APPENDIX

GENERAL LABORATORY TECHNIQUE

Only a short description of the apparatus and general technique adopted in the present work is given here.

Fig. 9. General apparatus.

Fig. 10. (Lt. to Rt.) Centrifuge, Waterbath, Hot-air oven.
Cleaning and sterilization of glassware: The laboratory glassware were meticulously cleaned. Gradwohl advises that all new glassware should be rinsed thoroughly in tap water, immersed in a 1:1000 nitric acid solution for one hour, and rinsed thoroughly in tap water to remove the acid. Glassware containing blood and serum were soaked as soon as they have been used in receptacles containing clean tap water. The receptacle was placed within reach of the work table. Care was taken to insure complete filling of tubes and pipettes which were immersed. After soaking, the glassware were cleaned mechanically and if necessary by immersing in sulphuric and chromic acid mixture. Composition of the mixture:

Saturated solution of technical grade sod. dichromate 35 c.c.
Commercial concentrated sulphuric acid 1000 c.c.

In making up the mixture the sulphuric acid was added cautiously to the dichromate mixture. After mechanical cleaning the glassware were rinsed thoroughly in tap water and finally in distilled water.

All the glassware were allowed to dry. The tubes for sterilization were plugged with cotton and wrapped in a paper and put for sterilization in a Hot-air oven (Fig.10). The temperature of the oven was kept at 140°C for 2 hours. After the oven has cooled down the tubes were taken out for use. Pipettes for withdrawing serum and cell suspensions were sterilised similarly after plugging with cotton and wrapping in a paper.

Pipettes: (Fig.9) For transfer of serum and red cell suspensions the simple ungraduated pipettes were used. They have the general shape of medicine droppers. Suitable ones with thick walled tips were made from glass tubing 12" - 18" long and 7 m.m. calibre.
The tube was heated over a Bunsen burner and drawn out to the desired length and thickness. By turning the tube in the flame, a uniform degree of heat was obtained. When at least three inches of the tube was well heated, under rotation, the tube was taken out of the flame and rapidly pulled straight on ends. It was then cut. The larger end of the pipette was fitted with a rubber teat. The length of the narrow portion of the pipette was slightly in excess of that of the test tubes so that the bottom of the latter can be reached.

For work on the secretor factor straws used for drinking purposes have been used. These could be procured easily. Each was cut into two. One half was used for each specimen of saliva and soon after it was discarded. A small glass pipette with a teat was attached to the straw to aid suction. Care was taken to apply only a little suction so that the saliva did not go into the glass pipette. The use of straws makes the work on the secretor factor easy, otherwise if a glass-pipette is used it should be rinsed very thoroughly after testing a sample of saliva and this consumes a lot of time. Preliminary tests showed that the straws do not vitiate the results.

Test tubes: (Fig. 9) For titration of sera and determination of blood groups and the secretor factor test tubes of the Kahn type were used, the size being 7 to 10 m.m. inside diameter and 100 m.m. long. For making cell suspensions and for collection of saliva test tubes 1" x 6" were used.

Test tube racks: (Fig. 9) For the Kahn tubes racks of the Wassermann type were used. These were made of sheets of copper and designed to permit shaking of the tubes in the rack. Each rack has three parallel rows of 10 holes each. To hold the larger test t
wooden racks were used.

**Glass slides and paraffin-ring slides**: Glass slides 1" x 3" were used for microscopic examination. For blood-grouping paraffin-ring slides (Fig.11) were used.

![Image of paraffin-ring slides apparatus]

**Fig.11. Apparatus for making paraffin-ring slides.**

They were prepared as follows: The glass slides were thoroughly cleaned and dried. The instrument used for making the rings was made as follows. A soft iron wire (S.W.G. 24) was wound twice around a test tube to form a double loop about 15 m.m. in outside diameter. The ends of the wire were twisted to form a doubled stem. The wire was covered by closely winding linen thread about it. To make the paraffin-rings the instrument was dipped into melted paraffin (about 120°C), drained quickly by touching the side of the dish and then the loop was placed on the slide and taken. Thus a paraffin-ring was made. Usually two rings were made on a slide.

**Incubator**: (Fig.12) This was used for work on the Rh factor. It was maintained electrically at 37°C.
**Fig. 12 Incubator.**

**Water-bath:** (Fig.10) This was operated by electricity and was automatically maintained at a temperature of 56°C. It was sufficiently large to accommodate the Wassermann racks. This water-bath was used to destroy the complement in the serum before titrating the serum.

**Refrigerator:** This was maintained at 15°C. It was used to store the blood specimens. The sera were stored in the freezing space.

**Centrifuge:** (Fig.10) A table model centrifuge was used. It holds 6 cups utilising the angle principle. It has four speeds. It can develop a speed of 7000 revolutions per minute. An optimum speed of 1000 to 2000 revolutions per minute was used for separating the serum from cells in clotted blood samples.

**Microscope:** (Fig.9) A compound microscope with low power and high power dry objectives was used. The low power gives a magnification of 100 diameters and high power gives a magnification of 440 diameters. The microscope has a moving stage as well. This makes the movement of the slide easy and expedites reading of agglutination.
results. The substage Iris diaphragm, Abbe condenser and mirror were used to give appropriate illumination of the field.

Other equipment used: (Fig.9) A small magnifying hand lens was used to look for agglutination when using the slides with paraffin rings. Wax pencils, gummed labels, slide box to hold paraffin-ring slides, petri dishes etc. were also used.

