flagellates, and (*iii*) that flies thus 'blocked' with heavy leishmania infection, although they attempted to feed when given an opportunity, were unable to draw any blood, despite very violent efforts to do so; during these efforts it was certain that portions at least of the flagellate block became detached and entered the wound made by the probosis.

Whilst it could truly be said that none of these observations was entirely new (*e.g.*, the standard method of keeping mosquitoes alive is by feeding them on raisins and the method has been previously applied to sandflies, the observations that subsequent blood-meals knocked out the flagellate infection had been made by Patton, a quarter of a century ago in his bed-bug experiments, and the 'blocking' had already been observed histologically by the workers of the Kala-azar Commission with reference to this sandfly and has of course long been recognized in the flea in plague transmission), yet Smith's perfection of the technique was undoubtedly the most important step in providing the final proof that the sandfly *P. argentipes* can transmit the disease to man; the next two steps followed naturally when opportunity arose.

Smith, who was now able to produce these blocked flies more or less at will, first placed them to feed on hamsters, and obtained a very high infection rate amongst his experimental animals, and he would in the ordinary course of events have conducted the final experiments with human volunteers, but was recalled to military duty. This experiment was entrusted to Swaminath, one of the earliest and most constant workers on the kala-azar transmission problem, who, with the administrative assistance—and no doubt expert technical advice—of H. E. Shortt and L. A. P. Anderson, Inspector-General of Civil Hospitals and Director of the Pasteur Institute, respectively, in Assam where these experiments were being conducted, repeated Smith's experiments with human volunteers. Six volunteers, who lived in a hill district in Assam where kala-azar does not occur, were selected, and infected flies were fed on them; a paper that is at present in the press reports that at least three of these volunteers have contracted kala-azar. The hypercritical might say that this does not prove that this is the way kala-azar is *always* transmitted. It is true that this isolated experiment would mean nothing more than that it *can* be transmitted this way, but taken in conjunction with all the rest of the observations that have been made on this disease, every scientist will, we believe, agree that this experiment has removed the last objection to the already generally accepted conclusion that the *usual* method of transmission of kala-azar is by the agency of the sandfly, *P. argentipes* in India and other allied species in other countries.

An attempt has been made to give a consecutive account of the investigations that led to the final solution of one of the few outstanding problems regarding the transmission of the major tropical diseases. A very fair criticism would be that only work in India has been mentioned. Very important and valuable work on this problem has been done by a number of foreign workers, for example, the Sergent brothers' very suggestive experiments with sandflies and oriental sore in North Africa in 1921, which were confirmed and developed by Adler and Theodor, the later work of Adler on kala-azar in Europe in which the *P. perniciosus* was incriminated as the vector, the collateral work in China by Young, Hertig, Meleney, Patton, Hindle, Faulkner, Zia, Yung Sun, and many others, which showed that *P. chinensis* of the *P. major* group of sandflies was an efficient carrier, and the more recent observations by Kirk and others in the Sudan; all this work has encouraged the Indian workers and led them to believe that they were working along the right lines, but it actually started no new lines of thought, and it bridged no gaps in the road to the final goal, as far as the transmission of kala-azar in India was concerned.

At least three of the active supporters of the sandfly hypothesis have not lived to see their beliefs justified; during the last ten years, Young, Knowles and Acton have died. Though the last-named took little active part in the work on kala-azar, his belief, based analogously on his observations on oriental sore in Iraq, that the sandfly was the transmitter inspired all these who were associated with him.

Special Article

FILTERS AND FILTRATION

WITH SPECIAL REFERENCE TO THE FILTRATION OF BLOOD PLASMA AND SERUM FOR TRANSFUSION PURPOSES

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Introduction

WITH the starting of blood banks in India certain commonplace laboratory procedures, such as filtration, have assumed considerable importance. Located as India is at present with her usual supplies from abroad cut off, workers in blood banks in this country are faced with the problem of obtaining suitable filters for their work. While drawing up proposals for the blood bank in Calcutta, it was found that use could not to be made *in toto* of filtration procedures adopted by blood banks in the West for want of appropriate material. Various alternative methods and materials had to be thought of. In connection with that a review on filters and filtration was prepared and in the hope that persons interested in filtration will find it useful it was submitted for publication.

Definition

Filtration is the process by which solids suspended in liquids and in an extended sense solids or liquids suspended in gases, are separated from one another by means of a false partition. This partition is called a filter and it blocks the passage of suspended matter while allowing the fluid in which it is suspended to pass through.

