VI. FURTHER CONTRIBUTIONS TO THE TECHNIQUE OF PREPARING MEM- BRANES FOR DIALYSIS.

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INTRODUCTION.

In a previous communication [Brown, 1915] a method was described by which a series of collodion membranes of differential permeability could be prepared. These membranes were for use in aqueous solution. It has been found that the method there described can be extended to the case of other substances (e.g. gelatin, agar, parchment, etc.) and to a large variety of liquids (ethyl alcohol, acetone, chloroform, benzene, etc.). Furthermore by a modification of the method, a series of graded gelatin membranes has been obtained which can be used in aqueous solution.

GENERAL PRINCIPLE.

By way of illustration we may take the case of the collodion technique described in the paper already cited. When films of air-dried collodion (celloidin) are immersed in water, only a negligible imbibition of the latter takes place. When similar films are immersed in absolute (or nearly absolute) ethyl alcohol, a rapid imbibition takes place, the film swelling up, and, if sufficient alcohol be present, disintegrating to form a uniform solution. By immersing the films in an alcohol-water mixture, an intermediate state is reached in regard to imbibition by the collodion, of such a type that the higher the percentage of alcohol used, the greater the resultant imbibition. When the films so treated are placed in water and thoroughly washed, it is found that while a certain amount of shrinkage takes place in the process, the effect of the differential treatment is still strongly marked,

and thus, corresponding to a given strength of alcohol, a collodion membrane possessing a definite permeability and having water as its continuous phase may be prepared.

In the above illustration, the function of the water may be described as being to restrain the imbibition of alcohol by the collodion. For convenience in description we shall speak of "restraining" and "swelling" liquids, the former being exemplified in the above illustration by water and the latter by alcohol.

The general principle may now be enunciated:

Suppose a system consisting of:—Liquid *A*—Membrane substance—Liquid *B*.

- If (1) the membrane substance only imbibes a negligible amount of *A*,
- (2) the membrane substance strongly imbibes *B*,
- (3) *A* and *B* are miscible in all proportions,

then by soaking films of the membrane substance in mixtures of *A* and *B* and subsequently washing in *A*, membranes of graded permeability can be obtained, and of such a type that the higher the percentage of *B* in the mixture the greater is the permeability¹ of the resultant membrane.

The membranes which have been produced by application of the above principle will be described under two headings:

A. Graded membranes for use in the restraining liquid, e.g. collodion in water, gelatin in alcohol. This class comprises the great bulk of the membranes prepared.

B. Graded membranes for use in the swelling liquid. Of this type, only one is at present known, viz., gelatin in water.

¹ In the paper already cited, the effect of the treatment with alcohol-water mixtures was shown by determining the change in the space relationships (e.g. increase in length, increase in thickness, diminution in percentage of dry to total weight), as well as by measurement of the rates of diffusion of particular substances through the membrane. In the case of the membranes dealt with in the present paper, it has not been found possible to carry out the experiments with the same amount of detail, partly on account of the large variety of membranes which have been produced, but mainly because of the impossibility of obtaining at the present time the glass-ware requisite for carrying out in the case of these membranes experiments on diffusion and dialysis. In the case of the collodion-in-water membranes it was found that high permeability was associated with a low dry weight/wet weight ratio, and *vice versa*, that is, that permeability increased with increased development of the external or continuous phase of the membrane. This correlation has been established in the case of gelatin-in-alcohol and gelatin-in-water membranes, and it is believed will be found to apply to membranes of all descriptions. In order therefore not to overstep the limits of experimentally ascertained fact, the statement in the text should be paraphrased into "the higher the percentage of *B* in the mixture, the lower is the dry weight/wet weight ratio of the resultant membrane, that is, the greater is the amount of its continuous (liquid) phase."

A. GRADED MEMBRANES FOR USE IN THE RESTRAINING LIQUID.

Preparation of the Air-dried Films—Practical Notes.

Gelatin. 100 cc. of a 2 % solution of sheet gelatin in water (40—50° C.) is poured over a clean mercury surface in a shallow circular dish of about 20 cm. diameter and allowed to dry completely. The method of preparation by pouring over a flat glass surface was rejected on account of the difficulty of removing the dried film from the glass surface.

Agar. 100 cc. of a 2 % solution is poured over a flat horizontal glass plate (20 cm. diam.) and allowed to dry; when slightly moistened, the film may readily be peeled off. It is then soaked in alcohol (this involving a certain amount of shrinkage) and dried in a current of air. The method adopted in the case of gelatin was found to be impracticable in this case as the drying film invariably broke away from the sides of the vessel and dried with considerable shrinkage in area.

