

XLVIII. THE DIFFERENTIAL DIALYSIS OF THE ANTINEURITIC AND THE ANTISCORBUTIC FACTORS.

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(Received May 3rd, 1921.)

It has been shown by Eijkman [1906] that the antineuritic factor is dialysable. Holst and Frölich [1912] also found that when fresh cabbage juice was dialysed for 3-4 days it lost the best part of its activity although all the salts did not disappear from the juice during that time. It is therefore evident that under certain conditions the antineuritic and the antiscorbatic factors will pass through a dialysis membrane. As it is now possible to calibrate the permeability of collodion membranes we set out to ascertain what degree of permeability of collodion membranes would permit the passage of these factors. Brown [1915] has shown that if air-dried collodion membranes, which are highly impermeable, are placed in solutions of alcohol and water they become permeable, that the permeability increases with the strength of the alcohol employed and that the degree of permeability of such membranes can be established by various methods based on certain properties which display a parallelism with the permeability. Utilising the above principles we have determined the permeability of the collodion membranes which permit the passage of the antineuritic factor of autolysed yeast juice and the antiscorbatic factor of lemon juice from which the citric acid has been removed.

EXPERIMENTAL.

The solutions were dialysed through collodion thimbles. These were prepared in the following way: a 14 % alcohol-ether solution of collodion (356A/9) supplied by the Necol Industrial Collodion Ltd was introduced into a test-tube 14 cm. long and having an internal diameter of 3 cm. The collodion was then either centrifuged or allowed to stand for about 12 hours in order to permit the bubbles of air to rise to the surface, the opening of the tube being protected by an outside tube in order to obviate the evaporation of the solvents. The tubes were inverted and the collodion was allowed to drain for 5 minutes after which time the tube with the collodion was immersed in water and the thimble was stripped off. It was then washed in water, dried for 24 hours in

the air and soaked for 24 hours in the alcohol-water solution of the requisite strength. After washing the thimbles were kept in water until required for use.

40 cc. of the active solutions were dialysed against running water. The thimbles were closed with indiarubber stoppers through which passed a glass U-tube containing mercury.

In order to ascertain the permeability of our thimbles, substances of various molecular weights were allowed to diffuse through them and the time and the degree of diffusion noted. The substances used—mostly dyes—were introduced into the thimbles which were immersed in beakers of water and the amount of the substance which diffused at various times was recorded. We have obtained the following results with thimbles soaked in 80%, 85%, 90%, 95%, 100% alcohol:

Sodium chloride had passed through all the thimbles in very considerable quantity after two hours.

Picric acid. After four days a certain amount of the substance had passed through the 80% membrane. As in the case of the following substances the amount which diffused in that time increased with the strength of the alcohol employed in the treatment of the thimbles, the 100% membrane showing the greatest permeability.

Potassium oxalate. None of the salt had passed through the 80% membrane in four days; a certain amount diffused through the 85% membrane.

Bismarck brown. None of the substance passed through the 80% and the 85% membranes; a certain amount diffused through the 90% membrane.

Methylene blue. None of the substance passed through the 80% and 85% membranes; a trace diffused through the 90% membrane.

Neutral red. None of the substance passed through the 80%, 85% and 90% membranes; a certain amount of it diffused through the 95% membrane.

Safranine behaved almost like neutral red.

Dextrin. None of the substance passed through the 80%, 85%, 90% membranes; a trace diffused through the 95% and a little more through the 100% membranes.

Litmus. None of the substance passed through the 80%, 85%, 90% and the 95% membranes, some through the 100% membrane.

Congo red did not pass through any of the above membranes in four days.

Autolysed yeast was used as the source for the antineuritic factor. The yeast after being washed free from wort was pressed out and placed in a large flask plugged with cotton wool in the hot room at 37° for a few days. The autolysed mass was filtered through a large Buchner funnel when sufficiently liquified and the brown filtrate thus obtained was dialysed for four days as described above and fed to rats which subsisted on a diet deficient in the antineuritic factor and which manifested signs of decline. Resumption of growth showed that the accessory factor had not totally diffused in that time. Failure to induce growth, on the other hand, showed that the active

substance had entirely passed through the membrane during the four days of dialysis.

As the source for the antiscorbutic factor lemon juice was employed which had previously been treated with an excess of calcium carbonate and filtered. This antiscorbutic solution, which was dialysed for three days, was tested on guinea pigs kept on a scorbutic diet of oats, bran and autoclaved milk. The administration of the doses was carried out about fourteen days after the animals had been put on the deficient diet, and in all the cases where the antiscorbutic factor diffused scurvy developed, in the other cases the onset of the disease was prevented.

A sample of the original material was always kept in a glass flask in the dialysing tank under the same conditions as the dialysing thimbles and was utilised for control purposes.