Filters

It is common knowledge that there are many kinds of filters in the market prepared from a variety of material. Some are simple while others are slightly more complex, but each type has its own special use. In order to be able to choose correctly the type of filter required for any particular work one must know the different basic materials used in making the different types of filters, and their advantages and disadvantages.

The materials commonly used in making filters are cotton, paper, glass, asbestos, sand, clay or other forms of earth, plaster of paris, charcoal, collodion and gelatin. A brief description of these materials and of the filters made from them and their uses are given below :—

Cotton.—Cotton is the carbohydrate, cellulose. On account of its filamentous nature, it is used as filter in one of two forms—(*i*) as cotton-wool and (*ii*) as woven cloth.

Cotton-wool is prepared in two forms—absorbent and non-absorbent. While absorbent cotton is a useful clarifying agent for liquids both the absorbent and the non-absorbent types are useful for filtering gases. The familiar cotton-wool plugs in test-tubes containing media for bacterial growth in fact act as bacterial filters for the air that enters the tubes.

Cloth, lint and gauze are forms of woven cotton which are sometimes used as filters for clarification and under suitable conditions they yield filtrates which are quite clear.

Recently, Bushby and Whitby (1941) have employed a patent preparation called 'perfecta pulp' for clarifying blood plasma by removing the suspended fat globules. It is said to be made of pure cotton.

Paper.—Paper, like cotton, is a form of cellulose. Unsized paper, *i.e.*, paper which has not been glazed, has a porous texture and is in principle filter paper. But good filter paper of constant porosity is prepared by more refined methods. Papers of graded porosities designated by numbers are on the market. The numbers are given by the makers and with each number its uses are also given. The number of paper chosen will depend upon the purpose for which it is needed.

Apart from the ordinary kind of filter paper, special forms are also available for special purposes. For example, ordinary filter paper is acted upon by strong acids and alkalies, and therefore a special kind of filter paper which is resistant to these has been manufactured. This is produced by changing cellulose into a gelatinous form called 'amyloid' which is resistant to acids at ordinary temperature. It is however relatively less porous. Parchment paper is also prepared in a similar manner, but it is gelatinized to a larger extent. As yet there is no method of standardizing this gelatinizing process to obtain different porosities. If that could be done, excellent bacterial filters could be made out of gelatinized paper. There are also filter papers specially prepared for other types of work. For example, a fat-free form is prepared for use in fat estimations and an ashless form in gravimetric chemical analysis. Special types are also available for filtering oils called 'Postlip thick grey' and for colloidal material called 'Chardin'.

Paper pulp is also used in filtration. It is supplied in the form of compressed slabs. These are broken up in hot water and ground up until the mass is of an even soft consistency. For use, the pulp is placed into a Buchner funnel of which the holes are covered by ordinary filter paper and by gentle hand pressure and suction the pulp is made into a pad. Such a filter is efficient in clarifying protein solutions.

Glass.—Glass, a mixture of silicates of calcium and sodium, is used in filtration in the form of glass wool or sintered glass discs. The former is a very familiar form and is used for rough clarification. Its main advantage is that it can be used with impunity with acids and alkalies. Glass wool is not difficult to prepare. A process known as 'the bow and arrow process' is employed in making it. In this process heated glass in the plastic stage is shot through with a bow and arrow type of arrangement and the long thin filaments formed are wound round a drum. By keeping the drum rotating all the time a continuous stream of glass fibres is drawn out from the plastic glass.

Sintered glass filters are also available in the market. These filters are made by spreading powdered glass of uniform degree of fineness into a uniform layer over mica plates and then heating them in ovens to a high temperature to enable the pieces just to fuse into a porous plate. This process is known as sintering. The surfaces of these plates are ground rough with emery and cut into proper discs which are later fused into suitable holders. Filters of varying porosities are available and recently some bacterial filters have also been produced. These have an average pore diameter of 0.9μ which though very much larger than the smallest bacteria (0.2μ) serve well as bacterial filters in practice, probably due to the clogging of the pores during filtration. The firms employ a code designation for the porosity of these filters. On the filter is stamped one letter between two figures, as for example, 1G3. The first figure indicates the type and size of the filter, the capital letter the quality of the glass and the last figure refers to the porosity of the filter disc. Sometimes the letter 'P' is added to the designation of the filter. This means that the filter disc is ground plane and polished. For bacteriological work the filter is designated G5 on 3 which means that 2 discs of porosities 5 and 3 are fused one over the other. These filters are easy to clean and sterilize and can withstand temperatures up to 120° and 150°C.