Starch. Only one membrane was prepared as it was found to be too brittle to possess any practical value. It was prepared in the same way as the gelatin membrane.

Collodion. The treatment is the same as in the case of agar films, the film being of course dried off without immersion in alcohol.

Preparation of Graded Membranes.

Films prepared as described above, together with vegetable parchment, were placed in various alcohol-water mixtures for 24 hours, and then transferred to absolute alcohol in which they were washed (with 3 changes) over a period of 2 days. The wet and dry weights of each membrane were then determined in the usual way and with the usual experimental precautions. The figures contained in Table I illustrate the type of result obtained.

TABLE I.

% Alcohol	(Dry weight/wet weight) × 100			
	Parchment	Starch	Gelatin	Agar
0	60.50	62.27	19.83	4.50
40	64.75	77.42	49.07	17.73
60	67.20	87.71	66.07	51.37
80	72.65	—	75.00	77.52
100	97.58	95.14	85.00	90.80

With regard to the above figures it must be remembered that in obtaining the wet weights of the membranes, it is impossible to remove completely the film of alcohol from the surface, and that therefore the figures obtained are all somewhat too low. The percentage error so introduced is obviously greatest in the 100 % alcohol membranes, and least in the case of those at the other end of the series. The error is greatest in the case of the agar and gelatin membranes as these were considerably thinner than the others.

The marked nature of the grading effected is sufficiently emphasised by the above figures not to require further comment. It is most pronounced in the case of the agar and gelatin membranes, and the latter have accordingly been selected as the most favourable substances for purposes of the present technique. Of these agar is preferred on account of the wider range it presents, and on account of the greater ease in its manipulation.

The figures obtained for parchment are anomalous in showing a very slow gradation over the greater part of the range of alcohol-water mixtures and a very sharp gradation towards the higher end of the series. This is considered to be due to the fact that parchment is not a homogeneous substance as it consists of two parchmented layers with a paper layer between. The observed wet weight of a textile fabric like the latter is higher than the true wet weight as it includes the liquid which lies between the fibres and which is not properly speaking imbibed in the substance of the paper. The high figure obtained for the 100 % parchment membrane is due to the fact that such a membrane is impermeable to alcohol and therefore the internally situated paper cannot be reached by the latter.

The graded effect produced in these membranes can be readily demonstrated by observing the rate at which they stain on immersion in a dye solution. Thus when 40, 60, 80, 100 % membranes of parchment, gelatin and agar are placed in a strong solution of night blue in absolute alcohol for a short time (e.g. 15 minutes), taken out and rapidly wiped with a cloth to remove excess stain, it is seen that in all cases staining of the 100 % membranes is negligible, strong in the case of the 40 % membranes, with intermediate conditions in the case of the 60 and 80 % membranes. *Pari passu* with this graded staining effect runs the capacity of the particular membrane to transmit the dye, though this relationship has, on account of the difficulty of obtaining the requisite glass-ware, only been as yet fully ascertained in the case of the gelatin membranes. Thus in diffusion tests of alcoholic night blue against absolute alcohol with a series of graded gelatin membranes, no trace of the dye had passed through in 24 hours in the case of the 80 and 100 % mem-

branes; a slight passage occurred in the same time in the case of the 60 % membrane; whereas in the 40 % membrane strong coloration of the alcohol took place within a few (e.g. 5) minutes. A similar behaviour can confidently be predicted for the other membranes.

Uniformity and Permanency of the Grading Effect.

With films prepared in the same way the results obtained are markedly uniform. The figures in the following table give as usual the percentage of dry weight in total weight of membrane. The membranes were prepared simultaneously, and all washed in absolute alcohol for 3 days; the wet and dry weights of the first lot *A* were determined immediately, while the corresponding quantities for the second lot *B* were determined after a further 7 days. The ratio of the thickness when wet to original thickness when dry was also determined, and the results are also incorporated in Tables II and III.

TABLE II.

Agar.

