THE PREPARATION OF LIQUID HUMAN PLASMA BY THE KAOLIN PROCESS

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The British Pharmaceutical Codex 1949 includes monographs on human blood and blood products. It is also proposed to include standards for these in the next Pharmacopœia or in an Addendum to the present Pharmacopœia (Report of the British Pharmacopœia Commission to the Pharmacopœia Committee of the General Medical Council, May, 1950)[1].

The Codex Monograph on liquid human plasma states that it is the liquid portion of whole blood. The plasma is removed from the red cells by aseptic methods and mixed aseptically with plasma from other samples of whole blood. Pooled human plasma so obtained is normally opaque due to the presence of fat and, on storage, clots and particles of fibrin may also develop. The cloudy appearance of the liquid makes it difficult to distinguish between sterile and infected plasma.

Two methods of treatment are available. Firstly, the plasma may be dried from the frozen state as soon as possible after collection. The alternative is to sterilise the pooled plasma by filtration through sterilising asbestos pads and this yields a clear fluid which, should it become contaminated, is self-indicating. The filtration of plasma through an asbestos filtering medium is made difficult by the activation of the plasma prothrombin by contact with the asbestos (Bushby and Whitby[2]). Clotting occurs in the filtered fluid and later, on the surface of the pads and filtration comes to an end.

Several methods are available for removing fibrinogen and prothrombin from plasma. McFarlane[3] has developed an ether-freeze process for the removal of fibrinogen and the unstable lipoid-globulin complex from plasma. This method, for technical reasons, has not yet been fully developed for large scale use. Fibrinogen may be precipitated by the addition of acids or by heating at 55°C. Both these methods need careful control during the process, and even then, precipitation is not always complete and the resulting fluid is not easily cleared by filtration. In addition, the fluid does not store well. Clegg and Dible[4] have suggested the addition of excess of calcium to the citrated plasma to remove fibrinogen. Our experience using this method has not been uniformly successful and the calcium content of the plasma is increased much above the physiological level. Maizels[5] has described a method using kaolin for the adsorption of fibrinogen and prothrombin and suggests that the product is less likely to produce toxic reactions than material untreated with kaolin.

A modification of the process suggested by Maizels has been used in our laboratories and is briefly as follows:— 400 g. of kaolin B.P. is weighed into a wide-mouthed bottle of 5 l. capacity. To this 400 ml. of

distilled water is added and well mixed. The addition of the water is necessary to ensure efficient sterilisation of the kaolin in the autoclave. The flask is closed with a two-holed rubber stopper fitted with suitable glass tubing and is then sterilised at 121°C. for one hour. After pressure in the autoclave has been released, 15 inches of vacuum is applied in order to dry the kaolin as much as possible. Plasma (approximately 4 l.) is withdrawn under aseptic conditions from 16 bottles of blood into each of the kaolin bottles. The pooled plasma and kaolin are well mixed by repeated shaking during 15 minutes and then stored frozen at −10°C. for not less than 5 days.

When required for processing, the plasma is allowed to thaw at 4°C. When completely thawed out, it is gently mixed and allowed to stand at 4°C. for 24 hours to allow the kaolin to settle. The supernatant is first clarified by passage through asbestos pads of clarifying quality and then sterilised by passage through sterilising asbestos pads in a frame filter. The sterile plasma is filled aseptically, in 400 ml. quantities, into sterile transfusion bottles. The processed plasma is incubated at 20°C. in a dark cupboard for 21 days. Before issue each bottle is carefully inspected for the presence of foreign bodies and bacterial or fungal contamination.

Plasma prepared in this way is clear and, being practically free from fibrinogen and prothrombin, will not form fibrin clots on storage for periods of about 4 to 6 months.

Standards for Liquid Plasma. The B.P.C. gives standards for liquid human plasma which include appearance, preparation, protein content and sterility. It is with the protein content of kaolin-processed plasma that this paper is mainly concerned. The standard laid down for protein is not less than 4·5 g. per cent. This is also the standard required by the Therapeutic Substances Act, 1925; Amendment Regulations, 1948. The object of the experimental work described below is to determine whether, by using the kaolin method, this standard of protein content can be attained.

