A METHOD FOR THE RAPID DIALYSIS OF LARGE VOLUMES OF PROTEIN SOLUTIONS

By H. A. ABRAMSON AND E. B. GROSSMAN

(From the Laboratory of General Physiology, Harvard University, Cambridge)

(Accepted for publication, January 30, 1931)

The removal of salts from solutions of proteins can be a laborious task. The method of Kunitz and Simms (1) was an important step in the direction of making this task simpler. We have found it convenient and necessary to be able to dialyze proteins rapidly, without undue loss due to denaturing, and without using a preservative. Methods of dialysis without circulation of the protein solution and of the outside liquid take several days, and frequently weeks, necessitating the employment of preservatives. Spontaneous denaturing of the protein is appreciable during long periods of dialysis. The method here described largely avoids these difficulties. It can be varied in certain ways. For example, the outside air can be excluded; the angle of the membrane-support can be changed; greater or less economy of distilled water can be effected. The whole apparatus can be placed in a cold room, or other methods can be employed to keep the temperature low.

Description of Apparatus

The design of the apparatus is indicated in Figs. 1–3. The outside liquid is contained in an ordinary metal wash basin (*A*), paraffined on the inside, fitted with intake pipe (*B*) and overflows (*C* and *C'*) to permit the circulation of water as indicated in Fig. 1. The cover of this vessel (*D*), on which a constant speed motor (*E*) is mounted, is just wide enough to support the motor and the casing for the drive shaft (*F*), which is inclined at an angle of approximately 70° to the horizontal. The supporting racks (*G*), of which one or two may be attached to the shaft by a sleeve and screw (*H*), can each support six membranes. The method of supporting the membranes on the rack is shown in Figs. 2 and 3. The membranes (*I*) are held in place by the pegs (*K*) on the rack, by two long rubber bands (*L* and *L'*) stretched completely across the pegs and passing over the membranes, and by glass rods (*M*) which pass through single-hole rubber stoppers fixed in the mem-

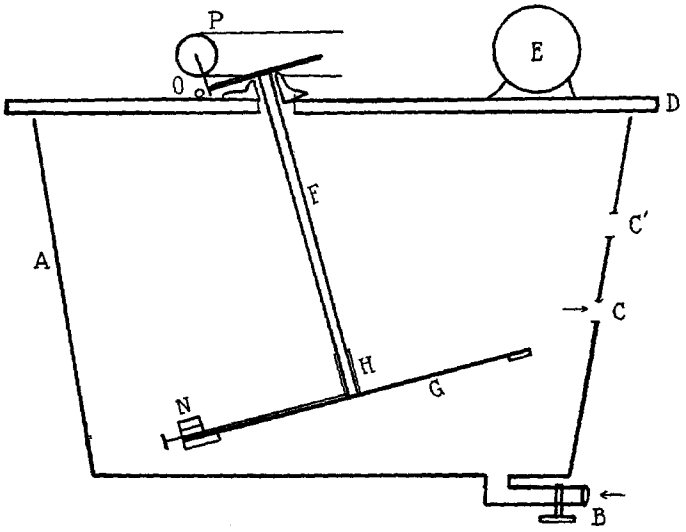

FIG. 1. Cross-section through entire apparatus. × 1/6 approximately. (See text.)

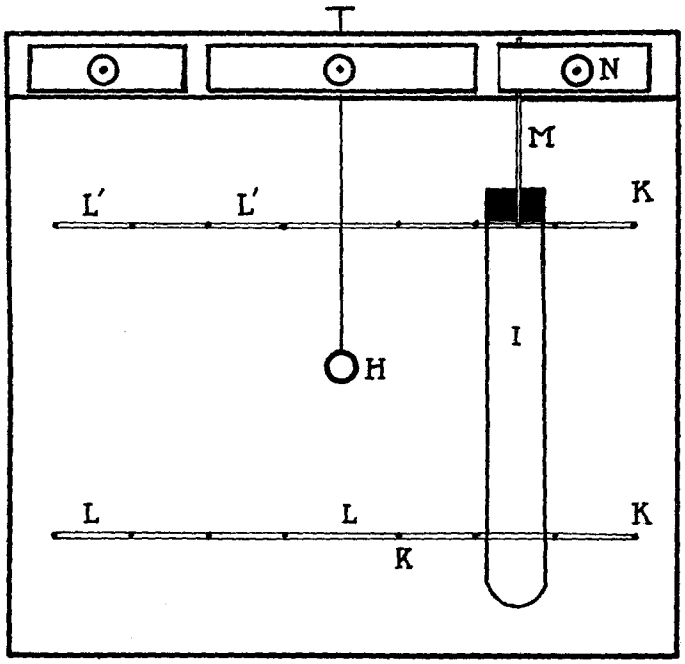

FIG. 2. Top view of rack (*G*) that supports membranes. × 3/10 approximately. (See text.)

branes. These rods in turn are fixed in place by the spring clamps (*N*) on the rack. The drive shaft is connected with the motor by the worm gear (*O*) and pulley (*P*).