% Alcohol	A (3 days in alcohol)		B (10 days in alcohol)	
	$\frac{\text{Dry weight}}{\text{Wet weight}} \times 100$	$\frac{\text{Thickness (wet)}}{\text{Thickness (dry)}} \times 100$	$\frac{\text{Dry weight}}{\text{Wet weight}} \times 100$	$\frac{\text{Thickness (wet)}}{\text{Thickness (dry)}} \times 100$
0	4.50	ca. 1900	3.13	ca. 2200
	4.55	ca. 1900	5.18	ca. 1600
40	17.73	ca. 510	19.44	ca. 500
	17.65	ca. 500	18.05	ca. 500
60	51.37	183	53.57	172
	53.40	175	54.14	168
80	77.52	120	78.90	114
	78.22	127	78.94	117
100	90.80	100 +	88.33	100
	90.23	100 +	87.50	100

TABLE III.

Gelatin.

% Alcohol	A (3 days in alcohol)		B (10 days in alcohol)	
	$\frac{\text{Dry weight}}{\text{Wet weight}} \times 100$	$\frac{\text{Thickness (wet)}}{\text{Thickness (dry)}} \times 100$	$\frac{\text{Dry weight}}{\text{Wet weight}} \times 100$	$\frac{\text{Thickness (wet)}}{\text{Thickness (dry)}} \times 100$
0	19.83	ca. 650	17.28	ca. 670
	19.30	ca. 630	18.53	ca. 700
40	49.07	220	52.21	235
	51.75	230	—	—
60	66.07	150	65.22	162
	64.28	150	66.67	160
80	75.00	120	74.47	123
	74.00	130	75.42	125
100	85.00	100	84.00	110
	—	—	—	—

It is thus seen that with similar treatment the membranes obtained show characteristic figures varying by not more than 2—3 per cent. With special precautions in the determination of these figures, it is certain that this range of error could be considerably curtailed.

Examination of the tables also shows that the membranes have not appreciably altered over a period of 1 week. More detailed tests by the staining method have failed to show any alteration over a period of 25 days. Similarly a 40 % gelatin membrane which allowed passage of the dye night blue in a few minutes was found to behave similarly after being kept for a period of 6 weeks. It is thus certain that these membranes preserve their properties with little alteration over a considerable period of time.

The degree of uniformity indicated in the preceding tables is not attainable unless the initial dry films have been prepared in a similar way. In the preparation of the solution from which the films are prepared, increased exposure to heat produces increased imbibition in the 24 hours' exposure to water and the alcohol-water mixtures, with corresponding differences in the dry weight percentage of the finished membranes. In the following table, a comparison is given of two films, one of which (*A*) was prepared by heating the original agar powder suspension to 100° for a few minutes; the other (*B*) by similar treatment at 120° for 20 minutes. It will be observed that the differential effect diminishes with increasing percentage of alcohol.

TABLE IV.

% Alcohol	Dry weight Wet weight $\times 100$		Thickness (wet) Thickness (dry) $\times 100$	
	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
0	10.24	4.50	450	1900
40	41.28	17.73	195	510
60	58.92	51.37	152	183
80	75.33	77.52	115	120

The corresponding figures for the ratio of dry to wet weight (in water) after 24 hours' soaking in water are: *A*, 4.4 %; *B*, 1.5 %.

An exactly similar phenomenon occurs in the case of gelatin. This is in agreement with the statement of Wo. Ostwald [1905] that increased heating confers upon gelatin an increased power of imbibing water. It is obvious therefore that in carrying out the present technique the process of preparation of the original films must be standardised.

Generalisation. Graded membranes having been obtained by the above process, the absolute alcohol may be replaced by any liquid which is miscible

with alcohol and which is not imbibed by the dry gel substance. Thus in the case of agar and gelatin, absolute alcohol as continuous phase may be replaced by acetone, benzene, petrol ether, etc., and thus graded membranes prepared for use in these liquids. The process of preparation consists simply of washing out the alcohol by immersion of the membrane in several changes of the particular liquid.

Table V shows such a gradation effected in a series of agar membranes. The figures represent as usual the percentage of dry weight in total weight. All the membranes were prepared by soaking in alcohol-water mixtures, with subsequent transference to absolute alcohol. The experiment was performed in duplicate so that an idea might be obtained of the degree of uniformity realised.

TABLE V.

