TABLE II.—Two-stage Operations

Treated			Control		
Maximum Weight Loss lb. kg.		Day of Maximum Loss*	Maximum Weight Loss lb. kg.		Day of Maximum Loss*
7	3.2	32	13	5.9	18
4.5	2.0	11	8.5	3.9	18
0	0	—	7	3.2	18
11	5.0	11	5.5	2.5	14
9.5	4.3	35	9.5	4.3	21
12.5	5.7	21	7	3.2	35
12	5.4	17	4	1.8	32
14	6.4	30	6	2.7	18
5.5	2.5	11	8	3.6	18
12	5.4	21	10	4.5	11
6	2.7	7	10	4.5	32
12.5	5.7	28	8	3.6	21
			12	5.4	14
			15.5	7.0	22
			7	3.2	56
Average 8.9	4.0	19	8.7	3.9	20

* Day after second stage operation.

is therefore an unwelcome feature of the post-operative phase, and its presence may well exert an unfavourable effect on the eventual course of the disease. Methylandrostenediol has proved disappointing in its effect.

We have made no attempt at direct measurement of nitrogen balance in this investigation. Even though part of the weight loss may be due to oxidation of fat, it is reasonable to attribute most of it to nitrogen loss. It seems justifiable to assess the efficiency of an anabolic hormone in the post-operative phase by the method we have used. We feel that only when a drug can be shown to influence the weight pattern can it be regarded as effective therapy. Our results do not indicate that methylandrostenediol has no effect on nitrogen metabolism, but only that its effects are not strong enough to be apparent clinically. It remains a possibility that a fivefold increase in dose might have some measurable effect, but at this level its virilizing effect, too, would be apparent, particularly in women treated for five weeks or longer. In most reports to date, the dosage has been in the range that we have used.

Summary

The pattern of the metabolic response after surgical trauma has been described briefly.

The considerable loss of weight that takes place after operation is an undesirable feature. This is particularly so in tuberculous patients undergoing multiple operations on the chest.

Methylandrostenediol has not been found to reduce this weight loss.

We have to thank many of the medical staff at Harefield Hospital for helping us in this investigation, particularly Drs. R. S. Francis, D. Levy, and R. G. May. We are grateful to the nursing staff for undertaking the extra work so willingly.

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RAPID Rh-TYPING

A "SANDWICH" TECHNIQUE

BY

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It is necessary to perform ABO and Rh blood-grouping tests before transfusion, and recipients who are Rh-negative should be transfused with Rh-negative blood of homologous ABO group.

Blood banks are usually short of Rh-negative blood of all ABO groups and it is sometimes necessary, in the first instance, to transfuse males who have never had any previous blood transfusion, or intramuscular injections of blood, with Rh-positive blood because of such shortage. This applies only to males and to their first visit to hospital, and such a transfusion should not be continued for more than a few days in succession, nor is it desirable that Rh-positive blood should be given to Rh-negative males who will obviously require multiple transfusions. I have seen Rh antibodies produced in an Rh-negative male patient seven days after primary transfusions of Rh-positive blood.

In the case of females, all those who are Rh-negative, of whatever age, should be transfused with Rh-negative blood. It is not generally appreciated that children who are Rh-negative can be immunized as easily as adults. I have seen an Rh-negative child, 5 years old, immunized by transfusions of Rh-positive blood. Once Rh antibodies have appeared in the blood of Rh-negative females they persist for long periods of time, probably for life. In another case recently observed, Rh antibodies were present in a woman aged 73 who had never received any blood transfusions or intramuscular injections of blood and whose last pregnancy was 38 years previously.

ABO typing can be rapidly and reliably performed, but with the Rh-typing sera available the recommended techniques often take a long time, and when a cross-matching test has to be done in addition to this there may be delay before the blood can be given. Consequently some transfusions, in the minds of clinicians, become "emergency transfusions," and they take the risk of giving blood without the necessary preliminary grouping and cross-matching tests being performed. If the period of time required for the preliminary grouping tests could be reduced then perhaps fewer transfusions would be regarded as being of an emergency nature.