Normal saline used was a 0.9 per cent sodium chloride solution, though De Grootin had shown that a hypertonic solution of 1.4 per cent sodium chloride gave optimal agglutination, both in the size of the clumps and speed of the reaction. The 0.9 per cent saline was prepared as needed by preparing and keeping a stock 10 per cent sodium chloride solution. Sodium chloride (Analar) and distilled water were used to make the saline solutions.

Collection of blood specimens: Blood was collected in sterilized Kahn test tubes from donors who came to the Blood Bank, Stanley Hospital. After bleeding the donor to collect blood for transfusion, about 2 c.c. were collected in a Kahn tube. Soon after, the specimen was labelled and kept in the refrigerator. The blood specimens were examined after 24 hours and within 72 hours of the time of collection.

Preparation of red cell suspension: The red cell suspensions were made from clotted blood as follows. The clotted blood was rimmed and centrifuged. The serum was pipetted off and the blood clot was shaken up with normal saline. Some of the blood suspension thus formed was pipetted off into another tube. The blood cells were washed as follows. The suspension was once more centrifuged and supernatant saline removed. Again fresh saline was added and the cells shaken up to form a suspension. The process was repeated two or three times. After the final washing enough to the cell sediment to get the required concen-
tration of cell suspension (i.e., a 2 per cent or a 5 per cent suspension). A 2 per cent cell suspension was prepared by adding 0.2 c.c. of the cell sediment to 10 c.c. of saline. The other required percentages were made accordingly. When a number of blood cell suspensions had to be prepared of the same (approximate) percentage, one suspension was prepared first and the other suspensions were prepared by adding saline to the cells (without measuring) till the colour of these matched the colour of the first.

Reading of tests: Agglutination tests were read first by the naked eye, then by the hand lens and finally by means of the microscope. Agglutination was marked by clumping of the red blood cells. Usually the clumps, in the M-N typing and in subgrouping groups A and AB, were large enough to be seen by the naked eye. When the clumps were not large enough the hand lens and microscope were used. Absence of agglutination was marked by lack of clumping. The red cells were discrete under the microscope. To the naked eye there was uniform turbidity. Figures 13 and 14 show the naked eye and microscopic appearances respectively of agglutination and absence of agglutination.

**Fig.13. Lt. Ring shows agglutination.**
**Rt. Ring shows no agglutination.**

**Possible sources of error:**

1. Rouleaux formation may give the appearance of clumps to the naked eye and under the microscope. Addition of a drop of
saline and agitation will disperse the R.B.C.'s if they are in rouleaux formation. Red agglutinates are not dispersed. The rouleaux formation is accentuated when doing the M-N tests and the subgroups of A and AB by drying of the blood and serum in the para-ffin rings on the slides. To prevent this drying the slides were covered with petri dishes and a moist filter paper was placed by the side of the slide under the petri dish. This enables one to read the results even after 30 minutes. The blood and serum mixture on the slides does not dry up before 45 minutes have elapsed after the constituents has been placed on the slide.

2. Cold agglutination will give rise to false agglutination readings. These cold agglutinins act best at 5°C. Because all the tests were done at room temperature (nearly 26°C) this source of error was eliminated.

3. Sera which are infected or not very potent will give rise to false reactions. This source of error was avoided by using sera from reliable sources. Anti-A and Anti-B sera have been supplied by the Blood Bank section, King-Institute, Guindy. This institute supplies the sera for all the Blood Banks in this presidency. Ab-
sorbed group B sera, anti-N and anti-H sera have been supplied by Dr. Mourant, Director, Blood Group Reference Laboratory, London. The anti-Rh serum was supplied from America. This serum was prepared under the supervision of Dr. Levine. Infection of the serum in the laboratory was avoided by using sterile pipettes to withdraw the serum. When not in use the sera were stored in the freezing space of the refrigerator.

4. Infection of the red blood cells makes them agglutinable by all sera. This is due to the unmasking of agglutinogen T by the action of bacteria. To avoid this blood was collected in sterile Kahn test tubes with all aseptic precautions and stored in the refrigerator. Cell suspensions were prepared just before use.

5. Errors due to labelling were avoided by scrutinising the labels at least twice before performing the tests.

Tile Technique of A-B-O grouping: High titre group A, group B and group O sera have been used. On a white porcelain tile 3 squares were drawn and into the first square 1 drop of high titre group A serum was put, into the second square 1 drop of high titre group B serum was put and into the third square 1 drop of high titre group O serum was put. To each of the squares 1 drop of a 5 per cent cell suspension of the unknown blood, was added. The two drops in each square were well mixed with a wooden applicator. After ten minutes agglutination was looked for. If there was no agglutination the reading was taken again after 20 minutes. Agglutination was marked as '+' and absence of agglutination was marked as '−'. If unknown blood was agglutinated by group A serum and group O serum, it belongs to group B. If unknown blood was agglutinated by group B serum and group O serum it belongs to group A. If unknown blood was agglutinated by all the three sera it belongs to group AB.
If it was not agglutinated by all the three sera it belongs to group 0. The group 0 serum was used as a control because if there was agglutination with group 0 serum the unknown should be agglutinated by at least one of the other sera. Table 31 shows the reactions of the A-B-O groups with the various sera.

**TABLE 31**

Reactions of the A-B-O groups with Group A, Group B and Group O Sera

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<th>Group A Serum</th>
<th>Group B Serum</th>
<th>Group O Serum</th>
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<tr>
<td>Group A</td>
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<tr>
<td>Group B</td>
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<td>Group AB</td>
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<td>Group O</td>
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