OBSERVATIONS ON THE BEHAVIOUR OF OPSONIN AND SERUM PROTEIDS DURING PRESSURE FILTRATION

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Martin (1) in 1896 introduced a method for separating colloids from crystalloids in solutions containing both. It consisted in filtering the fluid through a Pasteur-Chamberland filter, the pores of which had been blocked up with gelatine, under a pressure of forty to one hundred atmospheres. He found that if fresh serum or white of egg were passed through such a filter the filtrate was clear, colourless, and absolutely free from proteid. The crystalloids in solution on the other hand passed through the filter at the same rate as water.

Since the publication of the above paper, the gelatine filter has been employed on various occasions for the purpose of observing the behaviour of substances of physiological and pathological interest, when a solution containing such was passed through the filter.

Martin and Cherry (2) found that snake venom and diphtheria toxin passed through the filter and appeared in the filtrate, whereas the antibodies were retained.

Craw (3) made use of the gelatine filter in his work on the physical chemistry of the toxin-antitoxin reaction; he observed that while megatherium lysin passed through the filter, the megatherium antilysin was retained.

In the following experiments an attempt was made to observe the behaviour of opsonin when serum was forced through a filter similar in nature to the gelatine filter.

The method employed was that described by Martin *with the important exception that no gelatine was used to impregnate the filter*. A Pasteur-Chamberland filter was securely fixed into an iron jacket the inner surface of which was tinned. The upper end of this outer case, which served to hold the serum to be filtered, was closed and connected with a gas cylinder, which itself communicated with an engine by which it could be charged to a pressure of 200 to 300 atmospheres. The Pasteur-Chamberland candle used in the following observations was of a rather fine texture, its pores being comparatively small.

Fresh pig's serum was introduced into the metal case, and by means of a pressure of forty to fifty atmospheres was forced through the porcelain candle and the filtrate collected as it dropped through below.

At first little difference was to be noticed between the normal serum and the filtrate, which passed through the filter fairly quickly. After a short time, however, a very distinct difference was to be noticed, the filtrate, which escaped much more slowly, was observed to be clearer and more watery than normal serum. Ultimately the flow at the outlet of the filter became so slow that a pressure of eighty atmospheres sufficed to cause an escape of only one or two drops per minute, and sometimes the filtration was so retarded that only one drop escaped every three or four minutes.

Systematic examination of the filtrate showed that it contained less and less proteid as the filtration proceeded, until in the later stages it was found to be quite free from proteid.

Thus it is evident that by forcing serum through this fine porcelain candle one had been able to fill up the pores by the large proteid molecules of the serum, so that in a short time the filter had been converted into what was practically a gelatine filter.

Samples of the filtrate were taken at different periods as it escaped from the filter and their opsonic action compared with the normal serum and also with the contents of the outer case of the filter after about one-third of its volume had been forced through the filter.

The following are some of the experimental results :—

Experiment I.—In this experiment pig's serum, staphylococcus albus and human leucocytes were used.

	Number of bacteria phagocytosed by 100 leucocytes					
Control Serum	...	...	...	...	...	446
Filtrate after 5 minutes	...	...	...	...	...	430
Filtrate after $\frac{1}{4}$ hour	...	...	...	...	...	58
Filtrate after 1 hour	...	...	...	...	...	80
Specimen of the serum taken from inside the metal jacket when about one-third of its volume had passed through the filter	...	...	...	...	...	458

Experiment II.—Here also fresh pig's serum, staphylococcus albus and human leucocytes were used.

	Number of bacteria phagocytosed by 100 leucocytes					
Control	...	...	...	...	...	920
Filtrate after 5 minutes	...	...	...	...	...	938
Filtrate after 2 hours	...	...	...	...	...	164
Sample of serum taken from inside the metal jacket when about one-third of its volume had passed through the filter	...	...	...	...	...	916

Experiment III.—In this case B. Coli were used instead of staphylococcus albus.

	Number of bacteria phagocytosed by 100 leucocytes					
Control	...	...	...	...	...	414
Filtrate after 1 hour	...	...	...	...	...	60
Filtrate after $2\frac{1}{2}$ hours	...	...	...	...	...	32
Sample of serum taken from inside the metal jacket when about one-third of its volume had passed through the filter	...	...	...	...	...	460

Experiment IV.—Here Tubercle bacilli were substituted for staphylococcus albus.