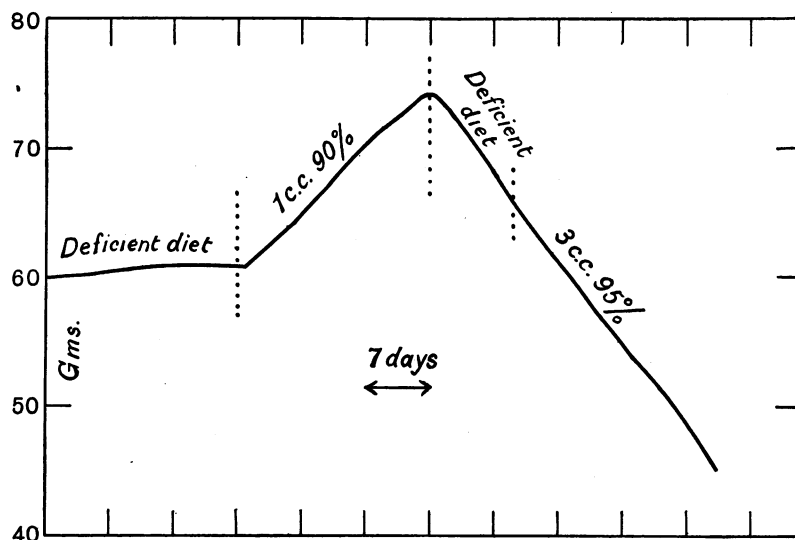


Fig. 1

Membranes of various permeabilities were tried until the one which allowed the free diffusion of the factors was obtained. In the case of both the antineuritic and the antiscorbutic factors it was found that the accessory factors would not pass through a 90 % membrane whilst they diffused entirely through a 95 % membrane in the time mentioned.

It will be seen from Fig. 1, which represents the growth curve of a rat kept on a diet deficient in the antineuritic factor, that an equivalent of 1 cc. of autolysed yeast dialysed through a 90 % membrane induced growth in the animal. As 1 cc. of autolysed yeast juice is approximately the minimum dose which is capable of inducing growth in a rat fed on a diet deficient in the antineuritic factor, one may conclude that very little of the factor diffused through this membrane in four days. The same animal was then placed again on the

deficient diet, and as was to be expected the rat declined in weight. The addition of 3 cc. of autolysed juice dialysed through a 95 % membrane for four days failed to promote growth, thus showing that the antineuritic factor had gone through entirely in this time. Other experiments have confirmed this observation: in all cases the factor failed to diffuse in any appreciable extent through a 90 % membrane but did so entirely through a 95 % membrane.

Similarly it will be seen from Fig. 2 that 7 cc. of decitrated lemon juice dialysed for three days through a 95 % membrane failed to prevent or even delay the onset of scurvy in a guinea-pig, whilst 3 cc. of the juice which had been dialysed through a 90 % thimble prevented the onset of the disease. The antiscorbutic factor therefore behaved much in the same way as the antineuritic factor. In this case also various experiments confirmed this result.

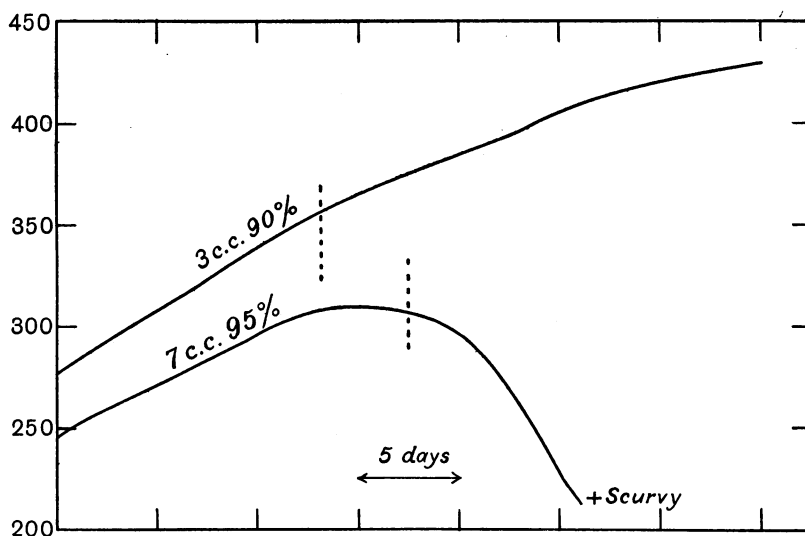


Fig. 2

A number of experiments with 35 %, 50 % and 70 % membranes showed that they were not permeable to the above accessory factors. These principles however passed freely through a 100 % thimble.

It is thus seen that the antineuritic factor in autolysed yeast juice and the antiscorbutic factor in decitrated lemon juice diffuse through membranes of such permeability as permit the passage of substances of such molecular dimensions as methylene blue, neutral red and safranin. From this one may conclude that the dimensions of the molecules of these factors or the molecules with which they may possibly be associated are of the order of that of a semi-colloid. There is, however, no evidence whether these molecules are simple or associated. It must also be pointed out that the above experiments were made without the application of pressure and only one source for each of the accessory factors was used. It would be of interest to investigate whether

factors such as hydrogen ion concentration, which influence the degree of dispersion, also influence the diffusibility of the antineuritic and antiscorbutic principles.

SUMMARY.

The antineuritic and the antiscorbutic accessory factors diffuse through a collodion membrane of such permeability as permits the passage of substances like methylene blue, neutral red and safranine. Membranes of lower permeability were found not to allow the diffusion of these factors. It is suggested that the active molecules whether simple or associated may be of a semi-colloid nature.

The expenses of this research were defrayed from a grant made by the Medical Research Council, to whom our thanks are due.

REFERENCES.

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Eijkman (1906). *Arch. Hyg.* 58, 150.
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