LXXXIV. THE DIFFERENTIAL DIALYSIS OF THE ANTISCORBUTIC FACTOR. II.

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ADOPTING the technique of Brown [1915], Zilva and Miura [1921] were able to show that the antiscorbatic factor of lemon juice diffuses through collodion membranes which permit the passage of substances of such molecular dimensions as methylene blue, neutral red and safranine. The vitamin of course was also found to diffuse through collodion membranes of higher permeability. In extending the investigation the authors hoped to utilise this method of differential dialysis for the purpose of separating the active principle from extraneous matter. Although the original intention could not be accomplished, other interesting information was obtained which is recorded in this communication.

The three main observations made in this investigation are:

(A) That the diffusion of the antiscorbatic factor proceeds differently from that of the sugar and nitrogenous substances present in decitrated lemon juice, which confirms the chemical observations made on the juice in this connection [Zilva, 1924, 1, 2].

(B) That the antiscorbatic vitamin in another source, namely swede juice, seems to diffuse through membranes of the same permeability as when it is present in lemon juice. This would suggest that the factor in both sources is more or less of the same molecular magnitude.

(C) That the vitamin will diffuse through collodion membranes of somewhat lower permeability than those which permit the passage of the dyes mentioned above.

EXPERIMENTAL.

The technique employed in this investigation was with the exception of a few modifications the same as that used by Zilva and Miura, which as already mentioned was based on that of Brown. The principle consists in soaking the dry collodion thimbles in alcohol of various strengths. The stronger the alcohol employed the greater the permeability obtained. The collodion used was a 14 % alcohol-ether solution (Necol 356/A9) to which 100 cc. of ether per litre were added. In order to obtain as great an accuracy as possible the viscosity

of the collodion was kept constant by frequent measurements and additions of ether if necessary. The thimbles were soaked in the respective alcohols for 24 hours at a constant temperature of 20° in an incubator. The alcohols were made up by weight in the following concentrations:

Table I.

	S. G.	% by weight	Approx. % by volume
.812	hydrometer	92.2	95
.8235	weighing bottle	88.1	92
.8280		85.8	90
.8340		83.8	88.5
.840	hydrometer	81.4	87

Exp. 1. As has already been shown the sugar in decitrated lemon juice consists mainly of invert sugar [Zilva, 1924, 1]. It was thought desirable to ascertain whether the diffusion of invert sugar in aqueous solution proceeds in the same way as that of the sugar in decitrated lemon juice. Decitrated juices, prepared by the old method of treating the neutralised lemon juice with two volumes of alcohol before concentrating, showed an average sugar content as estimated by Bertrand's reduction method of 0.45 %. The pure sugar solutions were therefore made up by using equimolecular mixtures of glucose and fructose giving a final mean concentration of 0.47 % estimated by the same method. The dialysis of the sugar in decitrated lemon juice and of the sugar in aqueous solution was compared on twelve occasions. The duration of dialysis in each case was three days. The following table gives the mean figures of the twelve experiments.

Table II. *Percentage of sugar diffused.*

Alcohol index of membrane	...	95	92	88.5	87
		%	%	%	%
Decitrated juice	...	97	49	9	0
Sugar solution	...	97	61	20	0

It will be seen from Table II that there was a deviation in the diffusibility of the sugar in the decitrated juice from that in the aqueous solution through the 92 % and 88.5 % membranes. The differences are beyond the limits of the experimental error of the method. It can, however, be shown that this deviation is due to the blocking of the pores of the membranes by matter in solution in the decitrated juice and not to any difference in the actual molecular dimensions. When the decitrated juice was diluted with three times its volume of water it was found that the diffusion of the sugar proceeded as in the case of the aqueous solutions of glucose and fructose. This is seen from Table III.

Table III. *Percentage of sugar diffused.*

Alcohol index of membrane	...	...	...	88.5	90	92
				%	%	%
Decitrated juice, original strength	...	...	...	7	21	50
Decitrated juice, diluted four times	...	...	...	18	43	61
Sugar solution	...	...	...	20	—	61

The experiment, therefore, shows that the sugar diffuses in the same way in both cases.

Exp. 2. In this experiment the relative quantities of the antiscorbutic factor, the sugar, and nitrogenous substances diffusing in three days through membranes of various permeability were studied; 95 %, 92 %, 90 %, 88.5 % and 87 % membranes were employed. A new series of the various thimbles containing about 20 cc. of the juice was placed in the dialysing tank every other day, so that no juice was fed to the guinea-pigs more than two days after dialysis. Some juice in a glass tube was kept in the dialysing tank with each batch as a control. Some succinic acid was introduced in each thimble to keep the juices on the acid side. Succinic acid was chosen on account of its low solubility in water and its non-toxic properties.

