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EDEMA

II. The Effectiveness of Ultrafiltration for Quantitatively Determining the "Free Water" Content of Blood Plasma, and for Estimating Physical-Chemical Changes of the Plasma Proteins in Edema

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The physicochemical condition of the blood plasma in edema has been the subject of much interest due to the attention which Fisher's (1) writings have drawn to the rôle of the colloids. In connection with a discussion of the causes of defective water secretion by nephritic kidneys, Fisher suggested that "acid acts upon the tissues of the body including the blood and lymph. The increased hydration capacity resulting from this makes the tissues hold more water in combined form (maintenance of body edema) while at the same time it prevents water becoming 'free' in the arterial blood stream." A review of the evidence for and against this theory is entirely outside of the scope of this paper. Our interests regarding Fisher's theory are chiefly directed towards the researches recently published by Beckmann (2).

By a process of ultrafiltration Beckmann studied the "free" water content of the blood plasma of seven cases of edema with varying etiological factors. In some instances he obtained a diminished amount of ultrafiltrate. Beckmann assumes that the amount of ultrafiltrate obtained from certain of these patients was diminished because the plasma colloids were swollen and held part of the water of the blood in combination so that it was not obtained by the process of ultrafiltration. In other instances where he obtained an increased amount of ultrafiltrate, he assumes that a converse physicochemical change had occurred in the plasma proteins, i.e., they were shrunken, thus lessening their ability to combine water, which would result in an increased amount of free water in the blood plasma.

Beckmann used the method of ultrafiltration described by Ellinger and Neuschlosz (3). He also made quantitative determinations of the dry residue of the blood plasma and of the plasma proteins. He assumes that quantitative differences in the dry residue may cause variation in the amount of ultrafiltrate delivered, independent of the shrunken or swollen condition of the plasma colloids and developed a formula to correct for this variation.

Our purpose in undertaking this research is not to utilize the formula developed by Beckmann, but simply to determine whether measurable differences from the normal can be found in the amount of water obtained by ultrafiltration from the plasma of patients with varying types of nephritis and edema, and to determine whether these differences, if present, bear any relationship to quantitative variation in the plasma proteins. Moreover, if in edema the plasma proteins become shrunken or swollen, such changes should be detected by using ultrafilters of graded permeabilities.

METHOD

The details of the technique employed in the process of ultrafiltration and the contributions of pioneer workers have been discussed by Bechhold (4); more recently Zilva and Muira (5) have contributed to the method by a different process for standardizing membranes. Cushny (6) in his researches directed toward determining whether or not the crystalloids of blood plasma exist in combination with colloids, reduced the high pressure advocated by Bechhold (1 to 20 atmospheres) to 21 cm. of Hg.

We employed the essential principles of the technique advocated by Beckmann, i.e., a pressure equal to 77 mm. Hg, and the gel for making membranes which was a glacial acetic acid solution of parlodion. Figure 1 illustrates the simple apparatus which proved entirely effective for producing and maintaining, at a constant level, low hydrostatic pressures. The pressure is raised by establishing a siphon which is not interrupted during the experiment, so that if a lowering of pressure occurs in the system by a slight leak of air or the filtering through of a few cubic centimeters of fluid, it is immediately restored with water from the reservoir.

The ultrafiltration sacs employed in the experiments of table 1, were made according to the technique used by Beckmann, i.e., paper Soxhlet extraction thimbles were impregnated with a glacial acetic acid solution of parlodion (DuPont) by suspending each with a loop of thread sewed through the upper part 1 cm. from the top and filling with the gel. The gel was of such consistency that complete impregnation (indicated by drops of gel falling from the outer surface of the sac) occurred in 3 to 5 minutes. The excess of parlodion was allowed to drain off,

then the sacs were fixed in distilled water and washed in running water for 12 hours. When not in use they were kept under distilled water in the ice chest. The membranes were brought into the pressure system by mounting each on a perforated rubber stopper of slightly less diameter than the membranes. Short glass tubing, one end of which was connected to the pressure system, the other end passing

