STUDIES ON THE BERKEFELD-FILTRATION OF COMPLEMENT.

By HANS SCHMIDT.

(*From the Bacteriological Department, Lister Institute, London.*)

Introductory.

In view of the divergent results obtained in recent work dealing with the effect of Berkefeld filtration on the haemolytic complement of serum, I think that the publication of the results which I have obtained during some investigations, which I have been carrying out on the inactivation of complement by shaking, may not be without value.

Muir and Browning (1909) found that by filtration through a Berkefeld filter the complement of a guinea-pig serum is retained by the filter to a large extent—sometimes indeed completely,—but if more serum is filtered it passes through. Neufeld and Andrejew (1909) and Andrejew (1910) found that out of all immune bodies in serum, complement is the most completely retained by filtration through Kieselguhr. Andrejew showed further that the relative loss of complement was considerably increased when the serum was previously diluted, no more complement passing through if the serum was diluted 1 : 10. A detailed investigation of the filtrability of complement was then undertaken by P. Schmidt (1911, 1912). He confirmed the observation of Andrejew in regard to the effect of dilution. His explanation was that the colloidal complement ferment, which he considers to be an entity, becomes less dispersed by dilution and therefore more easily retained by the filter. If undiluted, the serum proteins, especially the albumins, have a protecting influence upon the complement, which passes through the filter in the course of prolonged filtration, because the albumins cover the whole Kieselguhr

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surface, thus shutting off the adsorbent filter surface from the complement. When he filtered the same serum repeatedly through the same filter, he found a decrease of proteins corresponding with a steadily increasing complementing power of the filtrate.

The results of my first experiments proved to be very contradictory, and not until I had made the technique as uniform as possible did I get uniform results, which could be reproduced under given conditions. There is no doubt that in the filtration of serum an adsorption on the filter material takes place, and it is therefore evident that according to the relations between filter surface and the filtrans different results can be obtained. I think it therefore necessary to have the size of the filter stated as well as the quantity of the filtrans. The fact that these data are wanting in most papers, renders a comparison of the experiments difficult, and on the other hand may explain the divergent results which the literature contains. That *ceteris paribus* also, the velocity of the filtration, dependent on the pressure, the H·-concentration, and the presence of neutral salts play an important part, will be shown at the end of this paper.

Technique of experiments.

The Berkefeld candles employed were cylinders of about 40 mm. height and 14 mm. diameter. The filtration was directed from outwards to inwards and was effected by a suction water pump having a pressure of about 680 mm. Hg.

During filtration the whole surface of the filter was kept covered by the liquid. On every occasion the filter was cleaned by filtering a hot diluted solution of NaOH followed by distilled water until the filtrate was neutral to phenolphthalein. In those cases where the filter was not completely dried before use (dist. water or 0·85 % saline remaining in the filter) the first portion of the filtrate was rejected. The complementary function of the serum was tested for its haemolytic power in combination with sensitized sheep red corpuscles, of which a 2·5 % emulsion in 0·85 % saline solution was used. The haemolytic immune-serum was inactivated rabbit serum, the single lytic dose of which was 0·00125 c.c. for 1 c.c. of the red cell emulsion and 0·1 c.c. complement containing serum.

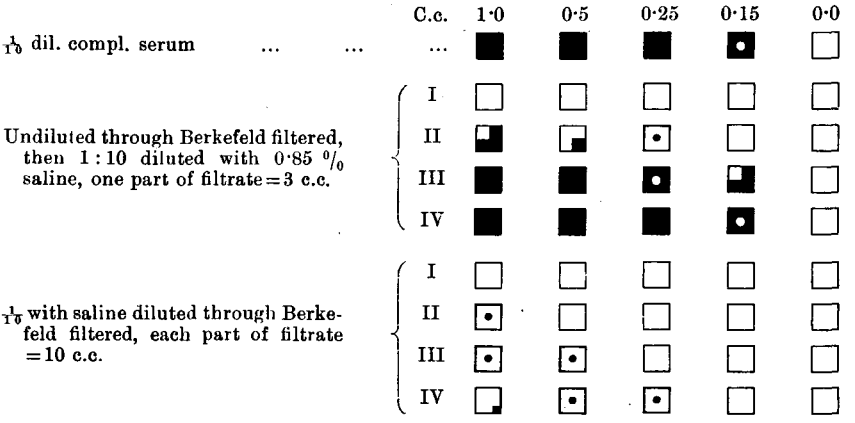
In the haemolytic tests the following schema has been adopted to illustrate different degrees of haemolysis.