The purpose of this paper is to describe a rapid method of Rh-typing which can be performed on a slide in ten minutes, using Rh-typing sera ordinarily available here.

Materials and Methods

Bovine Albumin.—Armour's 30% bovine albumin and bovine plasma have both been used in this test.

Rh-Typing Serum.—Undiluted anti-Rh serum is used, suitably absorbed to remove anti-A and anti-B agglutinins so that it is universally applicable. The sera employed had been kept frozen solid for several weeks. Strengths of the reaction of a typical serum used in this test are shown in the Table.

Red-Cell Suspensions.—The blood samples are collected either as clotted or as citrated blood. If collected as clotted

Titration of Serum 474, Using Group O, R₁R₂ Test Cells

Diluting Fluid	Dilutions of Serum									Control
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Saline	—	—	—	—	—	—	—	—	—	—
20% albumin ..	++++	+++	++	+	—	—	—	—	—	—

blood, the cell-serum mixture is poured or pipetted off the clot. Citrated or clotted cells are then spun and the serum or plasma is removed. The cells that remain are then washed once in a large volume of saline and packed tightly in the centrifuge. The supernatant saline is then completely removed from the cell pack with a pipette.

Methods.—The saline tube agglutination and albumin tube agglutination techniques were performed according to standard methods (M.R.C., 1952).

Technique

One volume, about 0.03 ml., of the Rh test serum, together with one volume of the 30% bovine albumin and one volume of the packed cells to be typed, is placed in the middle of a microscope slide. The human anti-Rh serum and the 30% bovine albumin may be mixed in equal volumes prior to the test, and two volumes of the mixture used together with one volume of washed packed red cells. The volumes are delivered on to the microscope slide by using a marked Pasteur pipette. On the right-hand side of the slide the

Preparation of the "sandwich."

reference number of the cells that are being typed is marked in grease pencil. The slide is then held in the left hand and another microscope slide held by the narrow edge with the thumb and first finger of the right hand, and the serum albumin and packed red cells are mixed together with the narrow edge of the slide, as in making a blood film. The mixture is spread out on the slide for two-thirds of its length. The slide with which the mixing was done is then gently lowered and pushed on top of the bottom slide so that a "sandwich" is made and the albumin-Rh-serum-packed-cell mixture is spread out in a thin layer between the two slides (see Fig.). When the "sandwich" is made the slides should not be pushed tightly, but only gently, together. Any excess liquid is gently wiped away from the edges of the "sandwich."

Control tests are put up consisting of group AB serum, 30% bovine albumin, and the patient's packed cells, and the sandwich is prepared as described previously. The sandwiches are then carefully placed on a heated plate or in the incubator at 37° C. They are left for ten minutes, removed, and read, using the low power of the microscope. Several fields in the centre of the slide should be examined. If the result is positive, large clumps of cells will be seen, and if the result is negative the cells will be freely suspended. It is always desirable to examine a moving film, and the thumb

of one hand is pressed on top of the sandwich as it rests on the stage of the microscope. As the sandwich is compressed the cells will be observed to move either as clumps in the case of a positive result or as cells which are freely mobile and not aggregated or clumped in a negative one.

Results

One thousand red-cell samples were tested by the tube technique at 37° C., using an anti-D saline agglutinating serum. The results which were negative with this serum were tested with another anti-D serum and also with anti-C and anti-E sera; D^u positive cells were identified and suitable control tests were included.

The 1,000 blood samples were thus divided into D-positive types (including D^u) and D-negative types. These blood samples were then tested by the slide-sandwich technique described above, using anti-Rh serum No. 474, details of the titration of which are shown in the Table. The results were as follows: There were 851 D-positive bloods—847 were typed as positive, 2 as doubtful, and 2 as negative. The remaining 149 were D-negative and all gave negative results using the sandwich technique. The two cells which gave negative results and the two which gave doubtful results, using the rapid sandwich test, were all of variety D^u—either cD^uE/cde or CD^ue/cde.