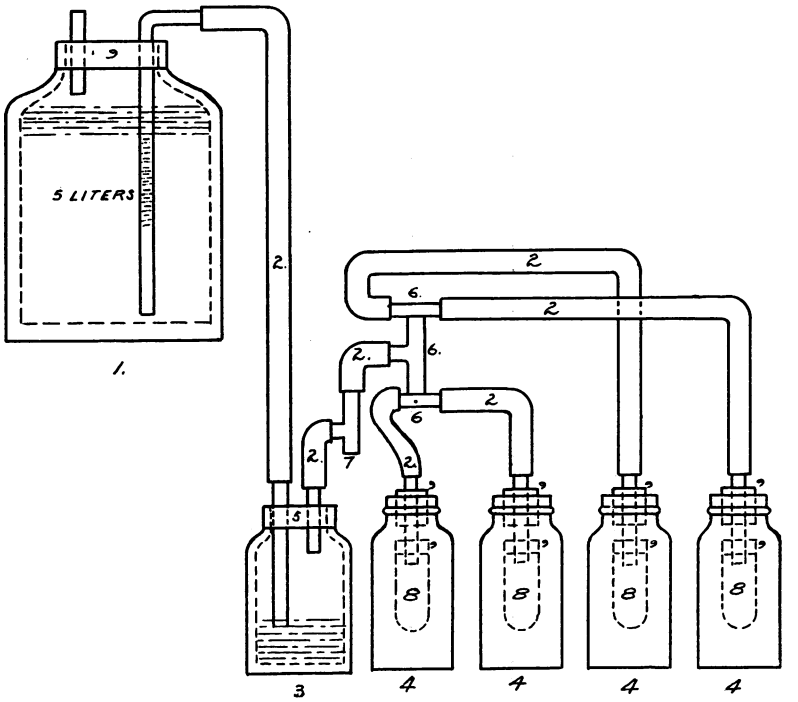

FIG. 1. PRESSURE SYSTEM WITH MEMBRANES ATTACHED

1, cistern; *2*, rubber tubing; *3*, pressure bottle; *4*, collecting bottles; *5*, metal top; *6*, "T" tubes; *7*, "T" tube for manometer; *8*, membranes; *9*, rubber stoppers.

through the perforated stoppers, admits the pressure from the system into the membranes. Above the filtration sac, the tubing passes through a large rubber stopper which loosely covers the receiving bottle. This prevents excessive evaporation of the ultrafiltrate, but admits atmospheric pressure to the outside of the membranes. The amount of blood plasma placed in each sac in all experiments was 5 cc. The duration of the experiment was 3 hours unless otherwise stated.

TABLE 1

Showing variations in amount of ultrafiltrates obtained when unstandardized membranes are used

Five cubic centimeters of blood plasma was placed in each ultrafiltration sac. Time of each experiment = 3 hours. Pressure 77 mm. Hg. (M) = male, (F) = female, (E) = palpable edema.

Case number	Sac number	Ultrafiltrate	Protein in ultrafiltrate	Clinical diagnosis
		cc.		
1(Su)	2	0.8	0	Acute nephritis (E) (M)
	4	2.6	0	Acute nephritis (E)
	5	1.9	0	Acute nephritis (E)
	6	2.2	0	Acute nephritis (E)
10(G. T.)	17	2.3	++	Acute nephritis (F)
19 (C. N.)	30	2.45	0	Acute nephritis (E) (F)
20 (C. N.)	31	1.9	0	Acute nephritis (E) (F)
	32	2.0	0	Acute nephritis
	33	1.4	0	Acute nephritis
	34	1.35	0	Acute nephritis
	35	1.3	0	Acute nephritis
	36	2.0	0	Acute nephritis
21 (J. C.)	37	1.7	0	Acute nephritis (E) (M)
	38	1.8	0	Acute nephritis (E)
	39	2.2	0	Acute nephritis (E)
	40	2.0	0	Acute nephritis
	41	2.0	0	Acute nephritis
3 (S. W.)	7	2.0	0	Normal (M)
4 (A. J.)	8	1.1	0	Normal (M)
	9	1.3	0	Normal
5 (Y. W.)	10	2.7	0	Normal (F)
3 (F. W.)	5	2.4	0	Normal (M)
2 (B. F.)	4	2.6	0	Normal (M)
	6	2.6	0	Normal
14 (C. J.)	22	1.6	0	Normal (F)
16 (A. J.)	24	1.5	0	Normal (F)
5 (Y. W.)	11	3.4	+	Normal (F)
15 (F. H.)	23	1.4	0	Nutritional edema (E) (M)
11 (N. N.)	18	3.2	+	Diabetes (M)
	19	2.0	0	Diabetes
12 (N. A.)	2	2.0	0	Postpartum 8th day
13 (V. C.)	21	1.5	0	Postpartum 9th day
6 (H. S.)	10a	3.8	++	Postpartum 7th day
17 (R. B.)	25	1.8	+	Toxemia of pregnancy
	26	1.3	0	Toxemia of pregnancy
	27	1.3	0	Toxemia of pregnancy
18 (R. B.)	28	1.3	0	Toxemia of pregnancy
	29	1.8	0	Toxemia of pregnancy
6 (H. S.)	12	3.4	+	Postpartum 8th day
8 (E. D.)	13	2.8	0	Hypertension (F)
	14	2.8	0	Hypertension
	15	1.6	0	Hypertension
9 (D.M.C.)	16	2.8	0	Chronic nephritis (M)