- No haemolysis.
- Faint trace of haemolysis.
- Very slight haemolysis.
- Well-marked haemolysis.
- Half haemolysed.
- Strong haemolysis.
- Very strong haemolysis.
- Nearly complete haemolysis [a slight trace of unlaked cells at the bottom].
- Complete haemolysis.

Experimental part.

Exp. 1. Filtration of fresh guinea-pig serum undiluted and 1 : 10 diluted with 0·85 % saline through a Berkefeld candle (50 × 14 mm.).

Haemolysis of 0·5 c.c. sensit. red cell emulsion, after 1 hour at 37° and 15 hours at room temperature. AB dose = 0·0025.



Exp. 1 shows that the loss of complementing power of undiluted serum seems to be relatively small compared with the nearly total loss in the case of the serum which has been diluted 1 : 10 with saline. It will be observed however that the absolute amount of native serum which passes the filter after filtration of 40 c.c. of the diluted serum is less than the amount of undiluted serum which has passed through the filter during the filtration of 6 c.c. undiluted serum. This experiment when repeated always gave similar results indicating that the relatively smaller loss of complement in an undiluted serum is only apparent.

If according to P. Schmidt the complement is supposed to be a ferment and an entity, it will be adsorbed on the Kieselguhr surface either

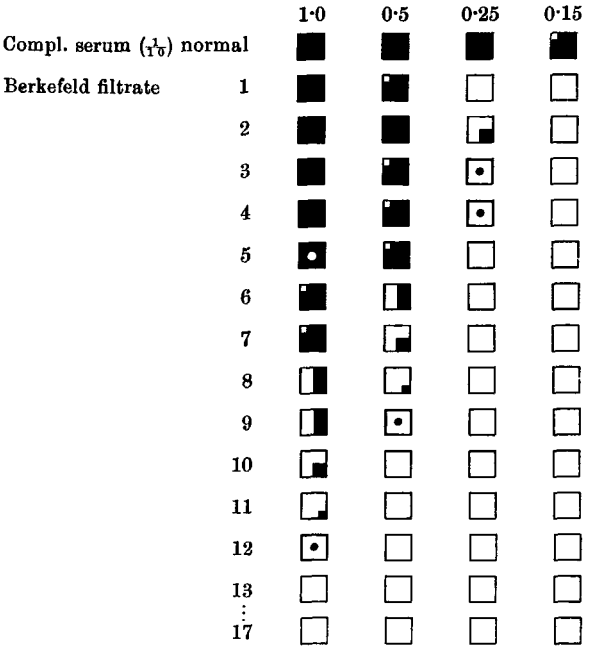
owing to its action on the surface tension or by electrical interaction [anomalous adsorption]. Adsorption is going on so long as the surface is not completely saturated, and saturation is more quickly reached in the case of undiluted serum. In the case of diluted serum much more liquid is required to saturate the adsorbing surfaces, so as to finally allow the complement to pass through. There is therefore no need to assume a lesser degree of dispersity of the complement ferment as an effect of dilution, for which there is also no other evidence. P. Schmidt supposes further that the complement already adsorbed on the filter surface will be freed in the course of the filtration by the protecting effect of the albumins, which accumulate at the filter surface. This seems to be difficult to understand, for there is no evidence that the albumins and the complement have a different action on the surface tension, and it is difficult to see why the albumins are not immediately adsorbed, thus preventing the complement from being adsorbed at all.

