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Capillary Tube Kjeldahl Method for Determining Protein Content of 5 to 20 Milligrams of Tissue Fluid.

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(Introduced by Soma Weiss.)

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A study of the protein content of tissue and edema fluid has been hampered in the past because of the difficulty in obtaining large enough quantities of fluids for satisfactory analysis. In many instances it is easy to obtain a few milligrams of clear fluid, but attempts to collect large amounts usually lead to bleeding. The pur-

pose of this report is to present a technic for collecting small amounts of fluid from the tissues and to describe a method for determining the nitrogen content of this fluid.

To collect the fluid, several 23-gauge 5/8-inch needles are inserted to the hilt into the subcutaneous tissue. If the part is not dependent at the time the needles are inserted, contamination with blood usually does not occur. The needles are then withdrawn about 5 mm and allowed to remain in place for 5 to 10 minutes, unless fluid appears in the hub of the needle before that time. If the needles are left in place for a long time, an inflammatory reaction may result and a fluid high in protein will be obtained. The needles are then withdrawn and attached to a syringe. Gentle pressure on the plunger of the syringe forces the fluid to the tip of the needle. From there the drop of fluid obtained is transferred by capillary attraction to a clean glass capillary tube of known weight. The fluid should be clear. If there is any question of contamination with blood, the fluid can be examined for red cells by placing the capillary tube under the microscope. Red cells are easily visible through the walls of the glass tube. After the weight of the filled capillary tube has been determined, the material is blown into a small pyrex test tube which contains 0.1 ml sulphuric acid (concentrated sulphuric acid diluted 1:1 with water). The content of the capillary tube is fairly quantitatively washed out by drawing the acid up and down several times. The capillary tube is then washed, dried and weighed. The mixture in the test tube is concentrated over a microburner until dense white fumes appear. Digestion is continued for 10 minutes. The flame is then removed and about ½ minute later, .02 cc of 30% nitrogen-free hydrogen peroxide is added. The heating is now continued for 5 minutes in order to complete the digestion. After cooling the tube, 4 cc of distilled water and 0.1 cc of gum ghatti solution are added. A color equivalent to the amount of nitrogen present is produced by adding 1.6 ml of Nessler's reagent (prepared according to Koch and McMeekin[1]). After mixing, the contents of the digestion tube are transferred to a Klett colorimeter tube and read with filter 42 (transmission limits 400 to 450 millimicrons). A blank is prepared in exactly the same way, except for the addition of the edema fluid.

The amount of nitrogen per 100 g is calculated as follows:

$$\frac{(\text{Reading of unknown} - \text{Reading of blank}) \times \text{calibration factor} \times 100}{\text{Weight of sample in milligrams}}$$

= mg nitrogen in 100 g of sample. It is not necessary to determine the amount of non-protein nitrogen in the fluid because the non-

[1] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

TABLE I.
Determination of Calibration Factor.
Ammonium sulphate solution 0.6630 g in 100 cc of water = 0.01405 g of nitrogen in 100 cc of water.

Weight of sample (mg)	Nitrogen in sample (μg)	Klett reading (reading of blank subtracted)	Calibration factor
9.0	12.7	250	.0508
6.9	9.7	181	.0536
7.6	10.7	200	.0535
8.3	11.7	230	.0509
9.9	13.9	260	.0535
		Avg	.0525

protein nitrogen of edema fluid and of plasma are practically the same.[2] The non-protein nitrogen of the serum or plasma of the patient from whom the fluid was obtained is then determined and this is subtracted from the milligrams of nitrogen in 100 cc of the sample. The concentration of protein in grams percent in the fluid is then calculated in the usual way. The calibration factor is determined by running ammonium sulphate solutions of known concentration through the entire procedure, including the transfer of a drop of such a solution from a capillary tube to a digestion tube (Table I).

The protein content of 5 different sera diluted 1:6 with saline was determined by a standard micro-Kjeldahl procedure,[1] with the Klett

TABLE II.
Protein Concentration of 5 Sera (Diluted 1:6) as Determined by Capillary Method and by Micro-Kjeldahl Method.

Sera diluted 1:6	Capillary Kjeldahl		Micro-Kjeldahl Protein (g%)
	Mg of fluid	Protein (g%)	
1	9.5	1.0	1.0
	9.0	1.0	
	9.1	1.0	
2	11.7	1.0	1.1
	8.7	1.0	
	11.2	1.0	
3	10.8	1.0	1.1
	10.5	1.0	
	7.6	0.9	
4	7.5	1.1	1.2
	10.2	1.1	
	8.7	1.2	
5	11.2	0.9	1.0
	11.6	1.0	
	11.5	0.9	

[2] Denis, W., and Minot, A. S., *Arch. Int. Med.*, 1917, **20**, 879.

colorimeter and filter 42, and by the capillary tube technic (Table II). The micro-Kjeldahls were done in duplicate and checked within 2%. The capillary tube Kjeldahls were done in triplicate, as shown in Table II. The results were either the same or slightly lower than those obtained by the micro-Kjeldahl method. The greatest difference was .2 g, and this occurred in only 1 determination out of 15. If sufficient fluid is obtained to make the determination in duplicate, it seems logical to take the higher of the 2 readings as the more nearly correct one.

Clear edema fluid can usually be obtained without difficulty if there is sufficient edema to cause even slight pitting. Table III gives protein content of the edema fluid of 6 patients.

Summary. A technic is given for obtaining 5 to 20 mg of subcutaneous fluid in subjects with minimal edema. A capillary tube Kjeldahl method for determining the protein content of small amounts of edema fluid is described.

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TABLE III.
The Protein Content of Edema Fluid of 6 Patients.

Subject	Diagnosis	Mg of edema fluid	Klett reading (reading for blank subtracted)	Protein (g%)
St	Chronic congestive failure	11.3	636	1.8
		9.0	586	1.8
W	Chronic congestive failure	10.4	128	.2
		13.4	185	.3
B	Cirrhosis of liver	12.4	141	.2
		8.7	95	.2
Sh	Myxedema and vitamin deficiency	7.5	328	1.3
		6.4	288	1.4
O	Emphysema	13.7	104	.1
		6.2	50	.1
C	Serum sickness	2.6	383	5.1