RESULTS

The data presented in table 1 show the amount of ultrafiltrate obtained from the plasma of normal individuals, patients with toxemias of pregnancy, normal postpartum patients, and patients with chronic and acute nephritis with and without edema of cardiac or renal origin.[1] The only group of this series that shows any degree of constancy in the amount of ultrafiltrate obtained is the toxemia of pregnancy group. The most extreme degree of variation occurs among members of the other groups. Obviously, attempts to estab-

TABLE 2

Variations in amount of ultrafiltrate obtained from the blood plasma of a dog, using different sacs

The duration of the experiment in each case is 3 hours. Amount of plasma placed in each sac is 5 cc. Pressure 77 mm. Hg. The ultrafilters are not standardized.

Sac number	Ultrafiltrate	Protein in ultrafiltrate	Percentile increase over least of this series
	cc.		*per cent*
1a	3.5	++	41
2a	3.1	Very slight	25
3a	2.8	None	16
4a	3.3	None	37
5a	3.6	++	50
6a	2.4	None	0
7a	2.9	None	20
8a	3.0	Slight	25
9a	2.4	None	0
10a	3.0	None	25
11a	3.2	None	33
12a	3.6	++	50

lish the amount of free water available from the blood plasma of normal individuals, or to classify the findings from pathological sera in clinical groups on the basis of these results is entirely futile. Either the available water of the blood in conditions of health and in these

[1] The clinical cases studied in this report were all patients at the Cook County Hospital, Chicago, Illinois. We gratefully acknowledge our indebtedness to the internes and resident physicians of this hospital. Thanks are especially due to Dr. H. A. Singer, whose coöperation made the clinical material used in this research available.

TABLE 3

The influence of adsorption by the membranes in reducing the amount of ultrafiltrates obtained from membranes standardized but once, and used repeatedly

The amount of plasma used, pressure and time, are the same as in tables 1 and 2. All studies excepting those marked * were on blood plasma obtained from patients of the pathological obstetrical ward.

Date, 1925	Ultrafiltrate	Total proteins in plasma	Case number	Clinical diagnosis	Condition of ultrafilter
	cc.	*mgm. per cc.*			
March 31	1.3	55	1 (R. B.)	Toxemia of pregnancy. Slight palpable edema	Used several times
March 31	1.3		1 (R. B.)		Used several times
March 31	1.3		1 (R. B.)		Used several times
April 8	2.1	73	1 (R. B.)	No edema	Used once
April 8	1.25		1 (R. B.)	No edema	Used once
April 23	1.25	88	1 (R. B.)	Slight edema	Used several times
April 23	1.3		1 (R. B.)	Slight edema	Used several times
May 2	1.2	89	1 (R. B.)	No edema	Used several times
May 8	1.25		1 (R. B.)	No edema	Used several times
May 8	1.34		1 (R. B.)	No edema	Used several times
May 8	1.4		1 (R. B.)	No edema	Used several times
May 15	1.4	71	1 (R. B.)	No edema	Used several times
April 3	1.15	67	2 (O. N.)	Toxemia of pregnancy. No edema	Used several times
April 3	1.2		2 (O. N.)	Toxemia of pregnancy. No edema	Used several times
April 3	1.1		2 (O. N.)	Toxemia of pregnancy. No edema	Used several times
April 21	1.5	82	2 (O. N.)	Toxemia of pregnancy. No edema	Used several times
April 24	1.5		2 (O. N.)	Toxemia of pregnancy. No edema	Used several times
April 24	1.45		2 (O. N.)	Toxemia of pregnancy. No edema	Used several times
August 22	2.8	68	3 (E. D.)	Toxemia of pregnancy. Marked edema	Used once
August 22	2.8		3 (E. D.)	Toxemia of pregnancy. Marked edema	Used once
August 22	1.6		3 (E. D.)	Toxemia of pregnancy. Marked edema	Used several times
August 22	2.8		3 (E. D.)	Toxemia of pregnancy. Marked edema	Used once
March 25	1.3	56	4 (A. B.)	Toxemia of pregnancy. No edema	Used several times

