, is recorded as "negative" (see Fig. 1).

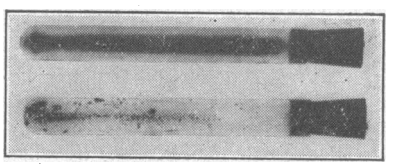

FIG. 1.—Above, negative test. Below, positive test

The tube is then warmed in the hand for a few seconds and re-examined to confirm that all agglutination has disappeared. If a negative result is obtained there is no point in repeating refrigeration, since in no instance has the delayed development of agglutination been encountered. On the contrary, floccular agglutination either appears with remarkable rapidity or not at all.

Relation of Test to Cold Agglutinin Titre

A satisfactory correlation having been demonstrated between a positive rapid test and the subsequent detection of an increased titre of cold agglutinins in a number of patients with virus penumonia, a systematic investigation was carried out on 92 specimens of serum to determine the range of titre associated with positive and negative results respectively.

Method of Titration.—Venous blood was obtained from the patient on the same day as the screening test was performed, and was allowed to clot at room temperature. Serum was separated after incubating the clotted blood for one hour at 37° C. Samples of serum were kept frozen solid until tested, within a fortnight of bleeding. Doubling dilutions were made in plastic trays, using 0.2-ml. volumes of serum in saline (isotonic sodium chloride). To each dilution was added 0.2 ml. of a 1% suspension of group O red cells freshly prepared and washed three times in saline. The same donor (D. H. G.) was used for all estimations. The results were read after overnight refrigeration. The tray was gently tapped to suspend the red cells, and the titre recorded as the reciprocal of the final dilution of serum showing definite agglutination readily visible to the naked eye, without special lighting. Agglutination which could be detected only with the hand lens or with difficulty was ignored. Although the impression had been formed that there was a positive correlation between the height of the

cold agglutinin titre and the size of the red-cell aggregates in the screening tests, no attempt was made to grade the latter, which were simply recorded as either positive or negative.

Usually the result was unequivocal, but borderline examples were occasionally encountered. As many as possible of these were included to determine the degree of overlap. The results are shown in Fig. 2. Screening tests

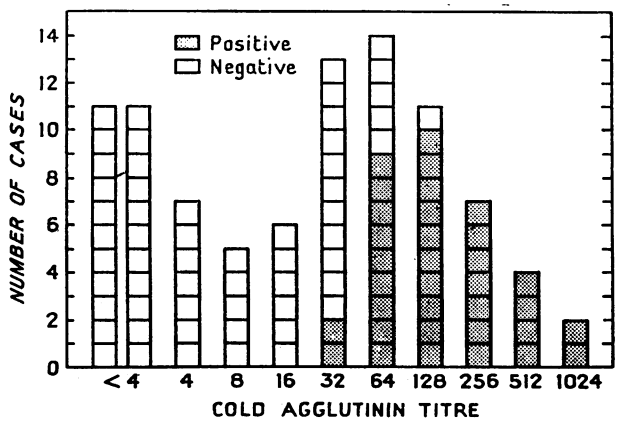

FIG. 2.—Results of rapid test compared with titre of serum obtained on the same day.

were usually negative at a titre of 32 and usually positive at a titre of 64. No negative result was recorded at a titre of 256 or higher, and no positive result at a titre of 16 or less.

Diagnostic Value of Test

This test has been found to be a convenient way of following the appearance and subsequent disappearance of increased cold agglutinins in children with virus pneumonia. With rare exceptions a positive result was found only in association with this condition. The accompanying Table

Rapid Test Results

Diagnosis	Positive	Negative	Total
Virus pneumonia or bronchitis	56	3	59*
Bacterial or non-specific pneumonia or bronchitis	0	226	226†
Bronchiectasis or asthma	0	26	26
Pulmonary tuberculosis	0	17	17
Measles	1	19	20
Infective hepatitis	0	7	7
Severe anaemia and leukaemia	0	9	9
Nephritis	0	15	15
Rheumatic fever	1	13	14
Other miscellaneous diseases	1	144	145
Healthy children and adults	0	45	45
Total	59	524	583

* Including 7 adults. † Including 59 adults.

shows the results of tests performed on 583 children and adults. Of the 59 children or adults positive on one or more occasions, 56 were considered to have virus pneumonia or bronchitis. In only three children was supporting evidence for a diagnosis of virus pneumonia not obtained—one convalescent from measles, one with rheumatic fever, and one infant admitted because of failure to thrive. In each of these the test was only weakly positive.

