Studies in Dialysis

2. CHANGES OF pH DURING DIALYSIS

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In Part 1 of this communication (Gilbert & Swallow, 1950) an experiment was described in which potassium chloride was dialysed through cellophan against distilled water. A small decrease in pH was observed, in agreement with previous observations by Schwarz & Huf (1932). Much larger effects are known to occur in electrodialysis, and have been extensively investigated in order to eliminate them (Manegold, 1940), but the much smaller effects in ordinary dialysis are usually ignored. It is probable that the irreversible loss in activity of acid-sensitive enzymes, which occasionally occurs in dialysis, is sometimes caused by a temporary and unnoticed fall in pH. An examination was therefore made of the pH changes occurring in the dialysis of salt and buffer solutions in common use under the normal conditions of the laboratory.

conditions of dialysis. Resin-impregnated cellophan was prepared by the method described previously (Gilbert & Swallow, 1950). Each experiment was carried out at least in duplicate.

Fig. 1. The pH changes on dialysing 0.01M-KCl, K_2SO_4 and $BaCl_2$ against distilled water through ordinary cellophan (A, B and C respectively) and resin-treated cellophan (D, E and F respectively). Initial pH approx. 5.5 in all cases.

METHODS

The apparatus used for dialysis against flowing distilled water was similar to that already described for dialysis against ion-exchange resins (Gilbert & Swallow, 1950). The water flowed through the central tube previously occupied by resins. Samples of 5 ml. were pipetted at intervals from the annular space, and their pH measured with a Cambridge Instrument Co. pH meter. No precautions were taken to exclude CO_2 from the dialysis apparatus, since it was intended to study the behaviour of solutions under the usual

Fig. 2. The pH changes on dialysing 0.01M- (A) and 0.41M- (B) KCl against distilled water through a cellophan membrane.

Fig. 3. The pH changes on dialysing 0.01M-phosphate buffer ($K_2HPO_4 + NaOH$) (A), phthalate buffer (KH phthalate + $NaOH$) (B) and $(NH_4)_2SO_4$ (C) against distilled water through cellophan.

RESULTS

Curves for ordinary cellophan and resin-treated cellophan in Fig. 1 show the pH changes observed when 0.01M solutions of barium chloride, potassium sulphate and potassium chloride were dialysed

against distilled water of pH 5.4 flowing at about 120 ml./min. The dialysis of 0.41M-potassium chloride is compared with that of 0.01M-potassium chloride in Fig. 2, and the dialysis of buffer solutions, each 0.01M with respect to their anions, is shown in Fig. 3.

Fig. 4. The pH changes on dialysing 0.01M- K_2SO_4 (A), KCl (B), $BaCl_2$ (C) and $(NH_4)_2SO_4$ adjusted to pH 7 (D) against Birmingham tap water through cellophan.

Fig. 5. The effect of changing from Birmingham tap water to distilled water during the dialysis of 50% (w/v) $(NH_4)_2SO_4$ (pH adjusted initially to approx. 7).

The result of dialysing against tap water of pH 7.0 flowing at 40 ml./min., is shown in Fig. 4, and the effect of changing from tap water to distilled water during the dialysis of ammonium sulphate in Fig. 5.

DISCUSSION

In view of the negative character of cellophan film, and the theories of membrane selectivity proposed by Teorell (1935), Meyer & Sievers (1936), Manegold (1940) and others, a lowering of pH during the dialysis of salts is to be expected. The specific

hindering of the movement of anions through the membrane leads to a difference in potential between the two sides of the membrane, and so to the attraction of hydrogen ions into the salt solution from the external water. Schwarz & Huf (1932) encountered this effect during the dialysis of 0.1M-potassium chloride solution through cellophan, and showed with bromthymol blue that the salt solution became acid.

A glance at the present results shows that they are in keeping with these theories, and reveals further that the fall in pH can be considerable. In particular it is seen that even buffer solutions are strongly affected, although here one might have expected the pH to be maintained. The operation of reducing the concentration of ammonium sulphate in a solution by dialysis against distilled water can lead to a change of pH which is quite sufficient to damage many proteins.

The pH change is dependent upon the nature of the ions of the salt. A comparison of the curves for potassium chloride and potassium sulphate illustrates the effect of an increase in the valency of the anion, again in accordance with expectation. The curve for barium chloride shows that the cation may exert a strong effect. In this case the rise in pH may be due to suppression of the negative charge of the cellophan by adsorbed barium ions. Where a small and rapid fall in pH is found at the beginning of dialysis, it is probably due to hydrogen ions arising from the ionization of the carboxyl groups of the cellophan by exchange with cations from the dialysing salt.