% of alcohol employed in grading	Eth. alc.	Acetone	Toluene	Benzene	Chloroform	Clove oil	Petrol
0	{ 4.10	4.21	3.89	4.26	2.15	5.00	5.21
	{ 4.23	4.42	3.84	7.93	2.28	5.24	4.49
40	{ 17.88	20.27	18.63	25.00	11.60	25.15	26.55
	{ 17.73	17.00	16.67	23.73	10.97	26.97	23.24
80	{ 74.60	78.22	88.20	78.67	73.21	68.22	87.00
	{ 74.08	76.92	88.42	79.00	74.42	68.60	88.90

It may be remarked that the figures in the horizontal rows of the above table are not comparable with each other. The low figures obtained in the case of the 0 % and 40 % membranes in chloroform are to be explained by the high specific gravity of chloroform. Even if the figures in the horizontal rows be corrected for the different specific gravities of the liquids concerned, it is not certain that they will even then be comparable, as it is possible that the volume of the continuous (liquid) phase of the membrane alters on transference from one liquid to another. An accurate investigation of this type has not yet been attempted.

A detailed determination of the permanence of the graded effect in these membranes has not been carried out. Membranes in clove oil and toluene have been kept for 6 weeks, at the end of which time the grading as far as could be judged from external appearances (thickness) was as pronounced as ever. It is tolerably certain that the permanency of grading demonstrated in the case of the membranes in absolute alcohol is characteristic of these membranes in general.

Membranes prepared by use of other Swelling and Restraining Liquids.

1. *Restraining Liquid.* Throughout the present section alcohol has been used as restraining liquid. Membranes of equal range can be obtained by a similar use of acetone.

In the earlier paper in which the technique of collodion membranes was described, water was employed to restrain the swelling action of alcohol. For this purpose it may be replaced by any other liquid which mixes with alcohol and which is not imbibed directly by the dry collodion. Thus graded collodion membranes have been prepared by use of chloroform, benzene, toluene, and petrol ether as restraining liquids. The range of mixtures available varies in the different cases, the maximum concentration of alcohol which can be used in each case being shown by the following figures:

Alcohol in toluene...	...	30—40 %
Alcohol in benzene	...	40 %
Alcohol in petrol ether...	...	75—80 %
Alcohol in chloroform	...	90—95 %
Alcohol in water		97—98 %

It is probable that the best series of graded collodion membranes for use in such liquids as benzene and toluene can be prepared by previous manufacture in chloroform and transference to the particular liquid to be employed.

2. *Swelling Liquid.* The scope afforded by the method under consideration is well illustrated by the variety of graded series of membranes which can be prepared from the acetate derivative of cellulose¹. This substance is soluble in acetone and acetic acid, and swells to a considerable extent in chloroform. It has been found possible to prepare graded membranes by use of any of the following mixtures:

Acetone—water,
 Acetone—ethyl alcohol,
 Acetic acid—water,
 Chloroform—ethyl alcohol,
 Chloroform—benzene,
 Chloroform—toluene,
 Chloroform—petrol ether,

the membranes after being placed in the mixture being finally set up in the

¹ This material was kindly supplied to me by Mr Cross of Messrs Cross and Bevan, New Court, Lincoln's Inn, W.C.

second liquid in each case. Whether, as is probable, these methods are of unequal practical value has not up to the present been determined.

While the method of grading put forward in the present paper is undoubtedly of very wide application, the writer is not prepared to state that where the conditions as laid down on p. 41 are fulfilled, a graded series of any particular membrane substance can always be obtained. Thus it is not certain that graded rubber membranes for work in alcohol can be prepared by use of benzene-alcohol mixtures. As far as preliminary observations went, it appeared that in all cases the differential treatment became obliterated when the membranes were washed in alcohol. The nature of the particular membrane substance therefore plays a part in determining the nature of the result obtained. It is noteworthy that all the substances with which the most marked success has been achieved—agar, gelatin, celloidin—are characteristically plastic substances, whereas rubber is eminently elastic. The difference in effects observed is believed to be correlated with this difference in respect of shear elasticity.

Again, as was stated in the earlier paper dealing with this subject, it was found impossible to produce any effective grading of celloidin by use of acetone-water mixtures. The range of mixtures here available was somewhat limited, 0–60 %, but in addition to that it was observed that the celloidin films towards the upper end of the series tended to disintegrate without the marked continuous swelling which is characteristic of similar films when placed in alcohol. The volume of the continuous phase of such a membrane up to the stage where it was still a manageable membrane was therefore too small to allow of its ever being converted into even a moderately permeable membrane.

At this point it may be opportune to summarise what is considered an ideal relationship in the system:—

Swelling liquid—membrane substance—restraining liquid,
from the point of view of the present technique.