Experimental

The protein content of pooled plasma was determined before and after treatment with kaolin and after clarification and sterilisation by filtration. All protein estimations were made by a modification of the micro-Kjeldahl method.

Method. 4 ml. of plasma was diluted to 100 ml. with water and carefully mixed. 5 ml. of this dilution (= 0·2 ml. of plasma) was pipetted into a micro-Kjeldahl flask. To this was added 0·2 ml. of a 7·5 per cent. solution of sodium molybdate and 0·2 ml. of 2/3 N sulphuric acid. The mixture was centrifuged in the flask for 15 minutes at 2,000 r.p.m. The supernatant liquid was decanted off and the flask allowed to drain on filter paper. Approximately 0·2 g. of potassium sulphate, 0·2 g. of copper sulphate, 1 ml. of concentrated sulphuric acid and a few glass beads were added to the flask which was then heated on a hot-plate until charring ceased and the liquid had a clear green colour. The flask was then fitted

to the micro-Kjeldahl distillation apparatus with the condenser delivery tube immersed in 15 ml. of N/70 sulphuric acid. 10 ml. of water and 5 ml. of 40 per cent. sodium hydroxide solution were then added and distillation by steam was carried out until the volume in the receiver had been doubled. The unused sulphuric acid was titrated with N/70 sodium hydroxide and the protein content of the sample calculated on the basis: 1 ml. of N/70 sulphuric acid = 0·2 g. of N and total protein per 100 ml. = ml. of N/70 sulphuric acid × 0·625.

Blank estimations on the reagents were carried out and any necessary adjustments were made.

Results. The results of estimations on 4 pools of plasma are shown in Table I. The figures are the mean of two estimations at each stage.

TABLE I

	Protein (g. per cent.) in Pool No.				Average (30 pools)
	1	2	3	4	
Raw plasma before treatment with kaolin ...	5·40	5·25	5·40	5·30	5·34
After treatment with kaolin	3·75	3·99	3·94	3·63	3·80
After filtration through clarifying pads ...	3·78	3·94	3·78	3·75	3·85
After filtration through sterilising pads ...	3·19	3·56	3·38	3·56	3·45

It will be seen from Table I that 1·54 g. per cent. of protein is lost by adsorption on the kaolin. Estimations of the protein on the kaolin sludge were carried out. Results corresponded to 1·40 g. per 100 ml. of plasma (i.e., 91 per cent. of the total adsorbed protein). The results shown in Table I have been confirmed by many estimations made during the routine processing of plasma.

Discussion

The use of kaolin-processed liquid plasma has a number of advantages. Firstly, the end product is liquid and does not require reconstitution with sterile water before use. Secondly, it is self indicating for sterility, and in addition, it is stable when stored for reasonable periods at room temperature.

The main disadvantages are in the lowered protein content, and in the fact that during transport over long distances there may be some precipitation due to breakdown of the lipoid-globulin complex. It may be inferred from the B.P.C. Monograph that the kaolin process is an "approved method." The results shown above, however, would indicate that plasma prepared by the kaolin process does not satisfy the official requirements for protein content. The official minimal protein content (4·5 per cent.) has, presumably, been decided upon using dried raw plasma as the standard.

The loss of 1·54 g. per cent. of protein on the kaolin is more than can be accounted for in terms of fibrinogen of which the average plasma content is stated to be 0·3 g. per cent. Whilst prothrombin and fibrinogen are selectively adsorbed by the kaolin there is evidence that other proteins are adsorbed at the same time. This fact has been pointed out by Maizels[5]. It is interesting to note that passage through the clarifying pads does not remove protein, but passage through the sterilising pads results in a further loss of 0·35 g. per cent. This may be accounted for by the much higher asbestos content of the sterilising pads. If the kaolin process is to be considered as a suitable method of treatment for plasma, then the official standard for protein in the finished product may have to be adjusted.