Reference to Fig. 1 shows that the substitution of a modified cellophan membrane, in which the negative groups have been counteracted by a basic resin, prevents the formation of a very acid solution. Alternatively the pH can be kept higher by dialysing against tap water, the pH of which in Birmingham is about 1.5 units more than that of distilled water in equilibrium with carbon dioxide of the air.

The examples in this paper illustrate the general effects to be expected during dialysis through cellophan, but in any specific case, other factors such as the nature and concentration of the non-diffusing substances present have to be considered. It is very clear, however, that if distilled water is used, one must expect the solution to become temporarily acid.

SUMMARY

1. The dialysis of salt solutions, including buffer solutions, is shown to result in a decrease of pH when the electronegative membrane cellophan is used. Treatment of the membrane with basic resin eliminates this decrease.

2. The changes in pH, arising from the ion-selectivity of the membrane, are often great enough

to endanger proteins or other substances which are sensitive to acid.

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The Cytological Interpretation of the Feulgen Reaction

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The chemical test for deoxyribosenucleic acid discovered by Feulgen (1914) and applied by Feulgen & Rossenbeck (1924) under the name of nucleal stain to histological sections has served many useful purposes in the hands both of its originator and of subsequent workers. It now finds much favour as a staining process for cytological preparations containing dividing cells, partly, no doubt, because of the particularly clear pictures of the chromosomes which result from its use, but mainly because of the belief that it specifically stains those nuclear structures which contain nucleic acid. Thus, because the chromosomes are the only structures in dividing cells which become stained by this method, it is assumed that these organs contain the whole of the nucleic acid of the nucleus, an assumption which is sometimes expressed by the statement that nucleic acid does not occur elsewhere in the nucleus than in the chromosomes. As an aid to studies of the morphology of chromosomes, nucleal staining may be unobjectionable, but when it is employed for ascertaining the distribution of nucleic acid within the nucleus it becomes open to serious criticism. For such a use is based on the assumption that the nucleic acid 'stains' *in situ*, an assumption which ignores the well known fact that the violet dye produced when isolated nucleic acid is submitted to Feulgen's reaction (hydrolysis with dilute hydrochloric acid and treatment of the neutralized product with Schiff's reagent) is soluble in water. Unless nucleic acid in the nucleus behaves differently from isolated nucleic acid, it follows that those nuclear structures which become stained by Feulgen's process do so because they are composed of non-diffusible material which adsorbs or otherwise combines with the diffusible, violet dye, and that

consequently nucleal staining can provide no certain information regarding the distribution of nucleic acid in the nucleus of the living cell. Evidence of an indirect nature supporting these conclusions has been adduced elsewhere (Stedman & Stedman, 1943, 1947). Direct proof is now provided that the hydrolysis of isolated cell nuclei under conditions used in nucleal staining gives rise to diffusible products which react with Schiff's reagent to form a soluble violet dye.

METHODS AND RESULTS

Isolation of cell nuclei. The method employed, which was evolved from that of Stoneburg (1939), has been used continuously in this laboratory since 1943 for the isolation of nuclei from the thymus glands, spleens, livers and tumours of various species. It has not hitherto been described in detail, although the principles on which it is based have been mentioned in publications of a general nature (Stedman, 1944; Stedman & Stedman, 1947). It differs from Stoneburg's method chiefly in two respects: the operations are carried out at a lower acidity, using acetic in place of citric acid, and the use of pepsin has been dispensed with. The detailed procedure is as follows. Ox liver (0.5–1.5 kg.) from a freshly killed animal is minced in a machine, first coarsely and then finely. The resulting pulp is poured into several volumes of 4% acetic acid. The exact proportion of acid to pulp is immaterial as regards the liberation of nuclei, but subsequent operations are facilitated if a ratio of not less than 3:1 is used. The mixture is, without delay, stirred vigorously with a mechanical stirrer for about 15 min., this operation being repeated four times at short intervals. It is then left for 2 hr. to allow froth to drain, a period which may be extended to 12 hr. if the timing of the experiment necessitates leaving it overnight. Much tissue fibre and other non-nuclear material, as well as viscous lumps of impure nuclear material, presumably originating from nuclei ruptured during the mincing process, are then