On the other hand it may be possible to assume that the complementing power of a serum is not bound on a certain substrate, but requires certain physical conditions of the serum, namely the relative state of the different serum proteins as regards their quantity and their degree of dispersity, which latter is closely connected with the amount of neutral salts present and the H·-concentration. If a serum which has complementing power is filtered through a Berkefeld filter, the whole filtering surface must first of all be changed by adsorption of proteins and its adsorptive power completely satisfied, before the serum can pass unchanged as regards its complementing power. This requires in a given filter a certain amount of proteins, and this condition of complete adsorption in the case of an undiluted serum is naturally more quickly reached. The first fraction of the filtrate is very poor in protein. With the increasing protein content the complementary function of the filtrate increases, finally reaching the original value of the serum before filtration. If such a serum is repeatedly filtered through the same filter, as P. Schmidt did 16 times, the protein content was found by him to steadily decrease, whereas the complement activity increased. The following Exp. 2 does not confirm this statement of P. Schmidt. But owing to the absence of detailed data in his paper the experiments can only be compared to a restricted degree.

Exp. 2. 30 c.c. of fresh undiluted guinea-pig serum was 17 times filtered through the same Berkefeld filter.

After each filtration 0·5 c.c. was taken and diluted 1 : 10 with 0·85 % NaCl.

Haemolysis after 1 hour at 37° and 1 hour at room temperature. 0·5 c.c. sensit. red cell emulsion. AB dose = 0·0025 c.c., the total volume being 1·5 c.c.



Exp. 2 shows that the complementing power of the serum, which is still well marked after the first filtration, is slowly but steadily decreasing with the number of filtrations. After the 12th filtration had been effected, the complementing power was lost and did not reappear on further filtration. The first part of the first filtrate was almost free of protein as in Exp. 1. Thereafter the velocity of adsorption is asymptotically decreasing, *i.e.* so long as filtration is going on there will be adsorption but finally to an indefinitely small extent.

From Exp. 2 either a steadily progressing adsorption of complement ferment may be assumed or the relations of the proteins may be steadily changing till the alteration is such that complementing power is no longer possible.

The saturation of the filter surface can also be effected by the previous filtration of a thermo-inactivated serum as has been done by Muir and Browning (1909). A similar experiment was made by Holderer (1912), who effected the filtration of diastase through a Chamberland candle after the previous filtration of an albumin solution.

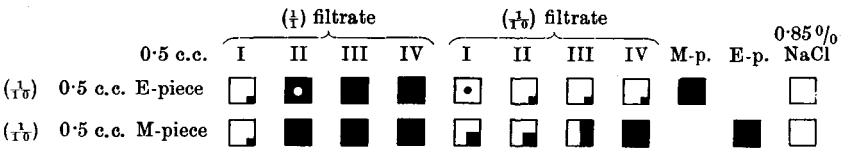
In order to decide whether the different proteins in the serum undergo a different adsorption on the Kieselguhr surface of the filter, a series of experiments was made to show how far an inactive Berkefeld filtrate can be reactivated by the addition of the serum fractions which have been obtained by the CO_2 method of Liefmann. For the sake of brevity I call the fractions M- and E-piece respectively, the M-piece containing the euglobulin and a relatively small part of the pseudo-globulin, the E-piece containing all the albumin and the main bulk of the pseudoglobulin.

Browning and Mackie (1912) found that no reactivation either by M-piece or by E-piece takes place, but later (1913) they found a restitution possible but not with regularity. P. Schmidt showed that a filtrate which was ineffective by itself could be reactivated by a small amount of fresh serum which, though inactive by itself, produced reactivation by a summation of effects. In accordance with his own conception that the albumin fraction contains always traces of the complement ferment, the main bulk of which is adsorbed on the surfaces of the euglobulin precipitated by dilution and CO_2, he concludes that a complete removal of the complement by means of adsorption on the filter is scarcely to be expected. The complement in the inactive filtrate is only in a state of subactivity. He found further that such a filtrate can replace an E-piece, *i.e.* that it can be reactivated by the M-piece.