	Number of bacteria phagocytosed by 100 leucocytes					
Control	...	...	...	...	...	396
Filtrate after 5 minutes	...	...	...	...	...	429
Filtrate after 1 hour	...	...	...	...	...	34
Filtrate after 2 hours	...	...	...	...	...	42
Sample of serum taken from inside the metal jacket when about one-third of its volume had passed through the filter	...	...	...	...	...	449

From these results it is apparent that, if any, only the merest trace of opsonin passed through the filter after it had been working sufficiently long for its pores to become filled up by the proteids of the serum. The enormous decrease in the opsonic power of the filtrate as compared with normal serum is even more pronounced, when the fact is recalled, that considerable dilution of normal serum by either '9 per cent. saline solution or by heated serum suffices to reduce its opsonic action, when measured by Wright and Douglas' method, to a slight extent only. Wright and Douglas (4) found that normal serum can be diluted twenty-four fold with only a comparatively slight lessening of its opsonic power when compared with undiluted normal serum. Then again the slight amount of spontaneous phagocytosis, which occurs when leucocytes washed free from serum are incubated with bacteria in the entire absence of serum, has to be taken into account when one is considering the significance of the above figures.

When, after about one-third of the serum had passed through the filter, the contents of the jacket were examined it was found that besides the comparatively unchanged serum, the opsonic power of which was given in the above experiments, there was a gelatinous substance which was adherent to the porcelain candle. This consisted of the contents of the serum which could not pass through the filter. An attempt was made to estimate the opsonic activity of this substance as compared with the normal serum. Beyond, however, ascertaining that it contained a considerable amount of opsonin one was unable to proceed, as, owing to its gelatinous nature, it was quite impossible by the ordinary method of Wright and Douglas to prepare specimens that were at all comparable with those made from normal serum.

In order to clean the filter, the candle was removed and the adherent jelly washed off, and then after being allowed to dry thoroughly in an oven the porcelain was heated red hot by means of a blow-pipe. By this means all organic substances in the pores of the candle were removed.

Since opsonins did not pass through a filter of this nature it seemed probable that they were of the nature of proteid.

The following experiments were performed to ascertain whether opsonin in normal serum was dialysable. Ten cubic centimetres of fresh serum were placed in a parchment tube and this was immersed in a somewhat wider glass tube containing an equal quantity of normal saline solution. At the same time a quantity of serum was put up in a Wright's capsule to serve as a control.

After allowing dialysis to proceed for twenty-four hours and forty-eight hours respectively, samples were drawn off from the inner tube containing the serum and from the outer tube containing the '9 per cent. NaCl solution into which the serum was being dialysed. The opsonic power of these specimens was then compared with the control serum. The following are the results :—

Experiment I.—Pig's serum, *Staphylococcus albus* and human leucocytes were used.

						Number of bacteria phagocytosed by 100 leucocytes
A. After allowing dialysis to proceed for 24 hours—						
Normal pig's serum	...	...	...	...	...	483
Dialysed pig's serum	...	...	...	...	...	470
'9 per cent. saline solution into which the serum was dialysed	...	...	...	...	...	4
B. After allowing dialysis to proceed for 48 hours—						
Normal pig's serum	...	...	...	...	...	268
Dialysed pig's serum	...	...	...	...	...	298
'9 per cent. saline solution into which the serum was dialysed	...	...	...	...	...	2

Experiment II.—In this case Tubercle bacilli were substituted for *Staphylococci*.

						Number of bacteria phagocytosed by 100 leucocytes
A. After allowing dialysis to proceed for 24 hours—						
Normal pig's serum	...	...	...	...	...	229
Dialysed pig's serum	...	...	...	...	...	241
'9 per cent. saline solution into which the serum was dialysed	...	...	...	...	...	8
B. After allowing dialysis to proceed for 48 hours—						
Normal pig's serum	...	...	...	...	...	326
Dialysed pig's serum	...	...	...	...	...	297
'9 per cent. saline solution into which the serum was dialysed	...	...	...	...	...	9

We have from these figures conclusive proof that opsonins are not dialysable.

Simon Tamar and Bispham (5) also find that opsonins are not dialysable, and that they are carried down with euglobulin when serum is treated by half saturation with ammonium sulphate. These observations also serve to support the view that opsonin found in normal serum is of the nature of a proteid.

In conclusion I desire to express my indebtedness to Professor Moore for his advice, and assistance in connection with the use of the filter.

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