Membrane substance—swelling liquid. The membrane substance should not go into complete solution, or if it does it should reach that state by a process of continuous imbibition. It should preserve a practicable degree of mechanical coherence up to a high degree of swelling. Only in such a case is it possible to prepare highly permeable membranes of practical utility.

Membrane substance—restraining liquid. The membrane substance should not take up anything beyond a trace of the restraining liquid. This condition is nearly realised in the case of celloidin in water, though not sufficiently so

to prevent the passage of water and the simplest salts. In the case of the cellulose-acetate membrane this condition is less closely realised, so that it is doubtful if this substance can be used for the preparation of so-called semi-permeable membranes.

B. GRADED MEMBRANES FOR USE IN THE SWELLING LIQUID.

Though only one series of such membranes is as yet known (viz. gelatin in water), and though the range of permeability of these is comparatively limited, nevertheless they are considered to possess such practical and theoretical interest as to merit a detailed description. It is known at present for instance that these gelatin membranes can be applied with great effect in the purification of certain enzymes and that they show properties which indicate that they may prove of considerable use in the treatment of phenomena of selective permeability.

Theory of Method.

In all the processes hitherto discussed, the films of membrane substance, once a state of differential swelling had been established by immersion in the series of "swelling-restraining" liquid mixtures, were without further treatment transferred to the pure restraining liquid. It was obvious on theoretical grounds (and was abundantly verified by experiment) that were transference to take place to the swelling liquid, all the membranes would rapidly swell up to much the same degree, and thus the differential effect would be obliterated. If however while the membranes were still immersed in the series of mixtures, the capacity of imbibing the swelling liquid could be suppressed or at any rate reduced to a small amount, it was argued that after such treatment the membranes could be transferred to the swelling liquid without obliteration of the graded effect. The method adopted in the case of gelatin is as follows.

Films of air-dried gelatin are placed in a series of alcohol-water mixtures at 20° C. After 24 hours, a given quantity (1 cc. to 10 cc. of alcohol-water mixture) of 40 % formaldehyde is added to each. After 24 hours' treatment in this solution, the membranes are transferred to water and thoroughly washed; they are then ready for use.

It will be noticed that the addition of the formaldehyde somewhat alters the alcohol-water ratio in the mixture. It is obvious that the process could be carried out without this ratio being altered, but such a refinement of the method has not as yet been deemed to be necessary.

A typical series of results is set forth in the following table.

The gelatin consisted of the ordinary sheet gelatin (as supplied for manufacture of culture media). After treatment as indicated above, the membranes were washed for two days in distilled water.

TABLE VI.

Treatment (% alcohol)	$\frac{\text{Dry weight}}{\text{Wet weight}} \times 100$
0	7.35
20	13.66
40	24.29
60	36.70
80	45.93
100	47.93

The grading may also be illustrated by measuring the increase in length and increase in thickness of the membranes. The following table gives the lengths and thicknesses of strips of commercial sheet gelatin after treatment according to the present technique, the corresponding quantity for the original aid-dried membranes being taken as 100 in each case.

TABLE VII.

Membrane	0 %	20 %	40 %	60 %	80 %
Length	112.5	105.4	103.3	102.2	102
Thickness	390	350	290	225	185

The grading is thus shown markedly by alteration in thickness of the membrane. The effect in lengthening the membrane is comparatively slight. In the case of membranes prepared from films which have dried under tension according to the method described on p. 42, an actual contraction due to the release of this tension has been observed when the membranes are soaked in the intermediate concentrations of alcohol.

By this technique membranes ranging from about 5 % to nearly 50 % of dry substance can be prepared. In no case has a membrane of greater content than 50 % in dry weight been obtained; in other words, it has been found impossible to formalise a membrane so strongly as to prevent it taking up on immersion in water at least an amount of water equal to its own weight. At the other end of the series a limit is placed by the increasing mechanical weakness of the membrane. Membranes of less content in gelatin can readily be prepared by carrying out the imbibition process at a somewhat higher temperature, but their extreme fragility renders them of little practical value.

Test of Uniformity. With similarly prepared dry films, and similar treatment, a very considerable degree of uniformity in the resultant membranes can be attained. This is illustrated in Table VIII which gives the results of an experiment carried out in triplicate.

TABLE VIII.