Asbestos.—Asbestos is a double silicate of calcium and magnesium. It occurs in the form of large sheets consisting of layers of long crystals. It has a filamentous texture and can be processed into a woolly consistency or ground into a pulp. This asbestos wool or pulp can be used as such or after being pressed into pads. Pads of asbestos incorporated with paper pulp are sold by the German firm of Seitz in two grades of porosities, K and EK; K for clarification and EK for bacterial filtration. They are available in different sizes—3 cm., 6 cm. and 14 cm. in diameter. There are also American and English equivalents of these pads. Suitable apparatus for mounting these pads for filtration are available in two types—(*i*) Manteufel and (*ii*) Uhlenhuth. These filters (particularly the latter) are becoming extremely popular in bacteriological work on account of their efficiency, cheapness and rapidity of action. They are also easy to handle and sterilize. It is this type of filter that is used in plasma and serum work both for clarification and sterilization. Further data regarding these filters are given later.

Sand.—Sand, the oxide of silicon, acts in nature as a filter when water percolates through the soil. It is used in the purification of community water supplies. The sand itself does not account for the whole of the filtering efficiency; it is the biological deposit of slime, that forms on the top of sand that acts as a true bacterial filter.

Clay.—Clay, the silicate of aluminium, when baked and unglazed as procelain is much used in filtration. The popular form in which porcelain is used as a filter is a hollow tube, thimble or candle. Chamberland, a French worker, was the first to use them and later Pasteur, and hence the name, Pasteur-Chamberland filters. They are prepared in different porosities, designated in increasing order of fineness, as L_1, L_1bis, L_2, L_3, L_5, L_7, L_9, L_{11}, L_{13}. The L_3 candle is the commonest in use for bacterial filtration. The English equivalent is known as the Doulton filter, the German as the Massen filter, and the American as the Allen filter. These are made in one porosity only, approximating to Chamberland L_3. Recently porcelain discs held between rubber gaskets in a metal holder called Jenkin's filters have come on to the market.

Keiselguhr.—Keiselguhr is a siliceous earth of diatomaceous origin obtained from the Keiselguhr mines. The story of the value of this earth for filtration is interesting. It was first found that water from the Keiselguhr mines was of excellent quality and bacteria-free. Investigation revealed that the earthy bed through which the water came was responsible for its bacterial purity. Use was made of this earth for the preparation of bacterial filters and these worked very efficiently. Filters made from this material were given the name of Berkefeld after the owner of the mines. They are shaped as hollow candles fitted to a metal nozzle and are sold in three grades of porosities, V, N and W. V or *viel* meaning coarse, N or normal, and W or *wenig* meaning fine. The English Berkefeld Company supplies filters of one grade only. These filters are extremely fragile and cleaning of the candles is a laborious and unsatisfactory procedure. There is also the danger of a leak developing at the junction of the candle with the metal nozzle. The American equivalent of these is known as Mandler filters, and the composition of these is slightly different. They are made of Keiselguhr, asbestos and plaster of paris. The proportions are varied to give three porosities which are graded according to the air pressure they withstand in water. They are called 'preliminary' (2 to 5 lbs.), 'regular' (6 to 9 lbs.) and 'fine' (10 to 16 lbs. per sq. inch). The Mandler filters are said to be stronger than Berkefeld.

Plaster of paris.—Plaster of paris, the semihydrate of calcium sulphate, becomes plastic on wetting with water and later sets. Recently, Kramer filters made of commercial plaster of paris either alone or combined with different quantities of magnesium oxide have come on to the market. They have been used for special purposes in which filters carrying a positive charge are required.

Charcoal.—Charcoal has been used in filtration, specially in connection with the clarification of sugar solutions and oils. In water purification for domestic purposes charcoal is, at times, employed.

Collodion.—The earlier filters made of collodion were those prepared by impregnating collodion on filter paper, but these filters were not of uniform porosities, nor could they be exactly reproduced when needed. The latest type of collodion filter is the Elford membrane. This is prepared from a solution of collodion in a mixture of absolute alcohol, anhydrous ether, acetone and amyl alcohol. The mixture is poured on to special plates and allowed to evaporate spontaneously under standard weather conditions and the film produced allowed to jellify by the addition of water. The resulting membrane is of uniform porosity and exactly similar membranes can be reproduced by employing the same identical procedure. The porosity of a membrane can be varied at will by the addition to the collodion solution of varying quantities of water to increase the pore, or acetic acid or ethylene glycol monomethyl ether, to decrease the pore. The actual pore size of any prepared membrane can be determined by noting the rate of flow of water through it and applying Poiseuille's equation. These membranes are sufficiently strong to stand handling and pressure and are of great use in certain types of bacterial and ultra-filtration work.

