normal individual these are hydrolyzed rapidly by pancreatic enzymes, whereas in cystic fibrosis patients such hydrolysis does not occur and the serum proteins remain detectable in the duodenal fluid.

Regardless of the exact nature of the material represented by the faster moving electrophoretic components of duodenal fluid from cystic fibrosis patients, the mere existence of these components is of clinical significance. If further study confirms the observation reported here that a difference regularly exists between the electrophoretic patterns of duodenal fluid in cystic fibrosis patients and those in normal subjects, an additional simple laboratory test will be available for use in establishing the clinical diagnosis of cystic fibrosis.

Summary. Duodenal fluids from patients with cystic fibrosis of the pancreas and from control subjects were studied by paper strip electrophoresis. Components with greater electrophoretic mobility than any in normal duodenal fluid occurred consistently in cystic fibrosis duodenal fluids. The most rapidly migrating of these fractions was identical in electrophoretic mobility to the albumin fraction of normal human serum.

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Adaptation of Calcein Titration Method for Calcium Determination in Tissues Without Ashing.* (24498)

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Determination of calcium in a large number of serum and tissue samples would be facilitated by a method that is simple and rapid, and does not require the cumbersome ashing of tissues in a platinum crucible. Recently several titration methods(1,2,3) have been published but they still require ashing of tissues. The present study was undertaken to adapt the calcein (a fluorescent dye) titration method of Diehl and Ellingboe(4) as modified by Ashby and Roberts(1) to determination of calcium in tissues and milky lipemic serum without the necessity of ashing. Since minor modifications were made and additional steps added, the entire procedure is described for the sake of continuity.

* The author is indebted to Mr. F. A. Smith for serum calcium determinations by the Clark-Collip method.

Materials. (1) Titration box. Titration end-point of light green fluorescence is seen in the dark under ultraviolet light as the only illumination. Since a dark-room is not always available, a cardboard box, 2 x 3½ x 5 inches in dimensions, has been modified for this purpose and is shown in Fig. 1. (2) A 1 ml microburette, reading to the fourth decimal place; (3) a long-wave ultraviolet lamp; and (4) a piece of PE 10 polyethylene tubing attached to 27 gauge needle which in turn is attached to compressed air line and is used for mixing during titration. All glassware is preferably rinsed in dilute acid followed by a thorough rinsing with distilled water to avoid calcium contamination.

Reagents. (1) Concentrated HNO_3, reagent grade; (2) 1 N HNO_3; (3) 1.5 N KOH; (4) saturated ammonium oxalate solu-

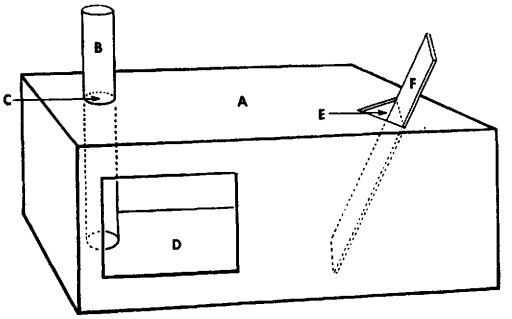

FIG. 1. A, cardboard box 2 × 3½ × 5 inches, painted black inside; B, titration tube; C, ½ inch diameter hole 2 inches from front of box, into which titration tube fits snugly; D, opening in front of box to admit ultraviolet light; ultraviolet lamp should completely cover opening so as to prevent entrance of outside light; E, opening in front of mirror; F, mirror to reflect titration.

tion; (5) concentrated NH_4OH; (6) methyl orange pH indicator solution; (7) approximately 0.001 M disodium dihydrogen ethylenediamine tetraacetate (EDTA) solution; (8) calcein indicator solution: dissolve 8 mgm calcein in 0.2 ml of 1 N NaOH and then add water to make total volume of 100 ml; (9) standard calcium solution for titration: 100 μg Ca ml; (10) 2 N NaOH solution containing 1% NaCN; (11) n-octyl alcohol. All reagents are prepared with redistilled Ca-free water.