Forty-two blood samples were also tested by the tube techniques detailed above and also by the sandwich technique, using five different anti-Rh sera (similar to No. 474) on each cell sample; the results were all in agreement. In order to determine whether cells which had been stored in A.C.D. mixture in a refrigerator could be typed by this method, one of these anti-Rh sera (No. 412) was used to test blood samples from outdated stored blood (average age, 21 days). Of 100 blood samples examined, 83 were D-positive and 17 D-negative, using the routine tube techniques, and identical results were given by the sandwich slide technique.

Discussion

Diamond and Denton (1945) introduced bovine albumin for the detection of incomplete Rh antibodies, and subsequently Diamond and Abelson (1945) introduced the open-slide test, using albumin for rapid Rh-typing. This test has been used in many laboratories and has been found to give satisfactory results, but for its success it is necessary to have an avid Rh-typing serum and to have considerable experience in the performance of the test. Such avid Rh-typing sera may mean reimmunizing human volunteers whose sera already contain Rh antibodies with injections of Rh-positive red cells. Such sera are not always available in this country. Another difficulty encountered during the performance of this test is the fact that the cell-serum-albumin mixture on the open slide tends to dry up towards the end of the test, making it difficult on occasion to read it. Furthermore, if people are not familiar with the methods of performing these tests, and if weak or low titre Rh antisera are used, a number of false-negative results occasionally occur, Rh-positive cells (often of type R₁r) being falsely diagnosed as Rh-negative.

Because of the need for a rapid slide test, the sandwich method was developed; it has a number of advantages. First, there is no trouble with drying up of the serum-albumin mixture, since although the mixture dries at the edges of the slides the mixture in the centre of the sandwich remains quite fluid. The drying of the edges is, in some respects, an advantage, since the sandwich becomes very stable, and even though dropped on the bench the slides will not come apart, and the patient's name or number cannot accidentally be rubbed off, as it is in the middle of the sandwich. Secondly, the anti-Rh sera that can be successfully used with the sandwich technique are readily available and need not be specially avid or hyperimmune. The test, furthermore, can be performed rapidly, and results were obtained at 37° C. in ten minutes.

It should be emphasized that the test is specifically designed for rapid Rh-typing before blood transfusion, and it

is not intended to be used as a routine typing method. A number of false-negative results were obtained, but these were all of the D^u variety of Rh, which are known not to be detected by the albumin technique (Renton and Stratton, 1950).

In using this test for the purpose for which it was designed, occasional false-negative results will not cause difficulty. False-positive results were not detected in the tests that were made. False-positive results do rarely occur in special circumstances—for example, in a recent case in which the cells from an Rh-negative patient suffering from acquired haemolytic anaemia were typed, they were found to be positive, but, similarly, the cells gave clumps when AB serum was substituted, as suggested in the technique, and used as a control. This false-positive result, therefore, was readily detected. Occasionally, slight rouleaux formation was observed, but this slight aggregation of cells is rapidly dispersed on compressing the slide and watching the cells under the microscope. A similar dispersal of cells does not so readily occur in the case of true agglutinates. No serious trouble was encountered in the interpretation of the results due to the presence of rouleaux formation. In avoiding rouleaux formation, care must be taken to see that the cell suspensions are washed properly, and, furthermore, that when the albumin-cell mixture is made on the slide it is not allowed to dry at all before the sandwich is made. An additional precaution is to avoid reading the edges of the sandwich. It is assumed, of course, that the Rh-typing serum has no rouleaux-forming properties, and it is often an advantage to keep these sera frozen solid for a week or two before bringing them into routine use. The bovine albumin should also be a reliable and non-rouleaux-forming batch.

If strong anti-D + D^u typing sera are used then some of the high-grade types of D^u can be detected, using the sandwich technique. One of the objects in employing this test, however, as stated previously, was to make it useful with weaker Rh-typing sera and with Rh-typing sera containing incomplete Rh antibodies, which would not normally be used for the saline agglutination test performed in tubes at 37° C.