Gelatin.—The use of gelatin as a filter material is now obsolete. But it is of historical interest in that many of the earlier investigations on ultra-filtration were carried out by depositing it on filter paper.

Purpose of filtration

It is common knowledge that filtration is widely employed for a variety of purposes in several branches of science. So far as bacteriology is concerned it is used at least for four purposes :—

(1) To obtain fluids free from bacteria as in the filtration of water for drinking purposes which is done with the object of removing bacteria and suspended matter.

(2) To obtain bacteria free from the fluid in which they are suspended as in the isolation of pathogenic bacteria from water samples where filtration is done with the object of concentrating the organisms in the filter as a preliminary to their isolation.

(3) To separate organisms of different types from one another as in the filtration through collodion membrane of fluids containing a mixture of bacteria and viruses for keeping back bacteria but allowing the viruses to pass through.

(4) To clarify material used in bacteriological work as in the filtration of culture media which is done with the object of removing gross particles and obtaining filtrates clear to the eye.

In serum and plasma filtration in which we are primarily interested, filtration is done with the object of 1 and 4, *i.e.*, sterilization and clarification. No doubt, theoretically it should be possible to avoid filtration of serum and plasma by the adoption of a strict aseptic technique and a closed system of collection, pooling and bottling. In fact, Dr. Strumia of the Bryn Mawr Hospital, Pa, advocates the aseptic closed technique and does not find any necessity to filter the plasma or serum he prepares. But most others in the West, as well as ourselves here, feel that, despite the adoption of a closed system and all-care in technique, contaminative bacteria do get introduced at some stage or other, and that these have to be removed. Under the conditions prevailing in India and particularly during the summer months, we find filtration essential and cannot be avoided.

Equipment required

In order to carry out filtration, four main things are required as shown in figure 1. These are (1) the filter and its holder, (2) the vessel for holding the fluid to be filtered, (3) the receptacle for collecting the filtrate, and (4) a mechanical pressure system which will ensure proper rate of filtration.

Choice of equipment

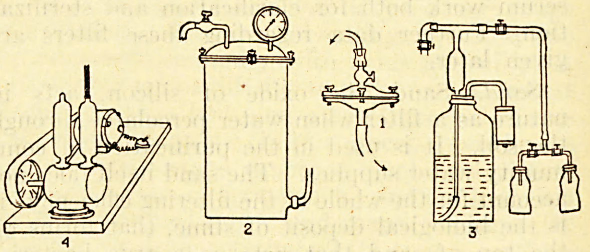
The choice of equipment for any filtration work will depend upon two important considerations—(*i*) the purpose of filtration, and (*ii*) the quality of the filtrate required.

As our main interest lies in the filtration of blood serum and plasma it may be pointed out that it is done for the purpose of clarifying and sterilizing the serum. And as regards the quality of the filtrate the requirements are that it should be free from bacterial and chemical contaminants and also have a chemical composition as near to its original as possible. From these two points of view let us consider the choice of equipment.

Choice of filter

A suitable filter is the first requisite for conducting successful filtration. From the long list of filters already given it will be clear that we have a wide range of filters to choose from for the two purposes mentioned. For clarification we can choose cotton-wool, gauze, cloth, lint, filter paper, paper pulp, asbestos wool or pads, glass wool, sintered glass, charcoal or sand. And for sterilization we can choose special types of filters, such as, Pasteur-Chamberland, Doulton,

Fig. 1.



Massen, Berkefeld and Mandler, Seitz or Kramer filters. But before making the choice we will also have to take into consideration the chemical composition of the fluid to be filtered, its pH, its viscosity, and the nature of the filtrate we wish to obtain. English and American workers are using Perfecta pulp for clarification and Seitz filter for sterilization. But in view of the fact that these are not available in India, it will not be out of place to say a few words as to what these are and what substitutes can be used in their place.

Perfecta pulp is a patent article sold by Messrs. Becker & Co., in cakes about 12 inches × 12 inches × $\frac{3}{4}$ inch, weighing $1\frac{1}{4}$ lb. each and priced at 1*s.* 6*d.* per cake. The exact method of manufacture of this pulp is not known, but from the description in the catalogue it seems to be made out of 'pure cotton without any admixture'. It should not at all be difficult to manufacture this locally. Further, for clarification purposes for which it is used, suitable substitutes can be improvised. It is a familiar practice in protein laboratories to use filter paper pulp for clarification. This pulp is made by kneading pieces of filter paper with water and then pressing a pad of them on Buchner funnels.

