lymphoid tissue of the fauces with subsequent ulceration of the overlying mucosa.

It has been seen that neutropenia of some degree was present in all our cases at some stage of the disease, and Kilham and Steigman (1942) report a similar finding. It has been suggested that this neutropenia bears a direct aetiological relationship to the occurrence of faucial ulceration—that the lesions are in fact of similar nature to those seen in agranulocytic angina. Against this view the following points may be mentioned: (1) The neutropenia is not confined to the anginous type of the disease. (2) In no case did it amount to the complete or nearly complete disappearance of neutrophils seen in agranulocytic angina. (3) Neutropenia of the same intensity as that seen in many of these cases—perhaps even a greater intensity—is observed in many conditions of which faucial ulceration is not a feature. (4) The stage of the disease at which it was observed in our cases varied greatly and in general could not be correlated with the condition of the throat: for example, Case I, one of the most severe, showed a neutrophilia during the worst phase, a moderate neutropenia appearing later; while in Case IV healing occurred despite the persistence of a severe neutropenia. (5) In agranulocytic angina there is evidence of long-continued neutropenia before the onset of faucial ulceration. The same may be the case in glandular fever, but in general the period of malaise before the faucial ulceration starts is relatively short, though it seems a more likely occurrence in the “double” or relapse type of illness seen in Cases II and V. If neutropenia does occur it no doubt facilitates the invasion of broken surfaces by secondary bacterial invaders, and so the initiation of a further degree of neutropenia by sulphonamides might adversely affect the outcome. Hence we consider that sulphonamide therapy is absolutely contraindicated in all cases of glandular fever, for a greater degree of neutropenia so induced may facilitate the development of the anginous form as the disease progresses. A possible example of this occurrence is Case V.

On the available evidence our conclusion is that neutropenia plays probably only a secondary part and is not in itself the cause of the ulceration. Further studies are needed for elucidation of this point. Blood and marrow studies during interim periods, such as were observed in Cases II and V, might yield information of interest.

Summary

Among patients with anginous forms of glandular fever are to be found some of the severest throat infections encountered in medical practice. Acute inflammation with purulent exudate, oedema, ulceration, haemorrhage, and mechanical interference with the action of the soft palate may severally be present.

Six patients suffering from anginous glandular fever were treated with intravenous arsenicals. Speedy and conspicuous benefit was obtained.

The haematological findings in these and other cases are tabulated and discussed. In cases seen before the 12th day a conclusive diagnostic titre of agglutinations had already been reached.

The general treatment and management of very severe throat infections are considered.

Diagnosis is reviewed briefly from the standpoints of history, glandular enlargement, throat inflammation, haematology, and response to drugs.

Neutropenia was present at some stage in each of our cases. Its significance is discussed. Since sulphonamides are apt to cause neutropenia they are contraindicated in this disease.

Our thanks are due to Major G. L. Robinson, R.A.M.C., for carrying out laboratory investigations in Cases IV, V, and VI.

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(It is regretted that the exigencies of overseas service have prevented fuller access to the literature.)

H. H. Clemens (*J. Pediat.*, 1945, **26**, 66) records his observations on 80 cases of exanthema subitum (roseola infantum), of which 42 were in boys and 38 in girls. Most occurred in February, March, April, and October. The disease is characterized by an abrupt onset, malaise, and termination in an afebrile exanthem. Fever, irritability, and slight enlargement of the lymphatic glands are typical in the eruptive stage. Complications are very rare. The blood shows relative leucopenia and neutropenia with lymphocytosis by the third day of disease.

POTENT ANTI-Rh AGGLUTININS DEVELOPED IN AN Rh-NEGATIVE FEMALE AFTER MULTIPLE TRANSFUSIONS OF Rh-POSITIVE BLOOD*

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In the case here reported iso-immunization to the Rh factor followed repeated blood transfusions and resulted in haemolytic reactions of varying degree and in the development of anti-Rh agglutinins of exceptionally high titre in the serum of the recipient. The recipient was Group O, as were all the donors. All ABO blood-grouping and cross-matching tests were performed in tubes (5 cm. by 1 cm.). In the cross-matching tests equal volumes (0.4 c.cm.) were used of serum, citrate-saline, and citrated cell suspensions of 0.5% strength in terms of cell sediment. Mixtures were stood five minutes and then centrifuged at 1,500 revs. per min. for two minutes. Readings were made with the naked eye after adequate agitation of tubes. Blood groups were always tested on cells and serum. In cross-matching tests the recipient's serum was tested against the donor's cells, and the donor's plasma against the recipient's cells. In Rh investigations the tube technique described below (on p. 586) was used.

