TECHNIQUE FOR THE FILTRATION OF HUMAN PLASMA AND SERUM FOR TRANSFUSION

BY

R. G. MACFARLANE, M.D.

J. C. MACSWEEN, B.Sc.Ed.

B. R. S. MAINWARING, M.A., B.Sc.

AND

H. J. PARISH, M.D., M.R.C.P.Ed.

In the spring of 1940 arrangements were made to prepare liquid and dried plasma and serum for the Blood Transfusion Service of the Medical Research Council. The following is an account of the technique used for preparing liquid plasma and serum:

The blood is received in the M.R.C. standard bottles, which contain 20 c.cm. of 15% glucose in distilled water, 100 c.cm. of 3% sodium citrate in distilled water as anti-coagulant, and about 420 c.cm. of blood (approximately 220 c.cm. of plasma and 200 c.cm. of cells). The method which is used to separate the plasma depends upon the time that has elapsed between bleeding the donor and arrival of the blood: (a) if less than forty-eight hours old the blood is run through an Alfa-Laval separator; (b) if older, it is allowed to stand for five days at 4° C. and the plasma is siphoned off by special apparatus, described below. The pooled plasma, derived from blood of appropriate groups, has normally a low agglutinin content (Loutit, 1941; Della Vida and Dyke, 1941; Jakobowicz and Bryce, 1941).

Separation and Collection of Plasma, using an Alfa-Laval Centrifuge

The separator is an Alfa-Laval Vickcen Type 50 machine. The bowl has a speed of 7,800 r.p.m. and a capacity of one pint, and is arranged so that cells and plasma are discharged continuously.

Assembly of Bowl.—This is carried out according to the instructions issued with the machine for assembly as a purifier. The size of the gravity disk is important, and the disk marked 0.95-0.98 is found to be the best, giving the highest yield of plasma consistent with negligible haemolysis.

Operating the Separator.—Blood of all groups is pooled in a 10-litre glazed earthenware vessel having a delivery outlet fitted with rubber tubing controlled by a screw clip. The machine is switched on and allowed to reach full speed. Citrate saline solution is led in until the bowl is full and the solution is issuing freely from the lower outlet. The citrate saline is then replaced by blood. (If blood is added to the empty machine without first introducing citrate saline much haemoglobin is present in the initial yield of plasma.) The rate of input is not critical, but 15 to 20 litres an hour seems to be optimal for a good yield of plasma containing few cells. The citrate saline solution is gradually displaced from the bowl and flows from the lower outlet with a small amount of plasma. After a short time this ceases, and diluted plasma begins to flow from the middle outlet. The plasma gradually becomes more concentrated as the citrate saline is displaced, and at the same time cells begin to issue from the lower outlet. From this point the process is practically automatic, although it is necessary to see that the small clots which occur even in fresh citrated blood do not accumulate and obstruct the rubber tubing or the strainer at the top of the machine. This would stop the flow and cause haemolysis on resumption. If the machine is stopped for any reason it must be taken to pieces, cleaned, and refilled with citrate saline to avoid excessive haemolysis when blood is run in. Up to 40 litres of plasma has been separated in a single

run, though a better product is obtained if the limit is 20 to 25 litres. It is unlikely that larger outputs will be required, but if they should be necessary a larger machine might be employed. The separated plasma contains a few red cells, which are removed at the next stage of the process. A small amount of free haemoglobin—usually 1 to 2 mg. per 100 c.cm.—is also present, and is enough to tinge the plasma, although it is clinically insignificant. Almost the theoretical yield of plasma is obtained—i.e., 65% of the volume of diluted blood.

Although all parts of the Alfa-Laval separator which come into contact with the blood are thoroughly cleansed no attempt is made to sterilize them. The plasma must therefore be sterilized by filtration as soon as possible after separation.

Collection of Plasma by Siphon Method

Blood more than forty-eight hours old on arrival is left to stand for five days at 4° C. and is then removed carefully from the refrigerator without disturbing the cells. Bottles showing excessive haemolysis or lipid material are worked up separately; as a rule the filtered plasma from these bottles is usable, although some of our batches have become opalescent on standing and have had to be discarded. The plasma is siphoned off from each M.R.C. blood bottle, using the apparatus shown in Fig. 1.

FIG. 1.—Apparatus used to siphon off the plasma.

