stantiate the hypothesis that the brainstem reticular formation might control acoustic input through direct reticulo-cochlear fibers.

Summary. 1. Electrical responses to clicks were recorded from the ventral cochlear nucleus in curarized cats, and the effect thereon of brain stem repetitive stimulation was investigated (stereotaxic method). 2. Clear-cut inhibition of the cochlear nucleus response to click was recorded when a critically-localized region of the posterior diencephalon was stimulated. 3. These and previous results provide the first physiological evidence for a specific extra-reticular descending pathway, presumably enabling higher levels of the nervous system to control acoustic input.

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Paper Electrophoresis of Duodenal Fluid from Patients with Cystic Fibrosis of Pancreas. (24497)

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Farber(1) suggested that the basic pathology in patients with cystic fibrosis of the pancreas resulted from abnormal mucous secretion. In patients with meconium ileus, a common manifestation of cystic fibrosis, the meconium has been demonstrated chemically by Rapoport and Buchanan(2) to contain an abnormal mucoprotein and electrophoretically by Green, Clarke, and Shwachman(3) to have an abnormal protein component. Recently di Sant' Agnese *et al.*(4), using precipitation with a benzene-alcohol mixture, have demonstrated an abnormal mucoprotein in the duodenal fluid in children with cystic fibrosis.

In our studies, samples of duodenal fluid from children with cystic fibrosis were examined by paper strip electrophoresis and the results compared with those observed in control children.

Methods. Ten children with cystic fibrosis were studied, 6 with this diagnosis considered but ruled out by laboratory studies, and 2 normal children. Following intubation, in most instances with fluoroscopic or x-ray verification of the position of the duodenal tube, duodenal fluid was collected in an ice bath. After centrifugation the fluid was frozen until used for electrophoretic study. A volume of .04 cc of the fluid was applied to Schleicher and Schuell (No. 2043A-mgl) filter paper strips in Spinco Model R electrophoresis cell. A veronal buffer of pH 8.6 and ionic strength 0.075 was used with constant current of 2½ ma applied for 16 hours. Following electrophoresis the strips were dried at 120°C for 30 minutes, fixed in absolute methanol for 6 minutes, stained with Bromphenol Blue Dye (Spinco) in alcoholic solution for 30 minutes, rinsed 3 times in 5% acetic acid, dried at 120°C for 15 minutes, and placed in ammonia fumes for 15 minutes-4 hours to enhance color development. Then they were read immediately with Spinco RA Analytrol, utilizing 1½ mm slit, 500 mu interference filters, and a B-5 cam.

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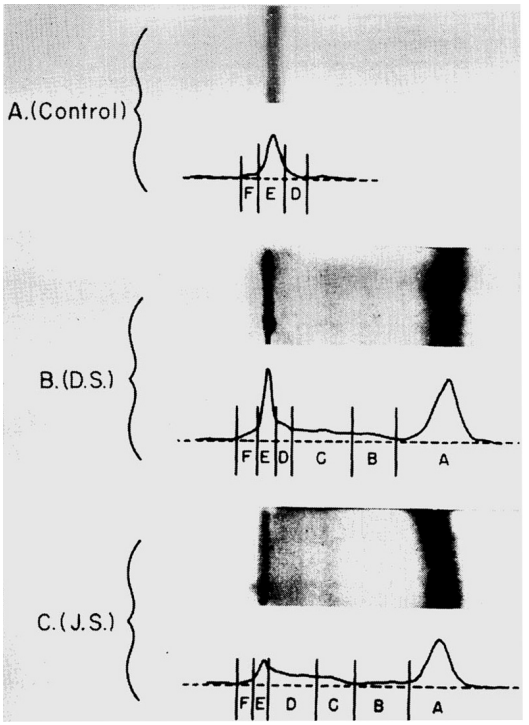

FIG. 1. Duodenal fluid protein pattern: (A) Normal, (B) and (C) cystic fibrosis.

Results. The duodenal fluid from each patient with cystic fibrosis had an abnormal electrophoretic component not present in any samples obtained from the 8 control subjects. Fig. 1 shows typical electrophoretic patterns obtained on duodenal fluid from a normal child and from 2 children with cystic fibrosis. In each instance a photograph of the paper strip and a reproduction of the analytrol pattern is shown. The peak labeled E represents the point of application of the sample to paper strip and the other peaks are designated arbitrarily as F, D, C, B, and A. In duodenal fluid samples from the 8 control children there was but little electrophoretic migration and only the peaks F, E and D were found. On the other hand, in each sample obtained from 10 patients with cystic fibrosis there was migration past the D fraction, the major portion of this more rapidly migrating material appearing in fraction A. The electrophoretic mobility of this material in fraction A was identical with that of the albumin fraction in normal human serum by comparing patterns obtained when samples of normal serum and cystic fibrosis duodenal fluid were submitted to electrophoresis simultaneously under identical experimental conditions.

The test of di Sant' Agnese *et al.*(4) for abnormal mucoprotein in duodenal fluid was performed on most of the cystic fibrosis patients and consistently was found to be positive. However, there was no correlation between quantity of abnormal mucoprotein and quantity of abnormal protein component A in individual duodenal fluid samples. In addition, periodic-acid-Schiff staining of strips revealed no difference between normal subjects and cystic fibrosis patients with regard to distribution of mucoproteins in the duodenal fluid.

Discussion. Although there is little information available regarding electrophoresis of human duodenal fluid, several electrophoretic studies of pancreatic juice in rats and dogs have been reported(5-9). In contrast to the small number of protein fractions in the duodenal fluid from human controls in our investigation, the studies in animals all demonstrated 4-7 fractions. Munro and Thomas (5) found that none of the fractions obtained by free electrophoresis of canine pancreatic fluid correlated with the albumin fraction in serum but that the fractions did appear to correlate with the globulin serum fractions, alpha-1, alpha-2, beta, and gamma respectively.

The fast moving fraction A in cystic fibrosis duodenal fluid may correspond to the abnormal peak found by Green *et al.*(3) in meconium of patients with meconium ileus; this latter peak also had a mobility identical with the albumin peak of normal human serum. In their study and ours evidence was obtained indicating that the rapidly migrating abnormal protein fraction demonstrated by electrophoresis does not correspond to the abnormal mucoprotein described by di Sant' Agnese *et al.*(4) in duodenal fluid of patients with cystic fibrosis. Although the identity of the material in fraction A with serum albumin has not been proved, the evidence strongly suggests that the proteins in duodenal fluid of cystic fibrosis patients are serum proteins. The possibility should be considered that all duodenal fluid originally contains serum proteins but in the

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.

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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-