Case History

An unmarried woman aged 47 sought medical advice in April, 1943, because of increasing lassitude and pain in shoulders and elbows. She had recently had a “chill.” On April 29 she had a severe bout of melaena and pain in her abdomen, left side of chest, and knees. There was no fever. The Widal reaction was negative. On the 30th she had a haemoptysis. Purpura now appeared on the limbs and trunk. On May 1 the blood count was: red cells, 1,552,000; platelets, 144,000; leucocytes 19,800. Haemoglobin 36%; coagulation time, 2½ minutes; bleeding time, 12 minutes. Her last menstrual period began on May 3, and lasted 16 days; the loss was profuse. The spleen was not palpable. Her previous medical history was unimportant. The patient had never had a blood transfusion and had never been pregnant. In spite of the platelet count a very tentative diagnosis of thrombocytopenic purpura was made. It was decided to give blood transfusions to raise the haemoglobin to a level high enough to permit of splenectomy. At this time the Rh group of the patient was not ascertained. Concentrated red-cell suspensions only were used in the 10 transfusions reported below. All transfusions were given at very slow drip rates. Blood older than eight days was not used. The standard mixture of donors' blood from which concentrated red-cell suspensions were prepared was: whole blood, 400 c.cm.; glucose, 20 c.cm. of 15% solution; and sodium citrate, 120 c.cm. of 2.5% solution. The sequence of events was as follows, dating from the first transfusion given on May 1.

1st Day.—First transfusion. Given 450 c.cm. (two donors). No febrile reaction. Later, one donor was found to be Rh+, but the other was never traced.

2nd Day.—Second transfusion. Given 450 c.cm. (two donors). No febrile reaction. Both donors later found to be Rh+. Cross-matching tests for the first two transfusions showed compatibility. Marked improvement followed these transfusions.

3rd Day.—The patient had a severe vaginal haemorrhage, but, despite this, was much better. On the 4th day the R.B.C. was 3,720,000 and the Hb 66%; next day the Hb had fallen to 60%.

6th Day.—Third transfusion. Red cells were prepared from two donors, both subsequently found to be Rh+. When 200 c.cm. had been given the patient had a rigor. Transfusion was promptly stopped. The temperature rose from 99 to 103° F. No improvement. On the 8th day the Hb had dropped to 46% and the R.B.C. to 2,480,000. On the 9th day the patient suffered from retinal haemorrhages. On the 11th day multiple ecchymoses appeared on the trunk and there was a profuse epistaxis. A specimen of blood

* This article came into our hands when the untimely death of one of the authors—Dr. G. L. Taylor—was announced.

collected from the recipient on the 9th day was now referred for investigation. In respect of the third transfusion a pre-transfusion sample of the recipient's blood had been conserved in cold storage, as had samples of blood from both donors. Cross-matching tests were repeated, using all these specimens, but no incompatibility was detected with the tube-centrifuge technique described above. The specimen of recipient's blood collected on the 9th day was tested for the Rh factor, and the cells gave a good positive reaction. No irregular agglutinin was detected in the serum.

13th Day.—R.B.C., 1,696,000; Hb 35%; platelets 52,000. Clinical condition suggestive of infective endocarditis.

14th Day.—Fourth transfusion. Given 500 c.cm. (two donors). No febrile reaction. The sample of recipient's blood collected on the 9th day was used in the cross-matching tests, which showed compatibility. General condition improved. The donors were never traced, so that their Rh groups are not known.

17th Day.—R.B.C., 1,992,000; Hb 46%. A specimen of the recipient's blood was collected and used in cross-matching tests for the next two transfusions; no incompatibility detected.

20th Day.—Fifth transfusion. A suspension of 500 c.cm. of red cells was prepared (two donors). Transfusion stopped when 250 c.cm. had been injected, because the needle slipped out of the vein. No febrile reaction. Later, one donor was found to be Rh-negative, but the other was never traced.

21st Day.—Sixth transfusion. The 250 c.cm. remaining in the bottle used the previous day was given. In addition 450 c.cm. of red cells (two donors) was given; both donors later found to be Rh+. No febrile reaction. Some hours after transfusion the patient had pains in her knees, but she had had similar pains previously. Two days later she had pains in the back and knees. A radiograph of the spine was negative.