A is a Winchester bottle with the bottom removed and the broken glass edges filed smooth. When in use it is held in position by a clamp round the neck. The rubber bung carries a length of glass tubing, to the lower end of which is fixed, by a rubber band, a metal disk, B, with three legs (like an inverted three-legged stool). This ensures that suction of the plasma is applied horizontally, thus enabling the operator to withdraw the plasma to within 3 to 5 mm. of the cells. C is an M.R.C. blood bottle

containing citrated blood and resting on a shelf, D, which is raised and lowered by means of a hydraulic lift. E is the main water supply to the lift. When the regulating valve F and the hand-operated tap H are both open the water runs to waste at G, and the piston is not raised. On closing tap H the piston carrying the shelf and bottle rises, and when H is again opened piston, shelf, and bottle are lowered. The apparatus allows of a perfectly smooth elevation of the bottle, and the lift can be controlled to a few millimetres. I is a spring clip which is fixed to the bench and controls the vacuum. M is a blood bottle used as a trap, and N a hand bellows which, by the manipulation of clips, enables pressure to be applied and thus reverses the plasma flow. If cells are accidentally drawn up past the disk B they can be returned by bringing the bellows into use. The inclusion of cells with the bulked plasma in the 10-litre collecting bottle O is thus avoided.

All apparatus in contact with the plasma is sterilized in the autoclave. For sterilization the suction dome with attachments is disconnected at K, which is fitted with a cotton-wool and parchment cover. A loose parchment cover is also placed over the dome. K to P constitutes a second section, and the 10-litre collecting bottle and its attachments, including a siphon cotton-wool filter, a third section. For convenience the entire "bulking" apparatus is thus sterilized in three parts.

Clarification of Plasma

To facilitate Seitz filtration the plasma is clarified by passing through a specially prepared filter of cotton pulp which removes the fat and the majority of bacteria, etc. The pulp filter is prepared by finely shredding 1 lb. of English "perfecta" pulp into 10 litres of water at approximately 50° C. and beating to a smooth suspension. The apparatus used for pulping consists of a double Winchester quart bottle (4-litre) with the bottom removed. The bottle is inverted and supported in an iron stand, one side of which is a gate for easy assembly. In preparing the filter a layer of zinc gauze (similar to desiccator gauze) is first placed in the inverted bottle. The next two layers consist of large round pebbles about 2 cm. in diameter. Additions of the soaked pulp are now made, the first of these consisting of enough pulp to cover the pebbles to a depth of 2 cm., enclosed in muslin and packed tightly on top of the pebbles. Moderate reduced pressure from the vacuum line or a water-pump is applied (through a rubber bung in the neck of the bottle) when the first addition of pulp is being made, and is maintained until the filter is ready for sterilization. When the pulp-muslin layer is completed

FIG. 2.—Method of clarifying plasma.

the remainder of the pulp mixture is then added in three or four amounts. Tight packing of each addition is facilitated by means of a beater, consisting of a large rubber bung on the end of a stick, and care must be taken to ensure that

there are no leaks down the side of the glass container. To avoid separation into distinct layers during sterilization it is important to keep the pulp wet during preparation.

When the filter is complete the total height of the pulp is usually about 10 cm. Distilled water is drawn through the pulp to wash it thoroughly, and the whole apparatus is then sterilized by autoclaving for half an hour at a temperature of 121° C. and a pressure of 15 lb. per sq. in. During autoclaving and until the filter is required for use, the open ends of the apparatus are covered loosely by parchment.

Immediately before use about 500 c.cm. of sterile citrate saline solution (1% sodium citrate and 0.85% sodium chloride) is passed through the pulp. The plasma is then

drawn through by suction, which is gradually increased, the filtrate being collected in sterile 10-litre bottles (Fig. 2). About 8 litres come through in half an hour, the negative pressure not exceeding 120 mm. Hg. About 30 to 40 litres of plasma can be passed through one preparation of pulp.