Alcohol %	0 %	20 %	40 %	60 %	80 %	100 %
Dry weight	12.66	17.86	23.86	34.76	44.07	26.43
Wet weight $\times 100$	13.05	18.08	23.69	34.60	44.09	26.87
	12.17	18.14	23.83	34.72	44.09	26.83

Throughout this series the maximum variation from the mean is less than 0.5 %. This degree of uniformity is however by no means attainable when gelatin films of different origin are employed. A comparison of Tables VI and VIII brings out the essential features of the variations experienced. In both cases crude sheet gelatin as supplied for the manufacture of culture media was employed, that in the latter series being of a different make from the former.

A considerable amount of experimental work has been carried out with a view to determining the range of variation and the means of experimental control. The results may be briefly summarised as follows.

Considerable variations may be shown at the lower end of the series (membranes prepared in water and 20 % alcohol) by different samples of gelatin. This difference must be ascribed to the previous history of the gelatin. Films prepared in the manner described on p. 42 show a higher degree of swelling in water and 20 % alcohol than is shown by the original commercial sheet gelatin itself. This sensitiveness of gelatin to previous heating has already been mentioned.

Within considerable limits, the figures obtained are independent of the initial thickness of the membranes.

In the middle region of the series (40, 60, 80 %) remarkable constancy in results is obtained, no matter what the type of gelatin employed may have been. Thus in several hundred measurements which have been made at one time or another with different samples of gelatin, a maximum variation of about 3 % only has been observed in the dry weight percentage of such membranes.

A remarkable variation is shown by different samples of gelatin in respect of the membranes soaked in 100 % alcohol. This feature is illustrated by

a comparison of Tables VI and VIII. In the former case, the membrane prepared in 100 % alcohol differs only slightly from that prepared in 80 % alcohol; in the latter table it has a much lower gelatin ratio. This is due to the fact that in the latter case only the outer layers of the membrane are formalised, the inner mass being highly swollen and unformalised. By lengthening the formalising period, this result can be avoided. In any case it may be noted that very little practical advantage is obtained by extending the series beyond the 80 % mixture.

Length of Imbibition Period. Though a slow imbibition in water and the lower concentrations of alcohol can be demonstrated over several days, nevertheless the effect so produced is so slight as to render an imbibition period of over 24 hours unnecessary.

Length of Formalising Period. It has been seen that in the case of membranes in 100 % alcohol, a period of 24 hours may be insufficient for formalising. In the case of membranes in 0—80 % alcohol, only a slight difference could be detected between membranes which had been formalised for 4 days and those which had had 1 day's formalisation. Thus 24 hours may be considered ample for the process. Table IX, giving a comparison of 6, 10, and 24 hours' formalising, shows that the period allotted (24 hours) cannot safely be reduced.

TABLE IX.

Formalising period	Membrane					
	0 %	20 %	40 %	60 %	80 %	100 %
6 hrs.	6.93	12.43	20.80	30.82	37.31	32.94
10 „	7.24	12.93	21.64	33.33	40.56	42.03
24 „	7.33	13.24	23.20	36.48	44.32	45.83

Permanence of Graded Effect. Unlike the membranes treated of in Section A, the membranes of the present section are known to undergo a definite and appreciable change when kept in water. This consists in a slow uptake of water, so that the figures representing the gelatin ratio undergo a steady decline. The amount of this effect will appear from Table X.

The membranes were prepared in the usual way, i.e. with 24 hours' imbibition in the alcohol-water mixtures, and 24 hours' formalising. The first wet weight readings were taken after 3 days; and then further readings taken at intervals of 7 days. From preliminary experiments it was known that the absolute dry weight of formalised gelatin films when kept in water does not appreciably alter. Therefore the relative gelatin content of the membranes at any time can be determined by measuring the wet weight at the particular time and the dry weight at the end of the experiment. The figures in the table are as usual the percentage ratio of dry weight to wet weight.

TABLE X.

Mem-brane	After 3 days	After 10 days	After 17 days	After 24 days	After 31 days	After 38 days	After 45 days	After 66 days
0 %	7.45	7.53	6.61	5.80	5.42	5.55	6.33	7.10
20 %	14.66	14.13	13.20	12.33	12.28	12.20	12.20	13.00
40 %	23.62	22.62	21.00	19.70	18.84	17.83	17.00	16.54
60 %	35.67	34.01	32.00	29.55	28.13	26.83	25.50	23.26
80 %	44.90	42.00	39.42	36.00	35.00	33.00	31.60	30.00
100 %	45.95	42.13	40.00	37.31	34.82	32.81	30.63	26.71

It will be seen that in the case of the 80 % alcohol membrane, the ratio of dry to wet weight decreases by about 2.5 % per week; that the weekly decrease in the case of the 40 % alcohol membrane is about 1 %. The membranes at the lower end of the series show a very gradual decrease. The slight increase shown towards the end of the experiment may have been due to a certain amount of bacterial contamination which appeared in the later stages of the experiment.