28th Day.—Acute pleurisy with audible rub developed on left side. No cough or haemoptysis. General condition fair. Some irregular pyrexia.

33rd Day.—R.B.C., 3,120,000; Hb 53%.

38th Day.—R.B.C., 2,560,000; Hb 50%.

43rd Day.—R.B.C., 1,696,000; Hb 26%. This fall was probably in part due to the very severe purpura present.

44th Day.—Seventh transfusion. Given 450 c.cm. (two donors). Later, one donor was found to be Rh-negative, but the other was never traced. The specimen of recipient's blood used in the cross-matching tests was collected the day before transfusion, and no incompatibility was detected. When nearly all the blood had been given the temperature rose sharply from 98.4 to 104° F. The patient had, however, been running a slight irregular pyrexia. The pulse rate rose from 128 to 136. The general condition rapidly deteriorated, and for some hours the patient was delirious. Next morning there was slight jaundice, which lasted a day only. The urine was a deep orange colour but apparently otherwise normal. Four days later the R.B.C. was 2,384,000 and Hb 34%.

51st Day.—Eighth transfusion. Given 480 c.cm. (two donors). Cross-matching tests, using a sample of the recipient's blood collected three days previously, revealed compatibility. Subsequent tests showed one donor was Rh+ and the other Rh-negative. Soon after transfusion the recipient became very restless and her condition deteriorated seriously. The temperature rose from 101 to 102° F. and the pulse became rapid and thready. A few ounces of dark urine were passed shortly after transfusion, and a note was made that blood was present in the urine. A second specimen of dark urine was voided half an hour after the first, but it was not so dark. Two hours later more urine was voided; though still dark, it was much clearer than the first two specimens. Subsequent specimens of urine were of normal appearance. Either the second or the third of the dark specimens of urine was examined with a spectroscope, but no haemoglobin was detected. It seems probable that the first specimen of dark urine contained haemoglobin. Tests for bile salts and pigments were negative. Three days after transfusion the Hb was still 34%, but the R.B.C. had fallen to 1,969,000.

58th Day.—Ninth transfusion. Given 900 c.cm. of red cells from four donors, all later found to be Rh+. Cross-matching tests, using a specimen of the recipient's blood collected two days previously, revealed no incompatibility. Nothing untoward noted during transfusion (which took over six hours), but soon after transfusion the recipient was restless, irrational, and incoherent. The temperature rose from 100 to 103.4° F. and the pulse became rapid and feeble. Next morning there was a suggestion of jaundice, but nothing definite. Urine normal. Three days later the R.B.C. was 1,616,000 and Hb 26%. The large-volume transfusion of red cells had been without beneficial effect. Patient now very anaemic. Numerous petechiae and ecchymoses present. Spleen just palpable.

65th Day.—Tenth transfusion. 450 c.cm. of red cells prepared (two donors). Both donors subsequently found to be Rh+. No cross-matching tests were carried out at the transfusion laboratory, since a recent specimen of the recipient's blood was not available. However, a sample of her blood was collected immediately before

starting transfusion, and a cross-matching test by the slide technique was performed while transfusion was in progress. Distinct agglutination of the donors' corpuscles was observed. A message was then received that 200 c.cm. of blood had been injected and that the recipient was having a rigor and had pain in the occipital region. Transfusion was immediately stopped. The temperature rose from 98 to 101° F. Next morning there was slight transient jaundice.

66th Day.—Further investigations requested. The pre-transfusion sample of the recipient's blood was referred for investigation. Specimens of the donors' bloods had been conserved. Cross-matching tests were carried out by the tube-centrifuge technique and the donors' cells were agglutinated into solid clumps which vigorous shaking of the tubes failed to disrupt. The donors' plasma did not agglutinate the recipient's cells. Recipient's and donors' groups were confirmed as O. The recipient's serum was then tested by the tube-centrifuge technique against the cells of 46 Group O persons, and 36 of these were strongly agglutinated. Relaxation of the rule that concentrated red cells should not be issued without preliminary cross-matching tests, using a fresh sample of the recipient's blood, resulted in the incompatibility being missed, and nearly had serious consequences for the patient. 'Blood urea, 75 mg. per 100 c.cm. Urine normal. Four days after transfusion the Hb had fallen to 22% and the red cells to 1,248,000.