Exp. 3. Reactivation of the filtrates of Exp. 1 by the fractions of the normal guinea-pig serum (CO_2 method).

Haemolysis after 1 hour at 37° and 18 hours at room temperature of 0·5 c.c. sensit. red cell emulsion.

AB dose = 0·0025 c.c. Total volume = 1·5 c.c.



Exp. 3 shows that in the case of the serum which has been filtered in the undiluted state no reactivation by any fraction can be obtained as long as the filtrate is inactive by itself. The filtrates of the diluted serum however could be reactivated by M-piece in an increasing degree, which means that with the progress of the filtration more albumin

passed through, which then increases the solubility of the M-piece. Such a Berkefeld filtrate acts therefore as an E-piece, just as P. Schmidt found. The addition of E-piece however to a Berkefeld filtrate of a diluted serum is not without effect. Either complement ferment passed through the filter in a state of subactivity, but becoming active by summation with the traces of complement ferment in the E-piece, or on the other hand the filtrate still contains globulins, which together with the globulins of the E-piece give a summation effect by the excess of albumin. In any way the restitution effect of the albumin fraction is very small.

In analogy with the result of Exp. 2, that in repeatedly filtering the same serum through the same filter a slow but steady decrease of complement occurs, the following Exp. 4 shows what happens if the same serum be filtered through a series of different filters.

Exp. 4. 20 c.c. of an undiluted fresh guinea-pig serum was filtered through a series of Berkefeld candles under similar conditions.

After each filtration 2 c.c. of the filtrate was removed, diluted 1 : 10 with 0·85 % saline and their haemolytic power as well as the effect of restitution by E-piece or M-piece investigated.

Control reactions with E-piece and M-piece worked satisfactorily.

Haemolysis of 0·5 c.c. sensit. red cells (AB dose = 0·0025 c.c.) after 1 hour at 37° and 15 hours at room temperature.

		1·0	0·5	0·25	0·0	1·0 c.c. E-piece + 1·0 c.c. filtrate	1·0 c.c. M-piece + 1·0 c.c. filtrate
$\frac{1}{10}$ normal compl. serum		■	■	■	□	■	■
Filtrate N_2O	I	■	■	■	□	■	■
	II	◪	◪	⊡	□	◨	◪
	III	⊡	□	□	□	◨	⊡
	IV	□	□	□	□	□	□

Exp. 4 shows that in this case the complementing power of the serum decreases rapidly with each filtration. The filtrate thus rendered inactive could not be reactivated by any means.

Filtration of the CO_2 fractions.

In connection with these experiments I undertook the isolated filtration of the CO_2 fractions and give in the following a record of some typical experiments.

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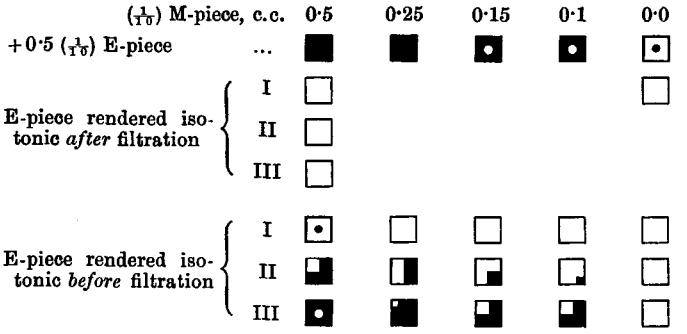
Filtration of E-piece.

Exp. 5. To decreasing amounts of M-piece diluted $\frac{1}{10}$ with 0·85 % saline 0·5 c.c. of filtered E-piece diluted $\frac{1}{10}$ is added.

Haemolysis after 1 hour at 37° and 1 hour at room temperature of 1 c.c. 2·5 % sensit. red cell emulsion. AB doses = 0·0025 c.c., the total volume in each tube being 2 c.c.