Sterilization of Plasma

Sterilization of the plasma presents a problem to which no satisfactory solution has yet been found. Filtration appears to be the only safe and practical method, but is a process that introduces its own special difficulties. The use of filter candles on a large scale is impracticable, since the plasma tends to clot during filtration and the interstices of the filters become blocked. The asbestos-pad filter allows the filtration surface to be renewed when necessary, and this type of filter was adopted. It has been found that fibrinogen is completely removed from the first sample of filtrate, but is present in later samples. Prothrombin is removed from a much larger volume, and when it finally passes the filter the filtrate clots spontaneously in the absence of ionized calcium. Therefore only a limited volume of plasma per unit area of filtration surface can be produced without fear of later clotting. Bushby and Whitby (1941) have found that clotting by filter pads is due to their asbestos content, a factor that unfortunately cannot be eliminated, as it is on this that the sterilizing qualities of the pads depend. The method of removing the prothrombin from the pads by washing with a solution of caustic soda (N/30) and sodium citrate (Bushby, Buttle, and Whitby, 1940; modified by Stanbury—method unpublished) has given unsatisfactory results in our hands, for reasons probably connected with the fact that the plasma we deal with is usually old.

By careful attention to the amount of plasma allowed to pass through the filter the difficulty of clotting can be largely overcome, though eventual clotting of some samples after several weeks has been observed. The underlying reasons for the clotting are extremely interesting, and it is hoped to publish the results of this study at a later date.

The following method, though expensive in pads and labour, gives a satisfactory sterile final product, which, provided it is kept at ordinary room temperature (above 12° C.), does not clot readily. Lower temperatures have the curious effect of hastening coagulation.

A multiple-plate Seitz "pilot" or "British" filter press is assembled in the normal way. The plates are numbered and should be in their correct order, with pads (Seitz EK or Ford's SB 20 by 20 cm. "sterilmats") between them. Eight or more pads may be used for each filtration.

Sterilization of the Seitz Filter.—This is carried out by autoclaving for half an hour at 121° C. and 15 lb. pressure per sq. in. For this operation the filter is correctly but loosely assembled, and all the ports are left open so that there can be free passage of steam between the filter pads and plates and through the ports. As soon as the autoclave is opened after sterilization the filter is tightened and the ports closed. About 10 litres of sterile citrate saline solution are now run through the filter via the inlet. This cools it very rapidly and prevents the formation of a vacuum, which would result from condensation of the steam, with the consequent risk of contamination by air leaking in.

Seitz Filtration of Pulped Plasma.—Up to three filtrations a day can be done with a single filter press. In practice it is convenient to have two filters, which are used alternately throughout the day. While one filter is in use the other is being sterilized. When required for filtration about 18 litres of plasma is placed in a tin-lined copper pressure-vessel connected to the filter press by rubber tubing. The cap over the outlet valve of the press is removed, and the outlet is connected, with aseptic precautions, to two sterile 10-litre bottles. The plasma is forced into the filter by air pressure of approximately 9 lb.

per sq. in., applied through the compressed-air line installed throughout the plasma laboratories. The remainder of the citrate saline solution used for cooling the filter comes through first and is collected in one of the 10-litre bottles. As soon as plasma begins to come through the outlet is connected with the second bottle, a third sterile bottle being substituted for the one containing citrate saline solution. About 8 litres of plasma is collected in each bottle. Two to three litres remains in the filter, and is blown out and included in the next batch.

The whole operation must not take more than twenty minutes, and not more than 16 litres can be passed through 14 pads if clotting of the plasma is to be prevented, although this amount represents only 3 c.cm. of plasma per sq. cm. of filtering surface.

Bottling the Filtered Plasma

A sterile siphon is fitted to the 10-litre bottle A, containing the plasma (Fig. 3). The plasma is filled into M.R.C. blood bottles, B, using air pressure at about 2 lb. per sq. in. (When using large glass vessels—e.g., 10-litre bottles—

FIG. 3.—Method of bottling the filtered plasma.

under pressure or vacuum, it is advisable to enclose each vessel in a strong wooden box.) Each of the M.R.C. bottles containing 10 c.cm. of citrate saline solution is autoclaved with the metal cap screwed down tightly. Details of the method of sterilization are given below. In readiness for filling, the cap of each bottle is removed in turn, the neck is flamed, and the bottle is placed immediately under the dome C, in such a way that the disturbance of air is minimal. The spring clip controlling the flow of plasma is then released, the bottle is filled, the neck is again flamed, and the cap is replaced.

The construction and the sterilization of a dome or hood with its attachments have already been described. Apparatus of this type is very effective in preventing contamination of filling-nozzles and of the contents of opened bottles.