At first the membranes were prepared by depositing in the usual fashion three layers of collodion, U.S.P.X, on the inside of 27 × 200 mm. test tubes, and drying in air for 20 minutes. Later this type of membranes was supplemented by the use of 25 mm. diameter "Visking" tubes,[1] which proved more satisfactory in that the necessity for the preparation of collodion membranes was eliminated. Using this apparatus and the "Visking" tubes, twelve membranes, each containing 100 cc. of crystals or of solution, may be dialyzed simultaneously.

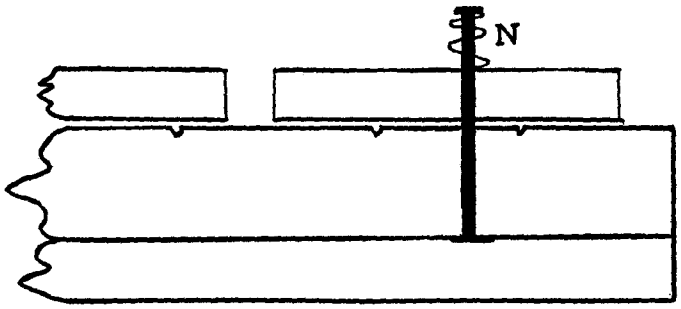

FIG. 3. Schema of arrangement for attachment of membrane. × 2/3

EXPERIMENTAL

The cylindrical membranes are filled to within a few cm. of the top with the solution to be dialyzed and are fixed in place on the rack. Tap water is then allowed to flow through the outer vessel at a rate of 1.5 to 4 liters per minute. As the inclined rack is rotated at 6 R.P.M. by the motor, the air bubble[2] in the membrane tube provides gentle stirring. When dialysis against tap water is complete the outer vessel is drained, the intake pipe shut off, and dialysis is continued against distilled water. In obtaining the illustrative data given in Table I only dialysis with one change of distilled water was used.

It was found that denaturing of protein was negligible during the short time required for dialysis. The loss of protein by leakage was

[1] Kindly supplied by the Visking Corporation. Two stoppers, one of them single-holed, are fixed with rubber bands into the ends of the Visking tubing. It is necessary to test each membrane for leaks.

[2] We are indebted to Professor Crozier for the suggestion that a smooth glass sphere may be more suitable than the air bubble since probably less denaturization would occur.

determined by the Koch-McMeekin method, and was found to amount to less than 5 per cent after 12 hours' dialysis. The rate of disappearance of salt was determined both with pure salt solutions and with solutions containing proteins. Half-saturated ammonium sulfate was dialyzed ammonia-free (as tested with Nessler-Folin reagent) within 12 hours. Some of the results obtained with the protein solutions are given in Table I. Not more than three membranes were employed in these experiments. The rate of dialysis against distilled water may be appreciably affected by the number of membranes used in each experiment.

TABLE I

These data illustrate the rate of disappearance of salts from cylinders of collodion and of "Visking" tubing under the experimental conditions described in the text.

Protein solution	Time dialyzed (hours)		Spec. conductivity $\times 10^5$ reciprocal ohms
	Tap water	Distilled	
1. Serum globulin	24	0	13.8
2. Egg albumin (10 per cent)	12	12	16.9
3. Egg albumin (2 per cent, approximately)	2½	0	857.0
	5½	0	96.3
	7¼	0	42.0
	8	0	36.9
	8	4	13.9

The conductivity of the tap water used was 12.0×10^{-5}; the conductivity of the distilled water (after exposure to the air) was 1.40×10^{-5} reciprocal ohms. In the dialysis of Solutions 1 and 2, collodion sacks were used. In the dialysis of Solution 3, "Visking" membrane was used. In all cases the solutions were half saturated in ammonium sulfate before dialysis.

These results, especially those obtained with Solution 3, compare favorably with the value of 18×10^{-5} obtained by Kunitz and Simms (1) after 24 hours dialysis against circulating distilled water, and the value of 16×10^{-5} obtained by them after 31 hours.[3]

[3] The apparatus can be obtained from Mr. J. H. Emerson of Cambridge, Mass.

SUMMARY

A method is described by which moderately large quantities of protein solutions can be dialyzed relatively free of salts within 12 to 24 hours. The use of "Visking" tubes instead of collodion bags is recommended, for speed of dialysis as well as ease of manipulation.

CITATION

1. Kunitz, M., and Simms, H. S., *J. Gen. Physiol.*, 1928, **11**, 641.