Exp. 5 shows first of all the fundamental difference in the filtrability of an E-piece whether rendered isotonic or not. The passage of the E-piece was impossible before the addition of salt. The salted E-piece behaved similarly to a complement serum in so far as filtration is concerned, that is, the longer the filtration the more it comes through.

Muir and Browning have already found that the permeability of the Berkefeld filter is increased by the addition of salt to the serum, thus rendering the serum hypertonic. The serum is then brought back to the isotonic condition by the dilution with water. This has been later confirmed by Manol and Nowaczynski (1910). The last mentioned authors succeeded in regaining the complement retained in the filter by filtering immediately afterwards a hypertonic solution of NaCl. Muir and Browning did not succeed in this experiment. It must however be mentioned that Manol and Nowaczynski worked with the Chamberland filter.

Before attempting to give an explanation of the part played by the presence of salts, I will refer to another experiment, which shows this phenomenon.

Exp. 6. Effect of Berkefeld filtration upon complement serum diluted 1 : 10 with distilled water.

Haemolysis of 0·5 c.c. sensit. red cell emulsion (AB dose = 0·0025 c.c.) after 1 hour at 37° and 15 hours at room temperature.

	C.c. 1·0	0·5	0·25	0·15
Guinea-pig serum after standing 24 hours, diluted $\frac{1}{10}$ with 0·85 % NaCl				
The same serum but diluted $\frac{1}{10}$ with aqu. dist. After 4 hours standing at room temperature, there was very marked cloudiness, which completely disappeared by rendering the serum isotonic				
The serum thus treated and rendered isotonic + 1·0 c.c. ($\frac{1}{10}$) E-piece				
The serum thus treated and rendered isotonic + 1·0 c.c. $\frac{1}{10}$ M-piece				
After 4 hours standing at room temperature and before being rendered isotonic, the serum was filtered through a Berkefeld candle and the clear-looking filtrate rendered isotonic				
The isotonic Berkefeld filtrate + 1·0 c.c. ($\frac{1}{10}$) E-piece... ...				
The isotonic Berkefeld filtrate + 1·0 c.c. ($\frac{1}{10}$) M-piece... ...				

Exp. 6 shows first of all the influence of the dilution with aqu. dist. upon a complement containing serum. As I have pointed out in another paper (1914) the inactivation phenomenon of Sachs and Teruuchi (1907) by 1 : 10 dilution with aqu. dist., and standing a certain time, occurs only in absolutely fresh serum. The serum in Exp. 6 was 24 hours old and was not yet inactivated after 4 hours standing at room temperature when diluted 1 : 10 with aqu. dist. The complementing power however was lessened. The complete restitution by E-piece and the absence of such an effect by M-piece are in favour of some alteration of the albumins. If the diluted and cloudy serum after 4 hours standing, and before being rendered isotonic, was filtered through a Berkefeld filter, the serum became clear, just as if it had previously been salted, but if the filtrate was rendered isotonic it proved to be inactive and incapable of being reactivated by E-piece. The filtrate however still contained traces of euglobulin because the passing of CO_2 through it gave rise to a slight opalescence. Only if the serum stood for many days diluted 1 : 10 with aqu. dist. did I succeed in completely filtering off the euglobulin. As in Exp. 5 the absence of a sufficient amount of salts inhibited the passage of the albumins, for the restitution by the addition of fresh M-piece to the filtrate proved to be very small, but still better marked than the effect of E-piece.

A comparison of the different effects of filtration through paper or through a Berkefeld filter upon the action of E-piece is given in the following Exp. 7.

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Effect of filtration upon E-piece.

Exp. 7. Guinea-pig serum after standing 24 hours in the cool room was diluted 1 : 8 with aqu. dist. and made acid by CO_2.

The precipitate was once washed and immediately before use dissolved in 0·85 % NaCl in 1 : 20.