The antiscorbutic factor could not of course be estimated with the same degree of precision as the sugar and the nitrogen. The accuracy obtained by the biological method was, however, found to be good enough to demonstrate the different degrees of diffusion of the three fractions. Three doses equivalent to 1.5 cc. 3 cc., and 5 cc. of the original lemon juice were used in estimating the vitamin in the decitrated lemon juice dialysed through each membrane and in the control juice. Two animals were used for each dose in the case of the 95 % membranes. All the six animals succumbed to scurvy within 30 days, showing that the whole of the vitamin had diffused. A similar set of animals was used for the 92 % series. All these animals died of scurvy, those on the lower doses within a month and those on the two higher doses within 35–38 days. It is, therefore, concluded that about 95 % of the vitamin has passed through this membrane. In the case of juice dialysed in the 90 % thimble the assessment was rather more difficult to make. Of the three animals on the daily dose of 1.5 cc. one died of florid scurvy within 35 days, the other two within 52 and 53 days respectively, showing signs of moderate scurvy at the post-mortem examination. On the 3 cc. dose one animal died within 56 days, showing marked scurvy, and another had to be chloroformed 46 days after the commencement of the experiment owing to intercurrent disease. This animal was also scorbutic. The animals on the 5 cc. dose survived for two months after which time they were chloroformed. Some signs of scurvy were observed at the post-mortem. It is estimated from the above that about 40 % of the vitamin diffused in these thimbles. The animals fed on the juice from the 88.5 %, 87 % membranes and from the control tubes all behaved in the same way. The animals on 1.5 cc. doses succumbed to scurvy, but after a delay of about 20 days. The animals on the 3 cc. and 5 cc. doses survived for two months after which time they were chloroformed. The similarity in the behaviour of the animals in these three sets shows that no appreciable quantity has diffused through the last two membranes. Table IV and Fig. 1 give the results of these experiments.

Table IV. *Percentage of substances diffused.*

	Alcohol index of membrane	%	%	%	%	%
Sugar		87	88.5	90	92	95
Mean of 12 estimations by Bertrand's method	{ Mean value	0	9	21	50	97
	{ Mean variation	0	± 2.5	± 4.5	± 4	0
Nitrogen						
Mean of 11 estimations by a micro-Kjeldahl method	{ Mean value	10	25	50	70	100
	{ Mean variation	± 5	± 10	± 10	± 7.5	—
Antiscorbutic factor		0	0	40	95	100

Fig. 1.

In analysing the results it is seen that the nitrogenous substances of the decitrated juice diffuse at different rates to the antiscorbutic factor, and this lends further support to the view that the vitamin is independent of the bulk of the nitrogenous matter. The sugar figures being an index of a definite substance and not of a probable mixture of substances as is the case of the nitrogen figures, definitely confirm the previous observation [Zilva, 1924, 1] that the invert sugar has no connection with the antiscorbutic activity. It is further seen that the 87 % membrane does not allow the passage of the sugar and the vitamin whilst the 88.5 % membrane permits the slow passage of the former but no passage of the latter. This would suggest that the dimensions of the active molecule and that of the hexoses cannot be far removed.

Exp. 3. This experiment was instituted with the intention of studying the rate of diffusion of the antiscorbutic factor in swede juice. The results were partly vitiated by certain unforeseen technical difficulties but they contribute enough information to be recorded. It was found that the animals

receiving the doses did not thrive well and mostly died before the end of the experimental period, probably through the action of some toxic principle which was produced in the juice during dialysis.

The swede juice was dialysed in the same way as the decitrated lemon juice. Swede juice as obtained from the root by pressing contains some matter in colloidal suspension even after filtration. It was, therefore, not advisable to dialyse it in this condition as there was a likelihood that the pores of the dialysing thimbles might be blocked by this matter and the results thus vitiated for comparative purposes. The juice was, therefore, previously treated with an equal volume of absolute alcohol which precipitated most of the colloidal matter. After filtration the alcohol was removed in a vacuum at 50° and the juice brought up to its original volume with water. Preliminary experiments had shown that this precipitation did not affect the potency of the juice. The minimum protective dose in the case of both the precipitated and the original juice was found to be 3 cc.

Table V gives a summary of the results obtained with the dialysed swede juice.

Table V.

Alcohol index	Dose	Days alive	Findings at p.m. examination
80 %	3	47	Very mild scurvy: death not due to scurvy alone
	5	46	Scurvy
	7	39	Death not due to scurvy
90 %	3	40	Very mild scurvy: death not due to scurvy alone
	5	54	Mild scurvy
	7	24	Death not due to scurvy
95 %	3	26	Died of scurvy
	5	24	" "
	7	26	" "

Reviewing these results one is inclined to conclude, in spite of the imperfections of the technique, that the 80 % and 90 % membranes did not allow the vitamin to diffuse to any considerable extent, since definite protection against scurvy was observed even in the animals receiving the lowest dose which affords protection when the undialysed juice is fed immediately after preparation. The intermediate doses showed even better protective power. No doubt can exist as to the inefficiency of the swede juice which was dialysed through a 95 % membrane.

It would, therefore, appear that the antiscorbutic factor of this source diffuses through membranes of the same permeability which permit its passage when present in lemon juice.

SUMMARY.

The diffusion of the antiscorbutic factor proceeds differently from that of the sugar and the nitrogenous substances present in decitrated lemon juice.

The antiscorbutic factor in swede juice and in lemon juice diffuses through membranes of the same permeability.

Indications are obtained which suggest that the size of the active molecule cannot be far removed from that of the hexoses.

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