Attempts were made to reduce the rate of change indicated above, but so far without success. Thus membranes which were exposed for 24 hours to the action of formalin of full strength (40 % formaldehyde) in addition to the ordinary formalising treatment were found to behave in very much the same way as those which had only received the ordinary treatment. This property must therefore for the present be regarded as inevitable, and its presence must be remembered in all applications of these membranes.

The rate at which these membranes are degraded is considerably increased by the presence of acid and alkali. Tables XI and XII illustrate this effect.

TABLE XI.

Effect of Acid.

Membrane	Membranes in water		Membranes in $n/100$ H_2SO_4	
	After 1 day	After 15 days	After 1 day	After 15 days
0 %	7.91	8.22	7.60	5.56
20 %	13.00	12.50	14.87	10.62
40 %	24.67	22.30	23.00	15.39
60 %	34.67	30.35	34.74	21.77
80 %	42.53	35.70	42.57	28.40

TABLE XII.

Effect of Alkali.

Membrane	Membranes in water		Membranes in $n/100$ $NaOH$	
	After 1 day	After 15 days	After 1 day	After 15 days
0 %	7.28	7.73	7.00	5.00
40 %	22.74	21.00	24.00	15.63
60 %	37.44	33.04	37.46	24.67
80 %	45.80	40.00	46.00	32.18

With increasing strength of acid or alkali, it is highly probable that the rate of degradation is further increased. The instability of the membranes under such conditions renders them therefore unsuitable for work in acid or alkaline media. It may also be mentioned incidentally that membranes of gelatin cannot be used in the presence of acid pepsin, as they are rapidly attacked and totally destroyed.

GENERAL REMARKS ON THE APPLICATION AND USES OF THESE MEMBRANES.

In applying these membranes to work on diffusion and dialysis it is to be kept in mind that in any series of membranes prepared from films of the same initial thickness, the more permeable members of the series are much thicker than those at the other end of the series. It will be observed that in this respect the technique of the present writer differs essentially from that described by Bechhold [1907]. The latter impregnated filter papers with solutions of various strengths of gelatin in water, or of celloidin in acetic acid, allowed to drain for a given time, and fixed, the former by immersion in 2–4 % formalin solution, the latter by immersion in water. It is obvious that by reason of viscosity the film which remains on the filter paper after drainage will be thicker in the case of the higher gel concentrations than in the case of the lower ones, and that therefore the apparent diminution of permeability of the membranes with higher gel content may be due in part simply to their greater thickness. If therefore the membranes described in the present paper be used in an investigation of the relationship between the "pore size" of a membrane and its capacity for allowing passage to substances in solution, it will be necessary to proceed in such a way as to ensure equal thickness in the resulting membranes. The initial dry films must thus be made correspondingly thinner when it is desired to use them for the preparation of the more permeable membranes. It is obvious that by a process of trial and error this experimental difficulty can readily be overcome.

The gelatin-in-water membranes described in the present paper compare favourably with the similar membranes prepared according to Bechhold's technique in respect of the much wider range (up to 50 % gelatin) of permeability and of the readiness by which they can be characterised. Bechhold's method was to standardise each particular lot in relation to its behaviour to a standard haemoglobin solution. The membranes of the present technique are standardised simply by determining the ratio of dry to wet weight of a

sample portion. The determination of this ratio is difficult if not actually impossible in the case of Bechhold's membranes on account of the presence of the supporting matrix of filter paper. It should also be noted that in Bechhold's technique, the concentration of gelatin in the final membrane may not be that of the gelatin solution originally employed. As the process of formalisation is a comparatively slow one, the gelatin membrane may swell considerably before that process is complete. It has for instance been found that membranes with as low a gelatin percentage as 5 % may be obtained by directly immersing air-dried films in 4 % formaldehyde at 20° C. Thus when in Bechhold's technique a filter paper impregnated with 10 % gelatin is placed in 4 % formaldehyde (even at 0° C.) it is not in the least certain that it formalises as such; on the contrary there is every probability that it swells during the process, producing a membrane of lower gelatin content and greater thickness. This consideration adds weight to the criticism as regards thickness emphasised above.