Sterility Tests on Human Plasma

For many months samples were taken for sterility tests at the start and the end of filling M.R.C. bottles from each 10-litre bottle. Each sample was tested aerobically and anaerobically, using glucose broth and meat broth respectively. Approximately 1 c.cm. of plasma was added to about 300 c.cm. of culture medium. The inoculated bottles of culture media were incubated at 37° C. for five days and inspected daily. Stained films were made from any bottles not remaining clear. In addition to the above sterility tests, every bottle of plasma was incubated for five days and examined during and at the end of this period. This provided a check on the sterility, since the plasma which remained clear was probably sterile.

It was eventually decided that the sterility tests were of little value, because it was found that in several cases a high proportion of the bottles of plasma were obviously contaminated after five days at 37° C. despite the fact that no organisms were detected in the samples taken for sterility tests. It is now our practice to fill the bottles with plasma as soon as it has been filtered and to seal them immediately with a viscap; the latter step was adopted when it was found that air could pass in and out of some of the bottles despite the apparently undamaged condition of the screw cap and rubber liner and the fact that the cap was screwed down tight. The plasma is then incubated for five days at 37° C. and inspected daily, an operation which is facilitated

by strip lights at the back of the shelves in the incubator. After removal from the incubator the plasma is stored at room temperature for fourteen to sixteen days, and inspected frequently. In this way it is possible to detect and eliminate contaminated bottles whether the organisms grow better at 37° C. or at a lower temperature. Occasionally a whole batch is found to be contaminated, but in general the amount of plasma lost through contamination is small.

Some Practical Details of Sterilization

The open ends of all tubing and other apparatus are covered with cotton-wool protected by parchment paper held by rubber bands. Blood bottles are prepared for use by washing with hot water and soap. After a thorough rinsing under the tap they are finally rinsed with distilled water, and 10 c.cm. of citrate saline solution (1% sodium citrate and 0.85% sodium chloride) is added.

For many months it was our practice to sterilize the bottles with the caps loose and covered with parchment paper. After autoclaving, the bottles were allowed to cool in the autoclave and the caps were screwed down as the bottles were removed. This method is efficient but rather time-consuming. Recently we have carried out a number of tests to check the efficiency of different methods of sterilizing bottles. 10 c.cm. of broth heavily contaminated with hay dust and *B. subtilis* spores was added to each of a number of blood bottles; these were divided into three groups. Some were plugged with cotton-wool, others were loosely capped and covered with parchment, and the third group had the caps screwed down tightly. All these bottles except two controls were autoclaved, and then incubated for five days at 37° C. and kept for a further sixteen days at room temperature in accordance with our routine procedure. In no instance was there growth in autoclaved bottles, while the control bottles gave copious growth overnight.

In consequence of these tests we now sterilize the bottles with the caps screwed down tightly. This effects a considerable saving of time and autoclave space, as the bottles can be removed before they are cold. A few bottles are found to have a vacuum inside, and these are discarded on account of the risk of contamination on opening. The method described above is very useful for checking the efficiency of other sterilizing processes, wet or dry. For example, we have applied it with satisfactory results to 10-litre bottles and siphons used in filling serum or plasma.

The 10-litre bottles are sterilized by a method similar to that used for blood bottles. 50 c.cm. of citrate saline solution is added to each bottle before sterilization, and the necks are plugged with non-absorbent cotton-wool and covered with parchment.

In autoclaving apparatus it is usual to pass free steam for twenty minutes and then to increase the pressure to 15 lb. per sq. in. (temperature 121° C.), which takes about ten minutes. The pressure is maintained at 15 lb. for thirty minutes, after which the autoclave should be left for at least another hour to cool. Immediately after removal from the autoclave all sterile apparatus is labelled with the details of sterilization. This prevents mixing with unsterile material. It is also advisable to keep records of the contents of the autoclave during each run and of the times of sterilization.

Immediately before the bottles are passed into the sterile filling-room they are swabbed with 0.5% phenol in water.

Preparation of Blood Bottles for Issue.—When the bottles of plasma have passed sterility tests they are labelled with (1) the batch and bottle number and date of filtration; (2) a warning in red not to store at a low temperature—a condition which favours the clotting of plasma once it has been filtered.