The supernatant fluid (E-piece) was treated in the following way :

I. The supernatant fluid was rendered isotonic and remained otherwise untreated.

II. The supernatant fluid was filtered through soft filter paper and then rendered isotonic.

III. The supernatant fluid was filtered through hardened paper and then rendered isotonic.

IV. The supernatant fluid was rendered isotonic and then filtered through a Berkefeld candle.

Haemolysis of 0·5 c.c. sensit. red cell emulsion after 1 hour at 37° and 15 hours at room temperature. AB dose = 0·005 c.c., the total volume in each tube being 2·5 c.c.

$\frac{1}{20}$ M-piece	1·0	1·0	1·0	1·0	1·0	1·0	
NaCl sol.	0·0	0·5	0·75	0·85	0·9	1·0	Half saturation with
C.c.	1·0	0·5	0·25	0·15	0·1	0·0	ammonium sulphate
Of: I	■	■	◪	◨	⊡	□	Heavy precipitate.
II	●	●	■	■	⊡	□	,, ,,
III	■	■	◪	⊡	⊡	□	,, ,,
IV	□	□	□	□	□	□	Only a slight dimness occurs, the liquid being almost clear.

Exp. 7 shows that a Berkefeld filtered E-piece is rendered incapable of giving an haemolytic effect with M-piece. The filtration removes about all the pseudoglobulins, which the albumin fraction contains, while the filtration through paper does not affect the pseudoglobulins. This is demonstrated by the effect of half saturation with sulphate of ammonium. In connection with this it is of interest to note that Browning and Mackie (1914) found recently that the pseudoglobulin represents the main factor as far as the complementing haemolytic power of a serum is concerned.

In regard to the filtration of the isolated M-piece, Exp. 6 shows that already without the presence of sufficient salts the euglobulins, being in suspension, are retained in the filter. Only a very minute portion passes with the diluted serum, probably owing to the small

amount of salts and to the protecting influence of the serum albumins. The filtration of the M-piece diluted 1 : 10 in 0·85 % saline has an effect similar to that of the filtration of the salted E-piece.

Exp. 8. M-piece obtained by the CO_2 method from a fresh guinea-pig serum was dissolved by 0·85 % NaCl solution in 1 : 10, and then filtered through a Berkefeld filter.

Each fraction of the filtration was of a volume of 10 c.c.

Haemolysis of 0·5 c.c. filtrate + 0·5 c.c. $\frac{1}{10}$ E-piece + 0·5 c.c. sensit. red cell emulsion (AB dose = 0·005 c.c.) after 1 hour at 37° and 15 hours at room temperature.

$\frac{1}{10}$ M-piece Berkefeld filtrate	I	II	III	IV	V	VI	VII	VIII
+ 0·5 c.c. E-piece $\frac{1}{10}$ ...								

Exp. 8 shows that the more prolonged the filtration the more M-piece passes through the filter. It behaves therefore just as the salted E-piece or the whole serum under similar conditions.

The effect of filtration upon the isolated fractions however must not be taken to represent what occurs if the two mixed together are filtered as in the case of a serum. Apart from other factors the different influence upon the surface tension of the medium by the different proteins would be already sufficient to vary the effect of filtration.

Theoretical part.

In attempting to give an explanation of all the phenomena observed in the Berkefeld filtration of serum it must be remembered that the filtration consists of several different processes, namely :

1. The mechanical retention of particles.
2. The adsorption on the filter material, in so far as surface tension is concerned.
3. The phenomena caused by the movement of the liquid in the capillaries of the filter causing either anomalous adsorption or coagulation.

The mechanical retention of particles occurred in the above described experiments only when the euglobulin was in suspension. It is however easy to demonstrate by means of an ultramicroscope the presence of many particles in the filtrate which escaped retention, but in view of the relatively large size of the Berkefeld filter pores (J. A. Craw, 1908) this is to be expected. It is also possible that in using a dry filter

the rushing of the liquid in the capillaries is so great that suspended particles may be carried through capillaries which otherwise would hold them back. This is shown by Grenet (1910) to occur in the case of filtration of bacteria. On the other hand, some of these small particles may consist of the filter material which appear especially when new filters are employed.