The method adopted by the present writer to characterise the collodion-in-water membranes was to state the concentration of alcohol in the mixture used in their preparation. In the case of the membranes dealt with in this paper (chiefly gelatin and agar), it has been shown that certain other factors (especially the thermal history of the gel substance) play a somewhat pronounced rôle. Unless therefore it be found possible and convenient to adhere to standard material, it would appear advisable to state, in addition to the alcohol concentration employed in the manufacture of the membrane, the ratio of dry weight to wet weight of the latter as determined on a sample portion.

Methods of attaching these membranes to glass-ware for purposes of diffusion and dialysis experiments will be described in a subsequent paper.

As regards the scientific or other interest of the large variety of membranes described in Section A of the present paper, it is obviously impossible to speak with certitude at the present stage, but it is plain that through them the scope of investigation by means of the processes of diffusion (in particular, dialysis) and adsorption is considerably extended. The present writer has begun an investigation by means of these membranes of the relation between the power of a membrane to adsorb a particular substance and its power to transmit it by diffusion, that is, of the relation existing between adsorption and permeability.

The practical aspects of this technique may also be mentioned. In the case of substances which are not readily separable from each other by dialysis

in water, it may be possible to find a solvent in which dialytic separation is practicable. An illustration of such is afforded in the case of Congo red and night blue dyes. Whereas no practicable separation can be obtained in aqueous solution, both substances being highly colloidal in water, the blue dye can be dialysed away with considerable ease in alcoholic solution, and thus a separation effected.

Lastly, these membranes can be used, not only in a large variety of liquids, but also in mixtures of the same. It is believed that in this feature there lies the possibility of an interesting investigation. Thus if a particular substance is crystalloidal in a particular solvent and colloidal in another, then it is probable that it shows intermediate characters in mixtures of the two solvents. It would thus be possible to vary the colloidal nature of the particular substance at will, and to determine its properties (diffusibility, degree of adsorption) in each case with a variety of membranes in equilibrium with the solvent mixture.

As regards the series of gelatin-in-water membranes treated of in Section B, it is already possible to speak with more definiteness. These membranes cover a portion only of the range covered by the collodion-in-water membranes, being, so far as is known at present, permeable to all electrolytes and to such simple colloids as safranin, neutral red, etc. As regards more colloidal substances, investigation has already shown that the order in which substances arrange themselves in reference to their diffusive capacity through gelatin is not the same as in the case of collodion membranes. One well authenticated case is afforded by the behaviour of starch and night blue solutions. It has been found possible to prepare a collodion membrane which completely prevents passage of night blue while definitely allowing passage to starch, whereas gelatin membranes have been prepared which allow slow passage of night blue but completely hold back a starch solution. It is hoped when this subject is further pursued that such a behaviour will be shown by a variety of substances (especially dye substances), so that a method may be evolved of attacking the problem of selective permeability in its purely physical relationships.

These gelatin-in-water membranes have been found to show properties which indicate that they may prove of service in the technique of enzyme purification. Membranes at the lower end of this series allow of the diffusion of the enzymes diastase and cytase, while those at the upper end hold them back. It is hoped to publish shortly an account of an investigation relating to the subject of enzyme purification.

Lastly, in relation to the phenomena of selective permeability, it would appear that certain dialytic processes can be carried out with greater effectiveness by means of these gelatin membranes than with membranes of collodion. Thus preliminary observations have shown that the dialytic separation of a starch-dextrin mixture can be carried out with greater rapidity with certain gelatin membranes than with any of the collodion series.

SUMMARY.

The general principle by which membranes of graded permeability may be prepared is as follows:—

Suppose a system consisting of:—Liquid *A*—Membrane substance—Liquid *B*.

- If (1) the membrane substance only imbibes a negligible amount of *A*,
- (2) the membrane substance strongly imbibes *B*,
- (3) *A* and *B* are miscible in all proportions,

membranes of graded permeability for use in the liquid *A* can be prepared by immersing films of the membrane substance in mixtures of *A* and *B* and transferring to liquid *A*. In this way are prepared graded membranes of collodion in water, chloroform, benzene, etc.; of agar and gelatin in alcohol, acetone, benzene, etc.; of cellulose acetate in water, alcohol, benzene, etc. Graded membranes for use in *B* can be prepared only when a method exists of suppressing the capacity of the membrane substance to imbibe *B*, this suppression to be brought about at the stage where the membranes are still immersed in the mixture of *A* and *B*. In this way graded membranes of formalised gelatin for use in water have been prepared.

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