TABLE 3—*Continued*

Date, 1925	Ultrafiltrate	Total proteins in plasma	Case number	Clinical diagnosis	Condition of ultrafilter
	cc.	*mgm. per cc.*			
March 25	1.3		4 (A. B.)	Toxemia of pregnancy. No edema	Used several times
March 25	1.3		4 (A. B.)	Toxemia of pregnancy. No edema	Used several times
April 2	1.3	70	4 (A. B.)	Slight edema	Used several times
April 6	1.0	74	4 (A. B.)	No edema	Used several times
April 6	1.1		4(A. B.)	No edema	Used several times
April 6	1.2		4 (A. B.)	No edema	Used several times
May 12	1.5	59	5 (B. M.)	Toxemia of pregnancy	Used several times
May 26	1.0	61	6 (L. W.)	Toxemia of pregnancy	Used several times
May 26	1.5	62	7 (E. G.)	Toxemia of pregnancy	Used several times
June 6	1.0		7 (L. G.)	Toxemia of pregnancy	Used several times
June 2	1.0		8 (L. B.)	Hypertension	Used several times
July 10	2.5	72	9 (L. C.)	Normal	Used several times
July 10	2.6		9 (L. C.)	Normal	Used several times
July 10	2.8		9 (L. C.)	Normal	Used several times
July 8	2.3	56	10 (A. B.)	Eclampsia	Used several times
July 8	2.15		10 (A. B.)	Eclampsia	Used several times
April 28	1.2	65	10 (V. L.)	Eclampsia. No edema	Used several times
April 28	1.1		10 (V. L.)	Eclampsia. No edema	Used several times
April 28	1.1		10 (V. L.)	Eclampsia. No edema	Used several times
May 6	1.1	67	11 (D. E.)	Eclampsia. No edema	Used several times
May 1	1.1		12 (N. N.)*	Normal pregnancy	Used several times
May 1	1.3		12 (N. N.)*	Normal pregnancy	Used several times
May 11	1.7		13 (A. A.)*	Normal pregnancy	Used several times
May 1	1.1	98	14 (P. B.)*	Normal pregnancy	Used several times
April 13	1.6		15 (N.P.)*	Normal pregnancy	Used several times
April 16	1.6		15 (N. P.)*	Normal pregnancy	Used several times
April 18	1.3	68	16 (F. F.)*	Normal pregnancy	Used several times
August 25	1.8		17 (F. U.)*	Normal pregnancy	Used several times
May 20	1.4		18 (C. J.)*	Normal	Used several times
May 27	1.5		18(C. C.)*	Normal	Used several times
May 28	1.0		19 (A. R.)*	Normal	Used several times
May 29	1.3		20 (M. B.)*	Normal	Used several times
June 30	1.2		21 (B. E.)*	Normal	Used several times
June 30	1.2		21 (B. E.)*	Normal	Used several times
June 30	1.2		21 (B. E.)*	Normal	Used several times
May 10	1.5		22 (E. G.)*	Normal	Used several times
June 1	1.6		23 (L. B.)*	Normal	Used several times

diseases is exceedingly variable, or sufficient uniformity in the permeability of the membranes made according to the method described above, had not been attained.

This second possibility was investigated, and the results of an experiment planned to test the uniformity of the ultrafiltration sacs are given in table 2. In this test 12 sacs were used; 5 cc. of blood plasma from the same dog was placed in each sac and the sac fitted to the pressure apparatus. At the end of 3 hours a 37 per cent variation was found in the total amount of protein-free ultrafiltrate delivered from the several sacs. Obviously this difference was due, not to a change in the "free" water content of the blood plasma of the dog, but to differences in the ultrafiltration membranes.