Preparation of Human Serum for Issue

Recently plasma has been largely replaced by serum, which is in some ways easier to handle, as clotting should not occur; hence the amount which can be passed through a filter in a single run is much larger. Delayed clotting does happen in some samples, however, and is a source of much trouble during filtration.

The serum is separated by various methods at the depots, the average yield being about 40% of the blood volume. The pooled serum is passed through a pulp filter and a filter press in the same way as described for plasma, with the important modifications that two sterilizing pads are used between each plate of the filter press, that a batch consists normally of 80 to 100 litres, and that a pressure of not more than $4\frac{1}{2}$ lb. per sq. in. is employed to force the serum through the press. As filtration can be carried out more slowly than for plasma, the serum is filled aseptically straight from the filter through two hooded pipettes. The rate of flow through the filter can be adjusted to a speed convenient to two fillers.

We are at present trying various modifications in this technique, including the use of clarifying pads in place of the pulp, and also of a pressure pulp with a very much larger surface area. The clarifying pads or the pulp precede the sterilizing filter and are connected in series with it, making the whole process continuous in an almost closed system and reducing the risk of contamination.

After every fifteenth bottle is filled a sample of serum is taken and tested for sterility by the method described for plasma. In the event of contamination of one or more bottles the tests are repeated on other bottles from the batch; the bottles are numbered in sequence, and the next bottles in the series are usually taken for sterility tests. If this second test also shows contamination the entire batch is discarded. It is unwise to re-filter the serum, as soluble bacterial toxins may be present which might produce undesirable reactions on injection.

It has been found undesirable to incubate all the bottles of serum, as this causes some change in the lipid distribution, leading to a slight cloudiness in the serum on subsequent storage. Incubation at 37° C., which is used for plasma, is therefore replaced by storage of the serum at room temperature for three weeks before issue. In our experience serum is more difficult than plasma to obtain sterile, but so far we have been unable to discover the reason. Another difficulty in the case of serum is that a number of bottles of serum, though sterile, showed changes varying from a slight deposit, detectable only by careful inspection in a good light, to general opalescence and even precipitation. It is extremely difficult, by visual inspection, to differentiate this phenomenon from contamination. The cloudiness generally begins to develop after about fourteen days, though it has been observed after much shorter and much longer periods. This problem is at present under investigation. The evidence so far points to the cloudiness being due to soaps apparently formed by the splitting of fats by serum lipase followed by combination with the calcium present.

The batch number and filtration date are entered on the issue label, which is affixed to all bottles of serum when the results of sterility tests are known.

Premises and Staff

The ground floor of a building 60 ft. by 40 ft., with an annexe 18 ft. by 15 ft., has been adapted for the preparation of plasma and serum for issue. There are pressure and vacuum lines in the processing-rooms. A large readily accessible incubator and cold room and abundant accommodation for storage at room temperature are essential for

the efficient working of the unit. The annexe contains three autoclaves and one steam sterilizer. Rooms for filling and other sterile work are fitted with large hatches with inner and outer doors, through which sterilized equipment can be passed into the room without using the normal entrance; air currents carrying bacteria into the room are thus avoided. The floors and benches of any room in which sterile manipulations are carried out are kept moist during the period of work, so as to allay dust. The bacterial content of the air is further reduced by the use of a "dynalysor" or other mist spray in the neighbourhood of the operation. Windows and, so far as possible, doors are kept shut. Outside the doors are placed mats in trays containing weak antiseptic to reduce the numbers of organisms carried in on the special canvas slippers worn by the staff. Only workers experienced in sterile manipulations are permitted in these rooms, and they change into sterilized gowns, caps, and slippers before entry; where possible a small ante-room should be used as a dressing-room. Hands and forearms are washed thoroughly. Transparent masks are comfortable to wear over nose and mouth, and are of considerable value in preventing droplet infection; the type which is bent backwards under the chin has the advantage that it presents a mechanical barrier to spray directed downwards as well as forwards.

Records

It is essential that a very complete record of every manipulation should be made at the time, and that all entries should be initialled by the individual responsible. Cards with headings of the information required are an advantage for ready reference, and they may be cross-checked with the details recorded in notebooks. The data considered important will of course vary from time to time. Thus, for many months prothrombin and protein estimations were made for every batch of plasma; but these examinations showed little variation between different pools, and were discontinued.