It would be worth while experimenting with such a substitute and determining its efficiency. Theoretically it should prove successful. Since writing this article experiments conducted at the A. I. I. H. have revealed that paper pulp made as above and sterilized by autoclaving is quite effective for clarification. In fact the Institute blood bank uses it regularly for clarification of serum.

Seitz Pilot filter.—The Seitz Pilot filters are special large types of Seitz filters which can take 20 cm. square filter pads. Into these 8 sets of paired pads (a clarifying Ford FCB pad followed by a bacterial Ford S.B. pad) can be fitted. Its advantages are : (*i*) that a large filtration surface is obtained and 140 litres of serum can be filtered in one day using a filtration pressure up to 12 lb. per sq. inch, and (*ii*) that both clarification and sterilization can be effected in one operation. These filters are not available in India and there is no knowing when they will arrive even if indents are placed. Although there are advantages in using these instead of the ordinary Seitz filters which are smaller, yet the latter will prove, if properly used, equally efficient. Another advantage in using a Pilot filter is that positive pressure can be employed in working it. Though the ordinary Seitz filters are meant for working with negative pressure, the Uhlenhuth models can be used for filtration both under negative and positive pressures. In fact it is this type that we have recommended for filtration of serum in the Institute.

The pads used in Seitz filters are of German manufacture. There are also English and American substitutes of these. India had a very poor stock of German pads when the war broke out. Since then all available pads have been sold out. Fortunately the Institute has stocked a fair quantity of the pads. But they may not last long, specially as large numbers of these will be used in serum filtration. Thus the problem of obtaining a large stock of pads still remains, specially as the English and American substitutes have not yet reached India. Under the circumstances it would be worth while considering the manufacture of these in India or at least attempting to renovate the used pads. Ordinarily the pads are rejected after being once used. A few preliminary experiments on renovating these pads, *i.e.* making new pads out of the cast off ones, have been conducted and there is every hope that it may prove successful. Provided the renovated pads are proved efficient for bacterial filtration, our temporary difficulty in obtaining these pads can be overcome.

In the choice of a filter the second factor to be considered is the quality of the filtrate to be obtained. Judging from the available reports on the use of serum and plasma filtered through Seitz Pilot filters, we find that there are two objections to the use of these filters. One is the adsorption by the filter of some of the important constituents of the plasma and the other is the presence of magnesium ions in the filtrate due to contact with the Seitz pads. As regards the first objection, it may be pointed out that all filters, other than glass and collodion filters, will adsorb a certain amount of the constituents in the serum, but that is not a very serious objection. As regards the second objection, it is important specially as the presence of magnesium ions in the serum has been held responsible for certain types of post-transfusion complications. These objections naturally raise the question as to whether other types of filters cannot be used with equal efficiency for serum and plasma filtration. It seems worth while investigating the possibility of using collodion or glass filters which approach nearer to the mechanical sieve. At least on theoretical grounds these should prove more satisfactory. They will certainly overcome the two objections raised against Seitz filters to a large extent.

Choice of vessel for holding the fluid to be filtered

In serum work as large quantities need to be filtered, suitable vessels for holding the fluid and feeding it to the filter are required. The ideal vessel for this purpose is one made of glass. Even where positive pressure is used glass bottles that can withstand the pressure would be the best. While some English workers use special aspirator bottles of 10 litres capacity (Greaves *et. al.*, 1939), others (Bushby and Whitby, 1941) use copper containers. It is generally agreed that as far as possible it is best not to use metal containers for fear of contaminating the serum. Tinning or enamelling of these containers have been resorted to, to reduce the risk of metallic contamination. In America, they use monel metal receptacles. The only objection to using glass is that it may break easily and may not be able to stand high pressures. If metal containers are used they must be properly tinned or enamelled. They must also be fitted with pressure gauges and air-tight covers which can be held down by wing nuts. All joints should be protected wherever necessary by rubber washers. The one in use at the Institute is made of copper and tinned inside.

Choice of receptacles for receiving and storing filtered serum

As regards the choice of receptacles for collecting the filtered serum here again glass bottles of good quality fitted with air-tight screw caps are the best and they should be carefully chosen. The bottles recommended by M.R.C. are 'U.G.B. medical flat bottles' of 12 ounces capacity, while the R.A.M.C. workers recommend 'pint blood-transfusion bottles'. If bottles of the above specifications are not available in India due to war conditions, suitable substitutes may be used after proper testing. In the Institute, bottles made by Scientific India Glass Co., Calcutta, according to army standard pattern are used. These are of non-alkaline glass, 540 c.cm. capacity and fitted with metal screw caps inlaid with rubber and having wire suspension hooks to keep the bottles in an inverted position during transfusion.