A more important effect upon filtration of serum is exerted by the adsorption of the Kieselguhr surface of the filter. According to Gibbs' thermodynamic principle those substances in the serum which lower the surface tension are adsorbed. These latter are principally the serum proteins, the albumins and the globulins. F. Bottazzi (1912), M. J. Gramenitzky (1913) and other authors have shown that the serum albumins as well as the serum globulins lower the surface tension of water, but the latter in a lesser degree. This may be the reason for the protective influence which the albumins have upon the stability of the dispersity of the globulins. I give below some data showing the influence of the Berkefeld filtration upon the surface tension of the filtered liquid. The figures represent the number of drops given by a stalagmometer of Traube, and calculated as mentioned in my former paper (this *Journal*, 1913, p. 316).

Table showing influence of Berkefeld filtration upon S.T.

Dist. water	100·0
M-piece diluted 1 : 10 with 0·85 % NaCl	102·16
,, ,, ,, ,, filtered through Berkefeld filter	100·17

E-piece	Untreated	Filtered through Berkef.
diluted 1 : 4, not isotonic	104·30	102·41
,, 1 : 8, ,, ...	102·77	100·76
,, 1 : 10, isotonic	104·95	102·51

Serum diluted 1 : 10 with	0·85 % NaCl	Aqu. dist.
,, untreated	103·67	101·57
,, heated ½ hour at 56° C.	106·87	106·38
,, filtered through Berkefeld filter ...	103·06	100·56

The table shows clearly that substances which lower the surface tension are kept back by filtration, thus producing a higher surface tension of the filtrate.

There are however other substances in the serum which lower the surface tension. Apart from the haemoglobin which according to Iscovesco (1911) lowers the surface tension of water, but the presence of which in the serum can be avoided, there are the pigments and the

lipoids of the serum as well as salts of fatty and gallic acids (J. Traube, 1908). The usual method of preparing a serum albumin by means of salting out with the sulphate of ammonium involves the pigments and the lipoids, the pigments coming down mainly with the albumin. It is therefore a matter of doubt whether the relatively large decrease of surface tension produced by the serum albumins is not at least partly due to the accidental presence of these substances, the more so as Iscovesco (1910) found that pure egg albumin even increases the surface tension of water. It would be of interest if egg- and serum-albumin are really different in their effect upon surface tension. That these serum pigments are readily adsorbed follows from the observation that the first parts of the filtrate are colourless; the serum colour reappears with further filtration.

As far as my experience is concerned, I agree with the statement of Bottazzi, that the serum albumins lower the surface tension of water (H. Schmidt, 1914). The albumins therefore tend to become adsorbed. If the serum has previously been heated, it has been shown by Traube, M. J. Gramenitzky, L. Berczeller, H. Schmidt and others, that a well-marked decrease of surface tension occurs. According to L. Berczeller (1913) this loss of surface tension is due to the production of albumoses and peptone.

From these observations it is easy to understand that a preliminary filtration of a thermo-inactivated serum (Muir and Browning) or of peptone (Holderer, 1912) facilitates the filtration of serum. Adsorption of the albumins diminishes their protective influence upon the dispersity and the solubility of the euglobulins. The dispersity especially of the euglobulins is decreased and as a consequence their filtrability is also decreased. But this may not be the only cause of the retention of the euglobulins. According to recent work of H. Chick (1913) the globulins form soluble compounds with the salt (NaCl) which are electrically neutral. As there is adsorption of the ions of the dissociated salts in the serum during filtration, as will be explained later, the globulin salt compounds tend to dissociate and become electrically charged, which renders them liable to anomalous adsorption. Quantitatively the amount of euglobulin in the serum is comparatively very small, and therefore its partial removal alters the mass relations of the proteins far more than a removal of a corresponding part of the albumins or the pseudo-globulins. This may help to explain the E-piece action of a Berkefeld filtrate.

In order to explain the influence of salts upon filtration through

a Berkefeld filter I would like to draw attention to a phenomenon, first described many years ago, but so far as I am aware not yet taken into account in the filtration of serum.