Bacteriological Notes

A few hundred bottles of blood have been examined for sterility on arrival from the depots. The contamination rate has varied from about 5 to 25% in different series: the former is probably a representative figure for present-day conditions of working with improved technique.

When the serum is withdrawn at the depots it is sent to us in Winchester bottles. As is inevitable, a small proportion of these bottles have become infected: the contamination rate during the past two months has been approximately 1%. As it is possible that some of the organisms present produce soluble toxins, we now examine all bottles for sterility and use only serum or plasma which is either sterile or only very slightly contaminated. The contaminants found in plasma and serum before filtration are various cocci and coccobacilli, and occasionally organisms of the *B. subtilis* group, *Pseudomonas pyocyanea*, diphtheroids, yeasts, and moulds.

It is unfortunate that the ideal preservative for plasma and serum has not yet been found (MacKay, 1941). Phenol and its derivatives are out of the question, as they are too toxic when several pints of plasma or serum containing antiseptic have to be injected. Merthiolate 1 in 10,000 is relatively non-toxic and does not affect serum proteins, but it does not prevent the growth of *Ps. pyocyanea*, which has appeared in filtered plasma on four occasions. Other organisms, including various cocci, coccobacilli, and moulds, have also grown after an interval in the presence of this antiseptic. Merthiolate is therefore regarded as untrustworthy, although undoubtedly it has considerable bactericidal and bacteriostatic properties. Its presence

must not be allowed to give rise to a false sense of security, and every possible care should be taken in handling material containing it.

Summary

Methods used for the production of human plasma and serum on a large scale are described.

Plasma is separated either by an Alfa-Laval separator or by suction after the cells have settled. It is then pooled in 10-litre bottles for convenience in handling.

Serum is separated by various methods at the blood supply depots and is sent to us in bulk for filtration and bottling.

The plasma and serum are clarified by passage through sterile paper pulp and are then sterilized by Seitz filtration.

Technical details are given of the methods employed to prevent contamination during the various stages of preparation and to ensure sterility of the final products.

Methods similar to the above have been used in and described from various laboratories. In the early days of the work information was freely exchanged between Dr. R. I. N. Greaves, Dr. W. S. Stanbury, Colonel L. E. H. Whitby, and ourselves, and it is impossible always to establish where particular processes originated. Bushby and Whitby (1941) have published an account of a similar procedure, but it was thought that a description of our methods might be of interest.

Our thanks are due to Dr. C. G. Anderson and Mr. J. G. C. Campbell for their helpful suggestions and criticism.

REFERENCES

- Bushby, S. R. M., Buttle, G. A. H., and Whitby, L. E. H. (1940). *Lancet*, 2, 131.
— and Whitby, L. E. H. (1941). *J. R.A.M.C.*, 76, 4.
Della Vida, B. L., and Dyke, S. C. (1941). *Lancet*, 1, 564.
Jakobowicz, R., and Bryce, L. M. (1941). *Med. J. Austral.*, 1, 318.
Loutit, J. F. (1941). *British Medical Journal*, 1, 729.
MacKay, M. E. (1941). *Ibid.*, 1, 747.

THROMBOCYTOPENIC PURPURA COMPLICATING ARSENOBENZENE THERAPY

BY

SYDNEY M. LAIRD, M.D., B.Sc., F.R.F.P.S.G., D.P.H.
Major, R.A.M.C.

Blood dyscrasia complicating arsenobenzene therapy, may, according to McCarthy and Wilson (1932), belong to one of three groups—the thrombocytopenic, the granulocytopenic, and the aplastic. While borderline cases do occur, haematological examination usually permits the placing of any particular patient with symptoms of blood dyscrasia in one of these three categories. Such classification is not merely of academic interest, but points the way to correct treatment and sound prognosis. This paper, in addition to recording another case of arsenical thrombocytopenic purpura, seeks to assess the present-day position of this serious complication of antisyphilitic treatment.