Choice of mechanical pressure system

As regards a mechanical pressure system all types of filtration do not require this. The gravitational force of the column of fluid in contact with the filter is, in itself, sometimes sufficient to effect complete filtration. Even in such cases the application of pressure renders filtration easier and more rapid. In some cases, due to the high viscosity of the fluid to be filtered and due to the fineness of the pores of the filter, filtration is very slow or is impossible unless pressure is applied. In such cases either positive pressure on the input side of the filter or a negative pressure on the output side can be applied.

For obtaining positive pressure one or other type of air pumps is used. There are in the market several types of such pumps. Mention may be made of the following: (1) The ordinary cycle pump giving up to 5 to 10 lb. pressure. (2) The bigger types of hand pump giving up to 20 lb. pressure. (3) The various types of foot pumps giving up to 40 lb. (4) Gas cylinders containing compressed N_2 giving up to 80 lb. (5) The rotary compression pumps giving up to 100 lb. or 200 lb. To any of these can be fitted a metal drum with a pressure gauge and regulator and from these the desired pressure can be got from one pound upwards.

As for obtaining negative pressure there are various types of exhaust pumps and mention may be made of the following: (1) The ordinary filter pump which can give a vacuum of about 25 mm. of mercury. (2) The hand Geryk pump giving a vacuum of about 0.02 mm. of mercury. (3) Rotary pumps single stage giving a vacuum of about 0.005 mm. of mercury. (4) Rotary pumps double stage giving a vacuum of about 0.00001 mm. of mercury. (5) Diffusion pumps using mercury or oil giving a vacuum up to 0.0000001 mm. of mercury.

In ordinary routine bacteriological work negative pressure pumps of the Geryk type are generally used, but in the filtration of plasma and serum there are certain advantages in using positive pressure, the most important of which are (*i*) avoidance of frothing of the filtrate, (*ii*) lesser chance of aerial contamination, and (*iii*) greater facility for bottling of the filtered product. It is on account of these that positive pressure is used instead of negative pressure. Here it may, however, be pointed out that when positive pressure is used the filtered serum or plasma will contain a larger percentage of dissolved gases.

For obtaining positive pressure one of the compressor types of rotary pumps giving a pressure of 25 lb. or more is recommended. This pump should also be capable of being regulated at will and being maintained without large fluctuations at any required level of pressure. With such a pump it has been found that rapid filtration of large volumes of plasma or serum can be effected by adopting the following procedure: The filter is started at 5 lb. pressure per sq. inch and the pressure is then gradually raised over a period of an hour to about 9 lb. per sq. inch. Filtration is allowed to proceed at this level until the speed begins to slacken (which is usual in less than 4 hours) and then the pressure is further raised by steps of $\frac{1}{2}$ to 1 lb. up to a maximum of 14 lb. per sq. inch in such a way as to keep the flow approximately constant. At the Institute a 'Kellog' compressor is available. The compressed air, before use, is passed through a bottle containing heavy machine oil and glass beads, then through a sterile filtration 'F' Pasteur-Chamberland candle and finally through a sterile cotton filter 12 inches long.

Filtration room

Apart from choosing the right type of apparatus required for filtration, it is also necessary to have a specially constructed dust- and draft-free room with a concrete floor, where filtration can be conducted under as sterile conditions as possible. This room should be so partitioned as to ensure a lobby, an outer room and an inner room. The lobby is to be kept as clean as possible but no attempt is made to sterilize it. But the outer and inner rooms have to be kept sterile. The lobby is used for changing into sterile garments, the outer sterile room for filtration and the inner sterile room for bottling and capping.

The question as to whether these rooms should be air-conditioned or not, has been raised, particularly in the tropics. In cold countries it is not done as it is not very uncomfortable to work in such closed draft-free rooms. But in the tropics, specially in summer, when 3 to 4 people have to work for a few hours inside these closed chambers, the question assumes a different colour. Although the process of filtration itself is not going to be greatly affected one way or another by air-conditioning, the comfort of the workers would certainly be affected. But it should be remembered that as these rooms have to be kept absolutely draft- and dust-free during filtration, either the air-conditioning will have to be stopped during the operation or the room in which the closed chambers are located will alone be air-conditioned or arrangements will have to be made for cooling the chambers without draft, or using cooled sterile air. At the Institute the air put in is 100 per cent fresh air which is cooled and filtered through a 'replacement filter' and again through a 'Visco filter'.