On the other hand, 524 children or adults failed to show a positive reaction when tested on one or more occasions. From the table it can be seen that a large proportion of these had pneumonia or bronchitis, usually of undetermined aetiology. Many acute infections of the upper respiratory tract have also been included under "other miscellaneous diseases," as have examples of other virus infections such as primary herpes simplex stomatitis, vaccinia, varicella, and infectious mononucleosis. Also included under this heading are examples of whooping-cough, fibrocystic disease of the pancreas, and gastro-enteritis.

These findings suggest that a positive rapid test is of value in the diagnosis of virus pneumonia.

Discussion

Clough and Richter (1918) noticed increased cold agglutinins when they attempted to perform a routine red-blood-cell count upon a patient almost certainly suffering from virus pneumonia. Upon drawing the blood into the diluting pipette it was found that the red blood cells became agglutinated so markedly that clumps were visible macroscopically as well as microscopically. They proceeded to carry out titrations of the patient's serum and to make a detailed study of cold agglutinins, but did not appreciate their clinical significance. The frequent association of the phenomenon with virus pneumonia was first recognized by Peterson, Ham, and Finland (1943) and by Turner (1943). These reports were soon confirmed and led to the adoption of cold-agglutinin titration as a routine laboratory procedure. The importance of a carefully standardized technique, however, was not at first appreciated. Each stage is a possible source of error. The temperature at which the serum is separated, the time it is stored, the strength of the red-cell suspension, the age of the cells, the donor from whom they are obtained, and the method of reading and expressing the end-point may all influence the result to a great extent. The rapid test avoids these sources of error, and has the additional advantages of being readily repeatable.

There is, however, another aspect of the problem to be considered. Even when accurately determined, titre as the sole measure of cold-haemagglutinating antibody has certain limitations. It takes no account of avidity, the term being used here as expressing the strength of red-cell adherence produced by a serum at a given dilution, as shown by the coarseness of the red-cell aggregates and their resistance to dispersal by agitation. Two sera may be defined as differing markedly in potency because of differences in avidity. Because of this, Crookston, Dacie, and Rossi (1956) used arbitrary "units" to compare the sensitivity to cold antibody of different red cells, allotting a score for the intensity of agglutination at each dilution and multiplying the sum of the scores by the number of tubes showing agglutination.

Turner, Nisnewitz, Jackson, and Berney (1943) observed that there was a definite correlation between high avidity (the formation of a solid disk of cells) at low dilutions of serum and the height of the cold agglutinin titre. Finland and Barnes (1951) observed that fine granular agglutination might sometimes be present in a variety of clinical conditions to quite high titre, but they regarded a lower titre of value in the diagnosis of virus pneumonia provided that floccular agglutination was present at a titre of 1:10 or higher. Whether or not estimation of avidity should take precedence over titre in the diagnosis of virus pneumonia, the rapid test (which is a measure of avidity) is a convenient way of obtaining an indication of the amount of cold antibody present in the blood of a particular patient.

Various methods have been used by different workers to simplify the estimation of cold agglutinins. Shone and Passmore (1943) observed in a number of patients with virus pneumonia that their blood rapidly formed clumps of red cells when a drop was left upon a slide at room temperature. They did not study the critical temperature at which this change occurred. Young (1946) used a presumptive test which, however, involved the separation of serum, the addition of a dilute suspension of red cells, and refrigeration in the usual way. Chang and Hou (1948) described a method whereby doubling dilutions of serum were made on a specially ground slide which could be examined directly under the microscope; agglutination was usually complete within two hours. Trönnberg (1950) described a simplified method of titration, using citrated plasma and a dilute suspension of patients' unwashed cells. None of these methods, however, is as simple and rapid as the test here described.

Summary

A simple and rapid screening test for the presence of increased cold agglutinins is described. A mixture of whole blood and sodium citrate in equal parts in a small

test-tube is chilled; under these circumstances an excess of cold antibody causes the red cells to agglutinate almost immediately, whereas a normal amount fails to produce agglutination even after prolonged chilling.