71st Day.—Further tests revealed that the recipient was in fact Rh-negative and that her serum contained a potent anti-Rh agglutinin with a titre of 1:512,000. The technique of titration was that described by Taylor and Race (1944).

Efforts were forthwith made to trace all donors used in the transfusions, in order to ascertain their Rh groups, and these have been given above. Independent serial titrations of the recipient's serum were carried out against the cells of three Group O Rh+ donors (see Graph 1). A maximum titre of 1:1,024,000 was attained on the 74th day—i.e., on the ninth day after the tenth transfusion. The effects of the various transfusions on the haemoglobin level are shown in Graph 2. In tests against the cells of 1,674 Group O

GRAPH 1.—Serial titrations of anti-Rh agglutinin in recipient's serum against cells of three known O Rh+ persons. The maximum titre was attained on the ninth day after the last transfusion of Rh+ blood, and thereafter steadily declined.

GRAPH 2.—The curve shows haemoglobin level during the period when transfusions of Rh+ blood were given.

persons the recipient's serum agglutinated 1,404 (84%) and reacted negatively with 270 (16%).

Subsequent blood transfusions were uneventful. A further 14 transfusions were given, involving the blood of 27 Rh-negative donors. Improvement followed every transfusion and there were no febrile reactions. The haemoglobin was raised from 29 to 70%.

These transfusions rallied the patient temporarily, but ultimately her condition rapidly deteriorated. Petechiae and ecchymoses constantly appeared in various situations, and profuse haemorrhage occurred at times per vaginam. Gradually the patient became emaciated, and ultimately, since treatment was of no avail, transfusion therapy was discontinued. The patient died on the 128th day.

Necropsy was performed by Dr. W. Panes, assistant county pathologist for Monmouthshire. The primary disease was lymphadenoma, the distribution of which was confined to the liver, gastro-hepatic lymph glands, and ovaries. Multiple ecchymoses were present in the tissues, which were oedematous. The spleen weighed 1 lb. and was engorged. The liver was not enlarged, and contained much deposit of iron. The kidneys were normal macroscopically, but were not examined histologically. The uterus was slightly enlarged and several fibromyomata were present.

Discussion

The first three transfusions were given on the first, second, and sixth days respectively, and five of the six donors used were Rh+, while the Rh group of the sixth is not known. The original Rh+ diagnosis was made on the third day after the third transfusion, and was certainly due to large numbers of surviving cells of the Rh+ donors in the recipient's blood. Differential agglutination tests (see Wiener, 1942, and Mollison, 1943) might have revealed the true diagnosis, but as so much Rh+ blood had been transfused this is doubtful. In the event, the Rh+ diagnosis induced a false sense of security, in no way lessened by the fact that the next three transfusions were not complicated by febrile reactions (although two at least of the six donors used were Rh+) and no incompatibility was observed in the cross-matching tests. The reaction complicating the third transfusion was possibly haemolytic in nature, since there was no subsequent rise of haemoglobin. Haemolytic reactions complicated the seventh, eighth, ninth, and tenth transfusions. However, for the above-mentioned reasons, and because of the clinical condition of the patient, the significance of these reactions was not at first apparent. Only in retrospect did the position become clear. It was because of the reaction and the agglutination observed in the cross-matching tests (by the slide method) in the tenth transfusion, and the failure of the patient to improve despite transfusions, that further investigations were requested. Only when the Rh-negative diagnosis was made and the anti-Rh agglutinin discovered in the recipient's serum, was the whole history reviewed and the facts as outlined above placed in their true perspective.

Cross-matching by the tube-centrifuge technique probably explains why incompatibility was not detected earlier. DeGowin (1938) reported a haemolytic transfusion reaction (Case 6) in which cross-matching tests by the tube-centrifuge technique did not reveal incompatibility. The recipient and donors belonged to Group A, and in the light of present knowledge it is probable that immunization to the Rh factor was involved. The tube-centrifuge technique is eminently satisfactory for rapid blood-grouping and cross-matching tests in the ABO system, but it certainly does not suffice in cross-matching tests when very weak anti-Rh agglutinins are present in the recipient's serum, and especially if tubes are stood only for a few minutes before being centrifuged; when, however, the Rh antibodies are strong, the tube-centrifuge technique is satisfactory (Drummond—personal observations). To detect incompatibility due to anti-Rh agglutinins the cross-matching tests should be performed by the following method.

