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Influence of Cystic Fibrotic and Heterozygous Serum on Rat Jejunum[1] (39993)

J. P. GILMORE, M. DAVIS, AND G. E. GIBBS

Departments of Physiology and Biophysics and Pediatrics, University of Nebraska College of Medicine, Omaha, Nebraska 68105

While the sweat test provides a highly reproducible and reliable test for the identification of the homozygous state of cystic fibrosis, assays which have been utilized for the identification of heterozygotes have provided less consistent results. Neither the rabbit trachea nor oyster gill assay have proven reliable when carried out under blind study conditions (1). The observation of Mangos and associates (2) that mixed saliva from homozygotes inhibited sodium reabsorption in the rat parotid gland suggested to Araki and associates (3) that cystic fibrotic plasma may contain a factor which inhibits sodium transport. Utilizing the rat jejunum, they observed that plasma from both homozygotes and heterozygotes, when added to the serosal surface just before mounting the tissue, inhibited short circuit whereas plasma from normals had no such effect. In a subsequent short communication Araki and associates (4) modified their technique in that the baseline short circuit current was recorded for 10 min, and the mucosal surface solution was then replaced by serum. Using this technique they found that sera from 48 of 50 cystic fibrosis patients and 40 of 46 heterozygotes decreased short circuit current. Two of twenty healthy controls showed false positives. The present study was undertaken to further study the reliability of the short circuit current method for distinguishing among cystic fibrotic, heterozygous, and normal sera.

Method. Short circuit current (Isc) and potential difference (PD) across the rat jejunum were determined according to the technique of Ussing and Zerahn (5). PD was measured using Ringer–3% agar bridges and calomel half-cells and monitored continuously employing a differential amplifier (Tetronics Model 3A9; input impedance, 1 MΩ) and displayed on an oscilloscope (Tetronics Model 561B). The output of the latter was recorded on a strip-chart recorder, and the calibration was checked using a Bioelectric Type CA5 calibrator. Current was applied through Ag–AgCl pellet electrodes and Ringer–agar bridges. Isc was determined using an HP 412A VTVM (internal shunt resistance <1 Ω), and the output was recorded on the strip recorder. Direct current resistance was calculated from PD/Isc.

The tissue was prepared as follows: Male Sprague–Dawley rats were anesthetized with pentobarbital sodium (60 mg/kg) ip, an abdominal incision was made, the jejunum was opened, and its lumen was washed out with bicarbonate–Ringer's solution henceforth referred to as Ringer's solution. The blood vessels to the jejunum were clamped with a hemostat, and a section was removed at a point usually 12–18 cm below the pyloric sphincter and placed in warm Ringer's solution. The tissue was cut longitudinally and mounted between two halves of a Lucite chamber which was then filled with Ringer's solution and oxygenated with a gas lift. The Lucite chamber was maintained at 37° using a warm-water jacket. Three minutes elapsed between clamping the blood supply to the jejunum and mounting the tissue. Sera were obtained from 10 normal, 17 cystic fibrotic, and 8 heterozygotic individuals and stored at 4° until testing which occurred at no later than 48 hr. Samples were assayed under blind study conditions after they were diluted by adding 2 parts of Ringer's solution to 1 part serum.

The protocol for assay was as follows: The rat jejunum was mounted, the tissue was allowed to stabilize for 10–15 min as indicated by the stability of the PD, and the control Isc was determined. The Ringer's solution on the mucosal side of chamber was then replaced with the diluted sera,

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and recording of the PD and determination of the Isc were continued for 15–20 min. The results were analyzed using a one-way analysis of variance and Scheffe's multiple comparison test (6).

Results. Figure 1 is an original tracing showing the influence of normal and CF serum on PD and Isc of the rat jejunum. The rapid upward deflections on the PD tracing indicate the times at which the current was applied. In this experiment, serum 9 (first arrow) was from a normal patient and serum 8 (second arrow) from a patient with cystic fibrosis. When the Ringer's solution on the mucosal surface was replaced with normal serum, a slight decrease in PD occurred, followed by an increase. This pattern was usually seen regardless of the source of the serum. The normal serum was replaced by Ringer's solution, the tissue was allowed to stabilize, and the Ringer's solution was then replaced by CF serum. The latter caused an initial decrease then increase in PD, followed by a more prolonged decrease.

Figure 2 is from an experiment in which the first unknown sample (arrow) that replaced the Ringer' solution was from a CF patient. Note the very substantial reduction in PD and Isc.