Recognizing the need of a more constant degree of permeability in our membranes, we attempted to eliminate variations in the thickness by using a gel (7 per cent glacial acetic acid solution of parlodion) of such consistency that the paper Soxhlet thimbles were completely impregnated in 2.5 to 3 minutes. They were then drained and dried for 8 minutes, immersed in the gel, drained again for 5 minutes, fixed in distilled water and washed as already described. The membranes were then standarized by placing 5 cc. of distilled water in each and fitting into the pressure apparatus. Only those were chosen for use that delivered 5 cc. of distilled water in 75 to 90 minutes, at a pressure of 77 mg. Hg. Such thimbles delivered 2.8 to 3.0 cc. of protein-free filtrate from 5 cc. of oxalated plasma from normal individuals or dogs in 165 to 180 minutes.

Table 3 shows the amount of ultrafiltrate obtained from a series of women, some of whom were in normal state of health, others were suffering from toxemia of pregnancy, or were normal postpartum patients. The membranes used in the experiments of this series were all standardized with distilled water and then tested with normal serum as mentioned above. After this, in most instances, they were used to test the amount of ultrafiltrate that could be obtained from the blood plasma of nephritics or patients with deferred diagnosis. They were then used in the series of cases reported in table 3. These data are important only in showing how consistently false results may be obtained because of adsorption by the gel of colloidal substances in the plasma.

TABLE 4

Data selected from experiments in which standardized membranes are used

Recalibration shows little or no change in the membranes during the experiment.* (M) = male; (F) = female. Time, pressure and amount of plasma are the same as in table 1.

Case number	Date, 1925	Ultrafiltrate	Total protein in plasma	Clinical diagnosis	Distilled water delivered after test
		cc.	*mgm. per cc.*		*cc.*
1 (R. B.)	April 8	2.1	73	Toxemia of pregnancy. Slight palpable edema	4.7
	April 8	2.1			4.7
2 (E. D.)	August 22	2.8	68	Toxemia of pregnancy. Slight palpable edema	5
	August 22	2.8			5
3 (L. C.)	July 10	2.6	72	Toxemia of pregnancy	5
	July 10	2.8			5
4 (A. B.)	July 8	2.3	56	Toxemia of pregnancy	4.8
5 (Su)	July 14	2.15	63	Acute nephritis. Marked edema (M)	4.7
	July 14	2.6			4.9
	June 18	2.6			4.9
6 (G. T.)	June 8	2.8	88	Acute nephritis	5
	June 8	2.7			5
7 (Mi)	August 19	3.0	100	Chronic nephritis and pernicious anemia (M)	5
	August 11	3.1			5
8 (C. N.)	March 14	2.5	83	Acute nephritis. Edema (M)	4.8
9 (J. C.)	May 18	2.8	52	Acute nephritis. Edema (M)	5
	August 22	3.0	51		5
	September 29	2.7	60		4.4
	September 29	2.5			4.4
10 (L. D.)	August 20	3.0	62	Chronic nephritis (F)	5
11 (N. N.)	August 19	3.2		(M)	5

TABLE 4—*Concluded*

Case number	Date, 1925	Ultrafiltrate	Total protein in plasma	Clinical diagnosis	Distilled water delivered after test
		cc.	*mgm. per cc.*		*cc.*
12 (C. T.)	August 24	3.1	68	Acute nephritis. Edema	5
	August 24	3.2			5
	August 24	3.1			5
13 (C. B.)	September 15	2.5	81	Hypertension (F)	5
	September 15	2.5			5
14 (Y. W.)	August 26	2.7		Normal (M)	5
	August 26	3.0			5
15 (H. S.)	August 31	3.4		Normal. Postpartum	5
16 (A. J.)	August 25	2.8		Normal	5
	August 25	2.8			5
17 (D. H.)	April 6	2.3		Normal (M)	4.7
	April 6	2.35			4.7

* Protein was present in the ultrafiltrates of cases 10 (L. D.) and 15 (H.S.).