Summary

In order to shorten the period of time required for the performance of the compatibility test, rapid methods for ABO and Rh typing are necessary. This paper describes a slide sandwich test for rapid Rh typing.

This test is intended to be used prior to blood transfusion and can be performed in ten minutes at 37° C.; it is not intended for routine use when large numbers of specimens have to be tested.

In this technique anti-Rh sera of moderate titre and avidity, such as are ordinarily available in this country, can be employed.

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In his annual report for 1953 Dr. V. Freeman, medical officer of health of Islington, refers once again to housing conditions in the borough. With an average of 76.2 persons to the acre it is one of the most densely populated boroughs in the county of London. Many of Islington's houses are out of date, being in the form of multi-story tenements, built during the early part of Queen Victoria's reign, or even earlier. Of the 82,000 private households in the borough, 53% share a water closet with other households, 42% share a piped water supply, 22% share a kitchen sink, 20% share a fixed bath, and 7% share a cooking stove. No fewer than 59% of the households are without a fixed bath. Many Islington houses are structurally sound, but it is uneconomic for the landlords to keep them in repair owing to the high cost of labour and materials.

A SIMPLE METHOD OF Rh-TYPING

BY

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It is generally accepted that the most reliable method of Rh-typing is that of Landsteiner and Weiner (1941), in which a suspension of red cells is incubated for two hours with an equal volume of anti-D serum in a small tube, the results being read microscopically. This method is too lengthy in cases in which blood is urgently needed, and shorter methods have been used.

The capillary-tube method originally described by Chown (1944) has been widely used, and yields results within thirty minutes. However, Poole and Williams (1951) have reported false-positive readings with this technique, and Chown and Lewis (1951) have advised that the cells should be washed for use in this test.

Poole and Williams (1951) have reported good results with a technique in which the cells and anti-D serum were first incubated at 37° C. for ten minutes and then centrifuged for five minutes at 2,000 to 2,500 r.p.m.

A modification of this technique (F. Stratton, 1952, personal communication) is called the double incubation and centrifuging method. The tubes of cells and serum are incubated and centrifuged, tapped to resuspend the cells, and then reincubated and centrifuged. This technique has been recommended by the Manchester Regional Hospital Board Advisory Committee on Pathology for use in emergencies. By this means, results can be obtained in about fifteen to twenty minutes. The method has the drawback of repeated manipulations, and it was thought that the number of manœuvres could be reduced if the centrifuge itself were heated to 37° C., thus acting as an incubator. It was hoped that this short and simple method, the "thermal centrifuging method," would yield results comparable to the standard two-hour incubation method.

Material and Methods

The cases were routine requests received in the laboratory of a general hospital. They consisted of a small number of midwifery cases undergoing routine typing and some emergency cases received in the casualty department, but the majority were composed of general medical and surgical cases admitted from the waiting-list.

Centrifuge.—This was constructed specially for these experiments by Laboratory Thermal Equipment, Ltd., of Greenfield, near Oldham, Lancs. It consisted essentially of an M.S.E. minor centrifuge, round the outer casing of which a lagged jacket was built, and between this and the casing a thermostatically controlled electrical heating element was fitted (see photograph). No special head was provided, the normal cups being satisfactory for the test. The tubes are removed from the centrifuge cups with forceps.

Cell Suspensions.—The addition of one or two drops of the patient's blood to 2 ml. of saline gives a suspension of approximately 2%. The final concentration of the test is therefore about 1% red cells.

Anti-D Serum.—For use during Experiment 1, special serum of average titre was supplied by the regional transfusion centre and used in all 64 cases. For the other experiments the routine sera supplied by the regional transfusion centre was used, and the titre of these varied. The same serum was used for all the tests made on any cell suspension. Each batch of serum was tested with known Rh-positive and Rh-negative cells.