Preparation of apparatus for filtration

This may be considered under three heads— (*i*) sterilization of the apparatus, (*ii*) sterilization of gowns and caps, and (*iii*) care of the filtration room.

1. Serum being an ideal medium for the growth of organisms it is imperative that all

apparatus coming in contact with it must be sterile. Sterilization of these is effected by steam at 15 lb. pressure for 40 minutes to one hour. Before sterilization they are properly cleaned and wrapped in muslin or cloth and packed in copper boxes to facilitate handling. During sterilization the cover of the box is raised slightly on one side to allow steam to enter, and it is closed when the autoclave is opened. The sterilization is done a day before filtration. If 48 hours or more elapse, the apparatus should be re-sterilized.

2. As sterile gowns and caps are worn by workers during filtration, these garments are placed in muslin bags and sterilized in the same way as the apparatus.

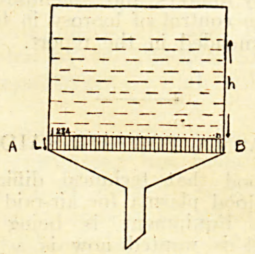
3. The room in which the serum is filtered must be kept clean and free from dust. It should always be subjected to a thorough preliminary cleaning on the day preceding filtration. Steam at 5 to 10 lb. pressure is blown in from a small autoclave for about 20 minutes to one hour. This produces a dense fog in the room which settles by the following morning. Immediately before filtration the table tops and other flat surfaces are wiped off gently with a sterile towel dampened in 1 per cent phenol or dettol solution. Check is kept on the efficiency of this sterilization by exposing a few blood-agar plates on the table while filtering and bottling are in progress.

Mechanism of filtration

It was stated previously that the chief function of a filter is to intercept particles in suspension or dispersion. Its action, therefore, from a theoretical point of view, may be considered as that of a mechanical sieve, but in reality it is not such a simple mechanical process. Due to the physico-chemical properties of the fluid undergoing filtration and of the material composing the filter the mechanism of filtration is a very complicated one. The theory of filtration may be explained briefly as follows :—

Imagine, as in figure 2, a filter AB with n straight pores, 1, 2, 3, 4, . . . n exposed to a

Fig. 2.



liquid standing above the filter at a height of 'h'. If the liquid is free from suspended particles, the rate of its passage through the filter is given by the classical formula of Poiseuille :—

$$V = \frac{\pi p r^4 t n}{8 L \eta}$$

where 'V' is the volume of liquid of viscosity η flowing through 'n' pores of average radius 'r' and length 'L' under a pressure head of 'p' in time 't'.

But since in a filtration the liquid contains suspended particles which are steadily being deposited on the filter and as a result the pores are steadily getting diminished in size, the above equation indicates the state of things at any given instant of time only.

Furthermore the filtration process in practice does not follow this simple rule because it is never true that the pores of a filter are straight and uniform and usually the form of the filter is such that it does not expose all filter pores to the filtration process to the same extent. Also the physico-chemical properties of the fluid including the suspended particles in it and of the material of which the filter is made influence the process; and particles much smaller in size than the pores of the filter are retained not by virtue of the impenetrability of the pores, but by electrical and other forces brought into action by virtue of the physico-chemical conditions prevailing. Thus, in certain cases even substances in solution may be retained by filters during filtration. It is on account of these reasons that when serum or plasma is filtered not only the bacteria and the suspended particles in it but also some of the important chemical constituents are removed from it.

Factors affecting filtration

From the discussion on the mechanism of filtration it will be clear that several factors other than the size of the filter pore determine whether in any particular instance filtration will be successful or not. The factors affecting filtration according to Rivers (1928) are :—

1. The electrical charge of the fluid undergoing filtration.
2. The electrical charge of the particles suspended in it.
3. The chemical nature of the substances present in the fluid.
4. The concentration in which they are present.
5. The pH of the fluid.
6. Presence or absence of electrolytes, oils, etc.
7. The temperature of the fluid.
8. The electrical charge of the filter.
9. The degree of pressure employed.
10. The duration of filtration.

In the filtration of biological fluids all these factors have got to be considered. In the case of plasma and serum many of the factors pertaining to the fluid to be filtered are known and are fairly constant. We need to be cautious only about (*i*) the choice of the filter, (*ii*) the pressure to be used in filtration, and (*iii*) the duration of filtration. Of these three factors the first two have already been dealt with and only the third remains to be discussed.