Tests were recorded as positive or negative respectively, and were compared with titrations in 92 instances; they were usually positive at a titre of 64, always negative at 16 or lower, and always positive at 256 or higher.

The diagnostic value of the test was assessed in 583 children and adults suffering from a variety of conditions. With 3 exceptions a rapid screening test was positive only in association with virus pneumonia.

The test has several advantages over titration as a method for estimating cold agglutinins: (1) Venepuncture is unnecessary; (2) no special equipment is required; (3) it is a measure of the avidity of agglutination as well as of the titre; (4) it takes less than a minute to perform; (5) the same sample of blood can be tested as often as desired. It has been found of considerable value in the diagnosis of virus pneumonia and could be performed as readily in general practice as in hospital.

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PRIMIDONE TREATMENT OF ATHETOSIS IN CHILDREN

BY

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Drugs have so far played little part in the treatment of congenital athetosis, and many that appeared promising on paper have proved disappointing in practice. One such drug was benzhexol ("artane"), which was shown to be not only useless but dangerous in a strictly controlled therapeutic trial, using the "double blind" method (Lorber, 1955).

As a result of a statement by Plum (1957) that small doses of primidone had helped 18 out of 31 athetoid children, it was decided to conduct a pilot trial with primidone in a selected group of athetoid children, and to follow this by a full-scale controlled trial if the results justified it.

Material and Methods

For the pilot trial nine patients were selected. All had already attended the out-patient clinic and physiotherapy department for at least four years, so that their abilities and the range of fluctuations in their condition were well known. With one exception all were educable and attended various special schools. Eight were between 6 and 12 years of age and one was a youth of 18. They had a variety of types of athetosis, which in six of them was the result of kernicterus. All had been included in the previous trial with benzhexol.

Primidone ("mysoline") was dispensed as an emulsion. The initial dose was 25 mg. twice daily, and was raised to 75 mg. daily if there was no therapeutic response or evidence of toxicity. The maximum duration of treatment was three months. A placebo, with similar taste and appearance, was then given to those children whose parents considered that improvement had occurred. None of the children had had primidone treatment before the present trial.

The parents were informed that a new drug was to be given to their children and that the drug was known to be helpful in a proportion of children like their own. They were not informed when the drug was changed to a placebo. They were asked to note with particular care any new features, good or bad, during the administration of the drug. At the same time the school-teachers were also informed of the new treatment through the school medical officers and were asked to report on the condition of the children during the trial.

The children were observed for a further period after discontinuation of the drug and placebo.

Results

A full course of three months was completed in eight children. Treatment was interrupted after five days in one hypotonic athetoid child aged 10, because he became so weak and unsteady that he could no longer stand. According to his mother's description "his legs went like jelly." His deterioration was also noted by his school-teacher. On the stopping of treatment he quickly returned to his previous condition. The response of this child to very small doses of benzhexol had been very similar four years earlier.

A second child, aged 6, was a little less steady and became drowsy during treatment. His teacher reported that he was "there in body, but not in spirit" during this period. He, too, regained his previous condition immediately at the end of the course of treatment. Five children showed no response whatever either subjectively or objectively.

Finally, the mothers reported appreciable improvement in two children on primidone treatment. They had more initiative, and attempted tasks with some measure of success which they had never tried before, and became generally steadier. Objectively there was no detectable change in their condition on neurological examination. They were then given a placebo, and they retained this improvement not only while they took a placebo but also subsequently when all drug treatment was discontinued.

Discussion

The results of this pilot study were so disappointing that it was not felt that a trial on a larger scale would be worth while. The doses of primidone were of the same order as those used by Plum (1957). A further increase in the dose was thought to be dangerous in view of the sensitivity of athetoid patients to certain drugs—for example, benzhexol—and of the toxic symptoms observed in two patients during this trial with small doses of primidone.

Summary and Conclusions

Primidone, in doses of 50 to 75 mg. daily, was used in the treatment of nine patients with congenital athetosis. In five no change was detected. Two showed subjective improvement while on the drug, and this improvement was maintained while receiving a placebo and after drug and placebo had been discontinued. In two children there were toxic symptoms: hypotonia and weakness in one and drowsiness in the other. It is concluded that primidone, in the dosage used in this trial, is of no benefit to children with athetosis.

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