Critical examination of the technique and further analysis and experimentation showed that the low values were due to lessened permeability of the membranes, and that these ultrafilters had changed during the process of using, and would no longer deliver 5 cc. of distilled water in 90 minutes. This tendency to change in permeability makes it necessary to know the degree of permeability of the membranes at the end of each experiment as well as at the beginning. We find that the membranes usually become changed as a result of the *first* ultrafiltration of blood plasma, so that instead of delivering 5 cc. of distilled water in 90 minutes, they deliver 5 cc. of distilled water in two hours; therefore two hours becomes the standard for subsequent recalibration tests. The membranes may become "set" at this point; if so they may be repeatedly used without a measurable reduction in the permeability. But they must be tested after each using with distilled water, since changes may occur after the second or subsequent

using. When sufficient adsorption has occurred to reduce the permeability of the membranes about one-half they may remain remarkably constant for a number of experiments, or the permeability may slowly continue to decrease or suddenly increase. A calibration test made with distilled water reveals their condition.

Table 4 gives the results obtained from a series of 33 ultrafiltration experiments made with the blood plasma of 17 subjects, including normal men and women, patients with nephritis with and without edema and hypertension, and patients suffering from toxemias of pregnancy. These results are chosen from experiments where recalibration of the membranes with distilled water showed that the permeability of the membranes had remained relatively constant during the experiment. The data show that there is no significant difference in the quantity of ultrafiltrate obtained from these patients or normal individuals, regardless of the amount of edema or nephritis present.

DISCUSSION

The amount of ultrafiltrate obtained by filtering serum through membranes made according to the technique advocated by Beckmann, is of no significance unless the limits of effectiveness of the ultrafilters are definitely known. The permeability of the membranes depends largely upon the amount of gel entering into the composition and at present no method for *exactly* controlling this suggests itself.

Walpole (7) showed, by chemical analysis, that the amount of gel in adjacent 5 cm. strips of membrane varies appreciably even where the membranes are made after the most accurate and painstakng method, and no paper support is introduced. Furthermore, micrometer measurements show considerable differences in the thicknesses of such strips. In the method used by Beckmann, no factor in the technique is controlled excepting the length of time taken to impregnate the paper Soxhlet thimbles, and that is given a leeway of 3 to 5 minutes. The paper thimbles vary at different points. This gives a base irregularly impregnated with gel and hence with differences in porosity at different points. Other conditions which we have found to cause changes which reduce the amount of ultrafiltrate are: first, the degree of drying of the membranes before fixing in water; second,

swelling of the gel which occurs in some cases, after 12 hours of washing in water, so that membranes used only for experiments with distilled water deliver lessened amounts of ultrafiltrate after several days; third, exposure to drying during the preparation for an experiment; fourth, the adsorption of colloidal substances by the gel.

The data submitted in table 2 show that uniform results cannot be obtained from blood plasma of the same subject where the above factors are operating in an unknown way. The 12 membranes used in the series of table 2 had not been previously used, so the 50 per cent difference found in the amounts of ultrafiltrate obtained from the same blood is due either to changes in the permeability that occurred during the process of making, or to differences in the amounts of adsorption which took place during the experiment.

Proper emphasis must be placed upon the *important factor of adsorption.* If our work had been interrupted at the point where the investigations of table 3 were completed, we could have advocated the false conclusion that the water available, by methods of ultrafiltration, from the blood plasma of normal women, postpartum patients and patients with toxemias of pregnancy, is 40 to 50 per cent less than that available from normal men. The results were obtained from membranes used several times without recalibration.

Comparable amounts of ultrafiltrate may be obtained from membranes of the same calibration, but recalibration is necessary after each experiment in order to make sure that the permeability has not changed during the experiment.