The period over which filtration can be allowed to proceed in any particular case is an important matter to decide. In filtration of protein solutions it is well known that the quality of the filtrate varies with time. It may improve with time in certain respects while deteriorating in other respects. This is true particularly of bacterial filtration of serum and plasma. In the earlier stages due to adsorption by the filter of important constituents the quality is poorer with respect to some of them; but later as the filter gets saturated the quality improves. But in the case of plasma long-continued filtration may lead to early clotting of the filtrate. Again, from the bacteriological point of view, if a filter is used over a long period bacterial growth may take place in the filter and pass by contiguity into the filtrate. On account of these clashing influences an optimum time period has to be fixed. In the case of serum and plasma 4 to 6 hours are the upper limit.

Conclusion

By way of conclusion it may be added that filtering is a process fraught with many possibilities of error and although filters of all varieties are guaranteed by the makers no filter can be called absolutely fool-proof. The process is by no means a simple matter and does not depend entirely on the size of the pores and the amount of pressure applied. Various physico-chemical processes come into play and only through a proper appreciation of these, can successful results be achieved. As such there is need, specially in an important matter like serum filtration for transfusion purposes, for the filtration to be carefully controlled by one who understands the subject, and can avoid its many pitfalls.

REFERENCES

Bushby, S. R. M., and Whitby, L. E. H. (1941). *J. Roy. Army Med. Corps*, **76**, 4.

Greaves, R. I. N., Gilson, H. C., and MacKay, M. E. (1939). *Notes on Production of Dried Human Serum for Transfusion Purposes.* Medical Research Council Serum Drying Unit, Cambridge.

Rivers, T. M. (1928) .. *Filterable Viruses.* Baillière, Tindall and Cox, London.

BIBLIOGRAPHICAL

Morton, H. E. (1938) .. *Amer. J. Clin. Path.*, Tech. Supp., **2**, 185.

Medical News

SUGGESTIONS FOR FIGHTING LEPROSY IN INDIA

A comprehensive report on the leprosy problem in this country has been made by the Committee appointed by the Central Advisory Board of Health to report on leprosy and its control in India.

The census figures, according to the report, give no true picture of the prevalence of leprosy. The 1931 census reported 150,000 cases, but leprosy surveys show the true incidence to be, on an average, about eight times this figure. In some highly infected areas the incidence may be from 5 to 10 per cent of the population surveyed.

While the problem of leprosy is primarily that of the disease in the general population, its presence amongst beggars causes much concern to the public. From the public health standpoint the problem of beggars with leprosy should not be ignored and the opinion that almost all such beggars are not infective is incorrect. The problem is most acute in Calcutta, Madras and Bombay.

The problem, it is suggested, needs to be tackled from several angles. Migration might be minimized by each province providing for its own leprosy cases. A profound change in public opinion is required to prevent the assembly of beggars with leprosy at festivals. There is a great need for a popular movement commanding the support of the public and of Indian religious and charitable organizations to work towards this end and for spending this money in constructive work, such as the provision of leprosy colonies, hospitals and clinics. A large increase in institutions for the isolation of infective patients is required.

An urgent need is the improvement of the teaching in leprosy given in medical schools and colleges; every doctor should know how to diagnose and treat leprosy and what precautionary measures should be taken against its spread. The report also gives a description of a model provincial leprosy institution and recommends that, in every province where leprosy is common, such an institution should form the hub of anti-leprosy activities.

The Central Advisory Board of Health has commended the report to all Provincial and State Governments in the hope that the various recommendations will materially assist them in the formulation of a co-ordinated policy for anti-leprosy work, with special emphasis on the preventive aspect of the problem.

While emphasizing the need for public co-operation the Board considers that provincial and local authorities should aim at providing for the isolation of those persons who are in an infective stage of the disease. In anti-leprosy propaganda more use should be made of schools and teachers' training institutions. The Board also recommends the establishment of a 'Leprosy Institute of India', the appointment of provincial leprosy officers and amendment of existing legislation for the control of leprosy in the light of the principles recommended in the report.

BLOOD PLASMA FOR AIR-RAID VICTIMS

It is understood that technical difficulties in the preparation of blood plasma for air-raid purposes have been overcome. Equipment is being manufactured locally and what is wanted now is a good response from the public to blood donor services in the Provinces and Indian States.

In a number of centres in India blood plasma is already being prepared. Dry blood plasma manufactured abroad is also being supplied to India.

The manufacture of dry blood plasma requires an elaborate unit which has recently been perfected in the United Kingdom and the U. S. A. Two dry blood plasma plants are on order from abroad and an experimental one is under construction in India.