Procedure. (1) *Tissue preparation.* Into a small test tube, graduated at 1 ml, place 0.5 ml of 1.5 N KOH and a known weight of tissue or volume of serum and heat in water bath at about 80°C until test material is digested. For normal rat tissues we used about 250 mg liver, 100 mg aorta and 0.1 ml serum/ml of KOH digest to obtain adequate calcium concentration for analysis. After the tissue is digested, add 0.1 ml of concentrated HNO_3 and sufficient redistilled Ca-free water to make a total volume of 1 ml, mix with a footed glass rod and let stand for about half an hour. Mix again and centrifuge. Transfer a known aliquot of supernate, 0.8 ml or less as required, to a clean test tube, 14 x 100 mm, add 1 drop of methyl orange indicator and make alkaline to the indicator by dropwise addition of concentrated NH_4OH. Add 1 ml of saturated ammonium oxalate, mix and let stand overnight. The next morning centrifuge and remove the supernate by aspiration, being extremely careful not to disturb the precipitate. Do not use an angle centrifuge because this makes it difficult to remove the supernate without losing some of the precipitate. Add 0.5 ml of 1.5% NH_4OH to wash the precipitate and again centrifuge and remove supernate as before. Add 2 drops of 1 N HNO_3 and heat gently to dissolve calcium oxalate precipitate. (2) *Titration.* Place tube into titration box and illuminate with ultraviolet light. Add 1 volume of EDTA solution and 1 ml of calcein indicator solution. It is absolutely essential that the identical volume of EDTA solution be delivered each time. This is readily accomplished with a ½ ml hypodermic syringe fitted with 24 gauge needle. Add 1 drop of n-octyl alcohol to prevent foaming and mix by bubbling air through the solution by the polyethylene tubing. Add 5 drops of 2 N NaOH solution containing 1% NaCN. If necessary, repeat EDTA addition until fluorescence disappears, indicating that all the calcium has formed a complex with EDTA. Add sufficient redistilled Ca-free water to make final volume of EDTA solution plus water 2 ml. Now titrate with standard calcium solution until a light green fluorescence persists, indicating an excess of free calcium in solution. Also titrate directly 1 volume of EDTA solution to standardize the EDTA solution and use a reagent blank carried through the entire procedure to check reagent contamination with calcium. The

TABLE I. Serum Calcium Determinations.

Calcium conc., mg/100 ml			
Normal		After parathyroid hormone	
Clark-Collip method	Present procedure	Clark-Collip method	Present procedure
10.40	10.42	14.60	14.60
10.60	10.98	13.40	13.68
10.90	10.57	15.40	15.06
11.30	11.36	15.50	15.98
10.90	11.38	14.00	14.36
10.90	10.86	13.80	14.02
10.90	11.39	15.00	15.48
11.00	11.52	15.00	14.50
10.00	10.17	12.70	14.01
11.20	11.76	14.50	16.01
10.81*	11.04	14.39	14.77
± .20	± .28	± .28	± .25

* Mean ± S.E.

amount of calcium titrated is then calculated by difference.

Results. For preparation of the tissue it was essential to obtain a relatively clear supernate and to insure complete solution of calcium present. To satisfy the latter requirement an acid solution was desirable. Trichloroacetic acid, sulfuric acid, and hydrochloric acid interfered with titration, but nitric acid gave satisfactory results.

For convenience, the validity of the present procedure was investigated in 2 steps. First, determination of calcium in dog serum was compared with the Clark-Collip method(5) as outlined in the USP XV(6) for assay of parathyroid hormone. The results (Table I) show good agreement. Secondly a tissue KOH digest was prepared. An aliquot of this was ashed in a platinum crucible in the usual manner. After ashing, the residue was dissolved in HNO_3, then analysed for calcium by the present procedure, starting with the oxalate precipitation. A second aliquot was processed as outlined above. The results presented in Table II show that tissue ashing was not necessary.

Further studies indicate that good recovery of calcium, added to the tissue just prior to KOH digestion, was obtained. These results are presented in Table III.

TABLE II. Tissue Calcium Determination.

Liver calcium, mg/100 g wet tissue	
With ashing	Without ashing
5.80	5.55
2.93	3.02
4.06	4.36
4.71	4.15
5.85	5.23
6.82	5.04
3.76	4.59
6.10	5.15
3.78	3.20
4.18	4.31
5.32	5.43
5.55	5.65
4.90 ± .33*	4.64 ± .25

* Mean ± S.E.

TABLE III. Recovery Studies.

Tissue	μg calcium added	With ashing: μg recovered	With ashing: % recovery	Without ashing: μg recovered	Without ashing: % recovery
Liver	10	9.0	90	8.8	88
		10.2	102	9.7	97
				9.0	90
				9.1	91
	20	19.6	99	19.8	99
				20.3	101
	40	39.5	99	39.1	98
		38.9	97	35.5	89
				37.9	95
				39.3	98
				37.1	93
Aorta	5			4.6	92
				4.3	86
	10			10.5	105
	20			18.8	94
	40			38.0	95
				33.2	83
Serum	5			5.5	110
	40			38.0	95
			97.4 * ±2.02		94.7 ±1.5

* Mean ± S.E.

Summary. A convenient and rapid procedure is presented for determination of calcium in tissues and lipemic serum without the necessity of ashing.

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