Standardized membranes, recalibrated, were used for the experiments given in table 4. These results indicate that the quantity of ultrafiltrate obtained in three hours with pressure of 77 mm. Hg from blood plasma of normal individuals, postpartum patients (both uncomplicated and of the toxemia group), chronic and acute nephritis with and without palpable edema, does not vary to any significant degree. Further evidence supporting these results was obtained from the following experiments which differs from those previously reported, only in that the time for the ultrafiltration was extended until no more fluid was delivered from the membranes (9 to 15 hours). The blood plasma from 10 subjects was tested for "free" water content according to the process of ultrafiltration. The subjects included 3 patients with

chronic nephritis (2 had moderate edema), 1 with acute nephritis accompanied by marked edema, 1 with essential hypertension and 5 normal individuals. Twenty ultrafiltration experiments were made in this series, using standardized and recalibrated membranes. The total amounts of ultrafiltrates delivered from the 5 cc. portions of plasma varied from 4.3 to 4.7 cc. Only one sac delivered as small an amount as 4.3 cc. and on recalibration with distilled water, the permeability of this sac was found to be markedly reduced. We believe the slight differences in the amounts of ultrafiltrate delivered by the other sacs to be due to undetectable changes in the permeability of the membranes. We do not offer these results as evidence that the available water of the blood plasma may not vary under some conditions. We believe, however, that such changes cannot be detected by this method.

Ultrafiltration, according to Bechhold, is not a method for detecting the *amount of water available*, but for separating colloidal particles of different sizes. The two processes are no doubt closely related, but not identical. For example, membranes of a given porosity will hold back all colloidal particles above a certain size regardless of the amount of fluid filtered through; the lower limit of permeability for a given colloidal particle is well defined. In measuring water the degree of refinement in the technique is infinitely more difficult, because molecules of water filter through when the porosity is entirely too small to pass other substances found in the blood plasma. If 5 cc. of distilled water is placed in a membrane of diminished porosity, only a small amount of ultrafiltrate is obtained at 77 mm. Hg and 3 hours time. If the same amount of water is placed in a membrane of slightly greater porosity, it will completely filter through (length of time and pressure as above). Using the same membranes and reducing the pressure to 33 mm. Hg (approximately the hydrostatic pressure of the capillaries) only 80 per cent of the distilled water passed through at the end of 9 to 15 hours. *Obviously this is not a test of the amount of free water in the contents of the filters*, but a *failure in some cases of the gel membranes to give rapid passage to free water under a given hydrostatic pressure because of the fineness of the pores.* The same condition holds to a great degree in experiments with blood plasma.

We find no indication of marked physico-chemical changes in the

plasma proteins in cases of nephritis with or without edema, at least as indicated by the ultrafiltration of the plasma. Results in table 4 were obtained by standardized membranes. No protein molecules pass through where the quantity of ultrafiltrate measures 2.8 to 3.0 cc. or less. If the permeability of the membranes is but slightly increased so that the amount of fluid delivered is increased by 0.4 cc. the ultrafiltrate gives a slight positive protein test[2] in all conditions tested. It does not seem probable that changes in size of the colloids through this small range could account for the binding of water by the blood protein described by Beckmann. However, one must consider the possibility of various proteins of the blood swelling differently. Hydrostatic pressure equal to 77 mm. Hg used in Beckmann's and my own experiments may tend to overcome the power of the slightly swollen colloids to hold water, hence at such pressure slight differences in amount of ultrafiltrate due to the hydration processes of the colloids may not be detected.

The nature of the proteins in solution influences the amount of ultrafiltrate delivered much more than do slight quantitative variations found in blood plasma. For example, 5 cc. of a 7 per cent solution of gelatin yields only about 0.7 cc. of ultrafiltrate from the standard membrane (pressure and time as in table 1), whereas a 7 per cent solution of colloids as found in blood plasma yields 2.8 to 3.0 cc.

SUMMARY

1. The amount of ultrafiltrate passing through a gel membrane in a given time and at a given pressure is of little significance unless the permeability of the membrane is definitely known.

2. Gel membranes should be standardized both before and after each experiment to detect changes which may occur during the experiment.

3. Ultrafiltration does not measure the free water of the substance filtered, but the speed with which the membrane can give passage to free water under a given hydrostatic pressure.

4. When standardized membranes were used in a system with a hydrostatic pressure equal to 77 mm. of Hg the amount of ultrafiltrate

[2] Nitric acid ring test (Heller).

obtained in three hours did not vary significantly whether obtained from the blood plasma of normal men and women or of patients suffering from nephritis, with or without edema, or toxemias of pregnancy, or of postpartum patients without complications.

5. We failed to find, by this means, evidence either supporting or refuting the theory that swelling or shrinking of the plasma colloids may occur to a degree sufficient to influence the free water content of the blood plasma in patients with and without edema.

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