Equal volumes (about 0.05 c.cm.) of serum and of red-cell suspension (0.5–1% in terms of sediment) are mixed in a small tube; 2 in. by 1/4 in. precipitin tubes serve well. In the great majority of cases agglutination due to Rh cannot be detected by the slide or tile method commonly used for ABO grouping. In addition to the cells of intended donors the recipient's serum should, whenever possible, be tested against, say, four or five lots of Rh+ cells known to be strongly reacting and against control Rh-negative cells. Serum and cell suspension are mixed by flicking the tube with the finger. Capping or corking the tubes will prevent evaporation. Readings are made after an hour at 37° C. Some of the cell sediment is carefully drawn up the stem of a Pasteur pipette and spread gently and evenly on a microscope slide and examined for agglutinates under a low power. In a negative reaction the cells are free and evenly distributed, but aggregation due to sedimenting may be mistaken for true agglutination.

The serum of the case here reported was of the ordinary 85% type, which contains one agglutinin, and had, in addition to complete or agglutinating antibody, some of the recently described "incomplete" (Race, 1944) or "blocking" antibody (Wiener, 1944). It is possible, but very unlikely, that the earlier direct tests of the patient's serum and the donors' cells failed to disclose incompatibility, because the antibody was then mostly or entirely of the incomplete sort.

There is another probable reason why incompatibility was not detected earlier—namely, that the anti-Rh agglutinins, initially only weak, would be completely absorbed by the substantial amounts of Rh+ cells transfused. Therefore, for an interval after each transfusion no anti-Rh agglutinin would be detectable in the recipient's serum.

Cases have been reported (Wiener and Peters, 1940; Boorman *et al.*, 1942; Diamond, 1942; Wiener *et al.*, 1942; Dameshek and Levine, 1943; Mollison, 1943; Beck *et al.*, 1944) in which serious or fatal haemolytic reactions complicated only one or a few transfusions of Rh+ blood in Rh-negative recipients. In the above case, despite the tremendously potent Rh antibodies (titre 1:1,024,000) present in the recipient's serum so soon after the tenth transfusion, and the substantial amounts of Rh+ blood transfused (14 donors were Rh+, 3 Rh-negative, while the Rh groups of 5 are unknown), symptoms during the majority of the transfusions were entirely absent. Thus, except for a rigor during the third transfusion, the first six transfusions were entirely symptomless, while the seventh was practically completed before symptoms, not especially severe, occurred. Symptoms were entirely lacking during the eighth and ninth transfusions, though they afterwards became manifest. Yet in the ninth transfusion no less than 900 c.cm. of red cells was given (4 Rh+ donors!). The rigor was not severe in the tenth transfusion. Nevertheless, haemolysis certainly occurred in the seventh, eighth, ninth, and tenth transfusions, as evidenced by jaundice or haemoglobinuria and the fact that there were no surviving Rh+ cells shortly after the tenth transfusion. It is also noteworthy that the haemolytic reactions were not followed by suppression of urine. The urine was alkalinized as a routine by large doses of potassium citrate given by mouth. It is likely that absence of alarming symptoms during practically all the transfusions was due to the fact that the transfusions were all given at very slow drip rates, the duration of each transfusion being usually anything from four to seven hours or more. The lessened severity, or indeed even total absence, of symptoms when incompatible blood has been transfused at a slow drip rate has been alluded to by Mollison (1943) and by Drummond (1944); see also reports by Boorman *et al.* (1942, Case 1) and Burnham (1930).

Conclusion

The above case illustrates the importance of (i) ascertaining the Rh group of a recipient as a preliminary when multiple blood transfusions are contemplated; (ii) the possible danger of being misled when an Rh-negative recipient's blood gives an Rh+ reading after a blood transfusion; (iii) never omitting cross-matching tests, which should be made in tubes by a reliable method.

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A. Bloxson (*J. Pediat.*, 1945, **26**, 32) records his observations on 40 persons—2 adults and 38 children—injected intradermally with recent convalescent measles serum. The serum was given in 0.4-c.cm. doses on successive days until 2 c.cm. had been given. The results were as follows: 35 children and 2 adults did not develop measles, 2 children developed uncomplicated measles before the course was completed, and 2 children developed attenuated measles fourteen days after the inoculations.