Case Report

The patient, a well-developed man aged 30, started anti-specific treatment on April 9, 1940, at which time he presented clinical evidence of early secondary syphilis, the Wassermann reaction and Kahn test being positive. The first course of treatment was irregular, and by July 25 he had received only three intravenous injections of neoarsenobenzene, five intramuscular injections of sulpharsenobenzene (total 5.4 grammes), and four injections of a bismuth preparation. The second course was started on November 13, at which time the Wassermann reaction and Kahn test were still positive. During this course he received six weekly injections of sulpharsenobenzene intramuscularly (3.45 grammes) and 1.4 grammes of bismuth, the last injection being administered on December 27. On reporting at the clinic on January 1, 1941, for treatment, purpura was noted, and on the following day he was admitted to hospital under my care.

On admission ecchymosis was widespread over the trunk and limbs, being most pronounced on the flexor aspects of the extremities and over the hip-joint areas. The purpuric patches varied from petechiae to large areas of ecchymosis, and there

was a subconjunctival haemorrhage affecting the left eye. Blood was oozing profusely from the gums around all the teeth, and this may have accounted for the marked melaena noted in the stool on the following day. Gross haematuria was present, but there was no evidence of haemorrhages into joints or pleural cavities, nor enlargement of liver, spleen, or superficial glands. The temperature, pulse, and respirations were within normal limits. Apart from discomfort and inconvenience from the bleeding gums, the patient had no subjective complaint and felt well.

Treatment comprised rest in bed, a milk diet, abundant fluids, the juice of two oranges daily, and a mouth-wash of liq. ferri perchlor. every three hours. In addition, 6 c.cm. of 10% calcium thiosulphate solution was given intravenously on admission and repeated the following day. On January 4 the patient's condition remained unchanged, and 1,000 mg. of ascorbic acid was administered by the intravenous route. The next day the clinical condition was still unchanged, and daily injections of calcium thiosulphate were resumed. On January 6 improvement began, the haematuria and bleeding from the gums being noticeably less. It was obvious on the 7th that the purpuric manifestations were abating rapidly, and on the 8th the bleeding from the gums and the melaena had ceased. There was no haematuria on naked-eye examination. From this time no further treatment was given, and convalescence proceeded rapidly, with early fading of the purpuric areas. The subconjunctival haemorrhage remained stationary for a considerable time, but eventually cleared up completely. The results of various examinations of the blood are shown in the table.

Table showing the Results of Blood Examinations

	3/1/41	7/1/41	13/1/41	23/1/41	28/1/41
Haemoglobin ..	102%	88%	89%	96%	—
R.B.C. (millions) ..	5.18	4.17	4.33	4.19	4.85
W.B.C. ..	9,200	5,200	6,100	5,700	—
Platelets ¹ ..	30,00	41,000	70,000	157,000	274,000
Bleeding time ² ..	+ 15 min.	—	9.5 min.	3.5 min.	—
Clotting time ³ ..	3 min.	—	3 min.	—	—
Clot retraction at 24 hours ..	Almost absent	—	Normal	—	—
Capillary fragility ⁴ ..	absent	—	30	—	8

¹ Estimated by the method of Evans (1928).

² Estimated by Duke's method.

³ Estimated by the method of Lee and White.

⁴ Expressed as the number of petechiae per unit area, at standard pressure and time.

Discussion

Aetiology.—The aetiology of the blood dyscrasias following arsenobenzene therapy is not yet clearly understood. In the granulocytopenic and aplastic groups impairment of bone-marrow function is obvious, but the actual mechanism responsible remains in doubt. All the arsenical preparations used in the treatment of syphilis contain a benzene radical, and the action of the latter in causing depression of the bone-marrow function is readily seen in chronic benzene poisoning. The prevailing view, which incriminates the benzene group, is supported by this analogy, but experimental evidence (Lancellotti, 1938; Montanaro, 1938) is inconclusive, and suggests that, although the arsenobenzenes can produce a small transitory depression of the haemopoietic tissues in animals, other factors, such as idiosyncrasy or sensitization, are necessary to bring about the agranulocytic or aplastic complications of antisyphilitic treatment in the human being. Wheelihan (1928) has reported granulocytopenia in a child after treatment with an inorganic arsenical preparation (potassium arsenite), but in this case infection may have played an important part in producing the depression of bone-marrow function.

Purpura develops only after a considerable number of injections, which in many cases have been received at irregular intervals, and this has led to the view that some sensitization mechanism produces the increased capillary fragility. The influence of sensitization is well illustrated in the cases described by Falconer, Epstein, and Mills (1940), who administered very small doses of arseno-