Quincke (1861) observed that in passing a liquid through a capillary tube a potential difference occurs between the capillary wall and the liquid. Helmholtz (1879) found an expression for the electromotive force thus developed according to which it is, apart from other relations, directly proportional to the hydrostatic pressure and inversely proportional to the viscosity and the electric conductibility of the liquid in question.

This has been confirmed later by J. Perrin (1904, 1905, 1906). Fr. Fichter (1911) and N. Sahlbohm (1910) drew attention to this phenomenon in explaining the precipitation which colloids with electro-positively charged particles undergo, when passing through capillaries, the diameter of which does not exceed 0·15 mm. N. Sahlbohm demonstrated this phenomenon to be a consequence of the movement of the liquid through the capillary and to be independent of the nature of the material of the capillary walls. Where there is no movement only adsorption takes place. The movement of the liquid produces a negative charge of the wall against the water, while in the case of electro-positively charged colloids, the colloidal particles are positively charged against the water as their dispersion medium. If the potential difference thus produced be large enough to overcome the positive charge of the particles the latter lose their charge and owing to surface tension aggregate and precipitate.

Now the unsalted E-piece obtained by dilution with water and acidifying with CO_2 has a distinct acid reaction and the protein particles in it are therefore positively charged (W. Pauli). It seems to me very probable that such an E-piece being an electro-positive colloid is to a large extent precipitated in the small capillaries of the filter and therefore not able to pass through a Berkefeld filter (cf. Exp. 5).

On the other hand, it is possible to assume that negatively charged protein particles will show a tendency to be repulsed by the negative charge of the Kieselguhr surface. This occurrence would inhibit the adsorption process and facilitate the passage. Positively charged particles would however show a tendency to adsorption, and the greater this potential difference the greater would be this tendency, but in the course of adsorption the potential difference is decreasing owing to the neutralising effect of the adsorbed particles and with that the adsorption decreases as well, till finally no more adsorption takes place.

The presence of electrolytes such as NaCl influences the phenomenon in the following manner: the NaCl is dissociated; its kation tends to neutralise the electric charge of the filter surface. This causes a decrease of the potential difference and therefore less repulsion of negative particles and less attraction or adsorption of positive particles. The influence of NaCl consists therefore in facilitating the adsorption of negatively charged particles and in diminishing the adsorption of positive particles. This is in analogy with the anomalous adsorption of dyes on filter paper described by W. M. Bayliss (1906) and may explain the results of Exp. 5.

It must however also be remembered that the addition of salts decreases a little the viscosity of proteins (W. Pauli), and increases the electric conductibility to a large extent. This latter factor will therefore, according to Helmholtz's formula, diminish the electromotive force produced by the movement in the capillaries, and in this way salts tend to prevent the precipitation of acid proteins. In addition to this the presence of salts (NaCl) decreases the degree of acidity and therefore the positive charge of the particles in an acid protein solution (H. Chick and C. J. Martin, 1913).

Finally I would like to draw attention to some experiments made by Holderer because these experiments, in spite of being made with Chamberland candles, verify to some extent the above described conceptions.

M. Holderer (1909, 1910) and M. Holderer and G. Bertrand (1910) found that many ferments which do not pass a Chamberland filter if in a medium neutral to methylorange ($H^{\cdot} = 10^{-4\cdot4}$) will promptly do so if the acidity is reduced to the neutral point of phenolphthalein ($H^{\cdot} = 10^{-8\cdot3}$). By the addition of neutral salts however Holderer (1910) succeeded in filtering the ferments even if the medium was only neutral to methylorange.

SUMMARY.

In the Berkefeld filtration of a fresh guinea-pig serum, adsorption on the filter surface takes place, and the serum passes unaltered, if the saturation of the adsorbing surface has become complete.

This adsorption involves most probably first the albumins, which process then secondly influences the filtrability of the globulins. It is also shown that the physical conditions of the serum as regards salt concentration and dilution as well as $H^{\cdot}$-concentration modify the effect of filtration to a large extent.

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