The kaolin process is a useful and safe method for preparing human plasma for use and it has been suggested by Maizels that pyrogens and other toxic substances may also be removed by the kaolin. The work of Chute and Vaughan[6] would appear to support this theory.

SUMMARY

1. The kaolin process for the preparation of human liquid plasma is described.

2. Results are given for the estimation of the protein content of the fluid at the various stages of the process. A loss of protein to the extent of 1·54 g. per cent. by adsorption on the kaolin is shown. A further loss of 0·35 g. per cent. is sustained by passage through sterilising asbestos pads.

3. It is suggested that, if the kaolin process is to be considered an "approved method," then the minimal standard for protein content of the fluid should be revised.

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REFERENCES

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2. Bushby and Whitby, *J.R. Army med. Cps.*, 1941, **76**, 4.
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4. Clegg and Dible, *Lancet*, 1940, **239**, 294.
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6. Chute and Vaughan, Rep. to Blood Transfusion Research Committee M.R.C., 1943.

DISCUSSION

The paper was read in abstract by Mr. G. M. TODD.

MR. G. SYKES (Nottingham) said that he was surprised at the enormous loss of protein on passing the plasma through the sterilising pads. He calculated that 14 g. of protein had been lost. That might be accounted for by the use of a large number of pads, but he imagined that not more than 1 or 2 14-cm. pads would be used to filter 4 litres of solution and it was difficult to believe that such an enormous amount could be

adsorbed on such a comparatively small pad. He wondered whether those figures could be accounted for by the fact that the whole 4-litre volume would not be filtered in the work reported, but only a comparatively small fraction. It was important from the manufacturing point of view because they were continually faced with the problem of the adsorption of all sorts of drugs, from protein to simple salts. In the case of the clarifying pads, there was comparatively no loss.

MR. F. H. OLIVER (Sunderland) asked whether the authors had any information on the ratio of globulins to albumins, because plasma was used largely for restoring the volume of the blood, and, since the albumins were more useful for the capillaries than the globulins, the proportion of globulins would be very important.

MR. G. R. MILNE (Glasgow) said that the B.P.C. monograph on whole blood gave details about the collection of blood into anti-coagulant solutions, and mentioned specifically three types of anti-coagulant. The first was 3 per cent. solution of trisodium citrate using 100 ml. to 440 ml. of blood. The second was 2 per cent. solution of sodium acid citrate with 2·5 per cent. of glucose, 120 ml. to 420 ml. of blood, and the third was a solution of 1·6 g. of trisodium citrate, 0·56 g. of citric acid and 1·5 g. of glucose, in 75 ml. which is used with 500 ml. of blood. In Glasgow, they used 100 ml. of the second solution with 440 ml. of blood. If one took the B.P.C. recommendation of 120 ml. of solution to 420 ml. of blood and assumed (1) that the volume of plasma to the volume of red cells was in the ratio of 55:45, which was normal, and (2) that the average protein content of human plasma was 6·75 per cent., then the amount of protein in the diluted plasma would be only 4·44, which was below the B.P.C. limit of 4·5, which was also given in the Therapeutic Substances Regulations. The other two methods were satisfactory, and gave figures of 4·77 and 5·4 respectively. The quality of kaolin needed investigation, as its adsorptive properties varied, although it satisfied the requirements of the British Pharmacopœia. With regard to the adsorption on the asbestos pad, the filtration of 4 litres of plasma had been described, but in point of fact they were filtering as many as 8 such quantities through a frame filter which would contain anything from 8 to 12 asbestos pads. There was no doubt that the ratio of the area of asbestos pads to the amount of fluid chemical being filtered was very important. They had satisfactorily prepared solutions of thrombin on a small scale, but when they tried larger quantities they lost the thrombin in the process, by adsorption on the large area of the filter pad. They had, therefore, reverted to the use of smaller pads which took more time, but the ratio of filter pad area to the volume being filtered was extremely important. The asbestos content of the pads was also an important factor and their quality was now being improved.

MR. G. M. TODD, also replying, said that the size of the pads used was 400 sq. cm. They had not yet done any work on the globulin-albumin ratio, but it was on their programme.