Figure 3 shows the influence of normal serum (first arrow) and serum from a heterozygous patient (second arrow) on PD and Isc. The experimental sequence was the same as that illustrated in Fig. 1. Note that the heterozygous serum had qualitatively

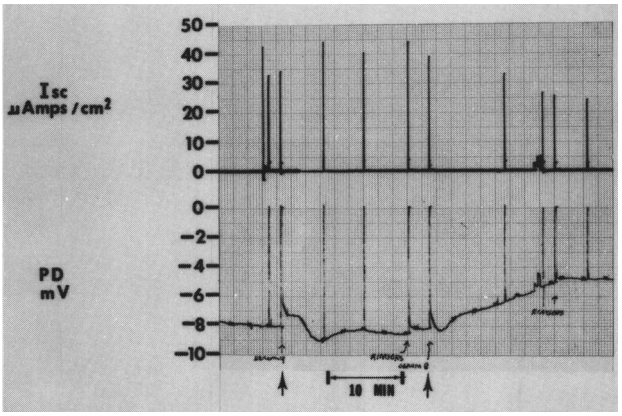

FIG. 1. The influence of normal (serum 9) and cystic fibrotic serum (serum 8) on the Isc and PD of the rat jejunum.

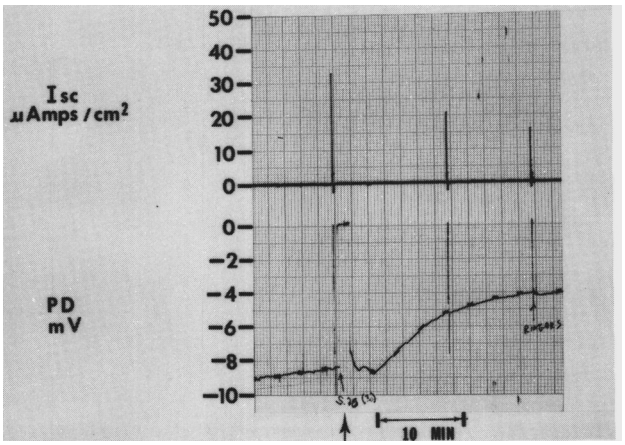

FIG. 2. Influence of cystic fibrotic serum (S.20) on the Isc and PD of the rat jejunum.

the same effect as serum from CF patients (Figs. 1 and 2). Figure 4 is from an experiment in which the first unknown sample (arrow) that replaced the Ringer's solution was from a heterozygote.

Before the replacement of the mucosal Ringer's solution with any sera from the 35 tissues studied, the mean potential difference was 7.44 ± 0.28 mV, the mean Isc was 37.6 ± 1.65 μA/cm^2, and the mean resistance was 203.9 ± 3.57 $\mu\Omega$/cm^2.

Figure 5 shows the individual changes which occurred in PD and Isc as a result of replacing the mucosal Ringer's solution with the various sera. Note that there is no overlapping between the normal serum and serum from either the CF or heterozygous patients. The mean change (±SE) in PD caused by normal serum was +0.29 ± 0.52 mV; for heterozygous serum it was −1.5 ± 0.31 mV, and for CF serum −2.38 ± 1.16 mV. With respect to Isc, normal serum increased it by 2.6 ± 4.5, heterozygous serum decreased it by 8.75 ± 3.58, and CF serum decreased it by 13.82 ± 5.84 μA/cm^2. The changes in the heterozygous and CF groups are significantly different from those of the normal group, but are not significantly different from each other. Thus while these experiments did not distinguish between CF and heterozygous serum, they did distinguish between normals and these two groups. The changes in PD and Isc were in general proportional so that none of the sera produced significant changes in membrane resistance.

Discussion. The results of the present experiments confirm the finding of Araki

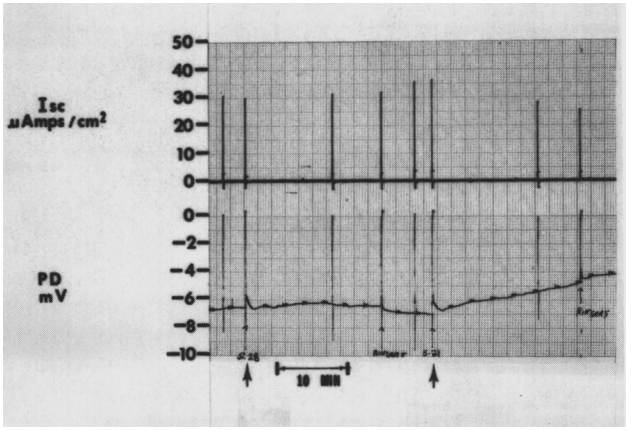

FIG. 3. Influence of normal (S.28) and heterozygous serum (S.21) on the Isc and PD of the rat jejunum.

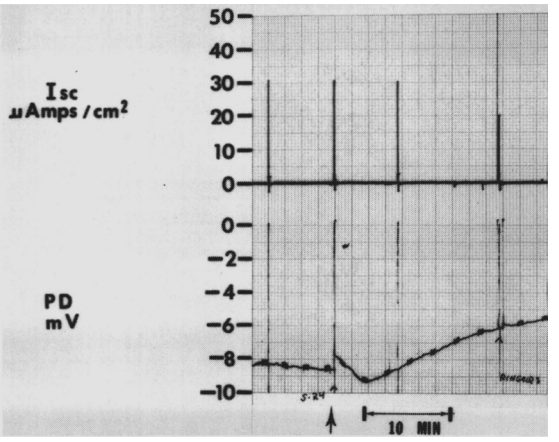

FIG. 4. The influence of heterozygous serum (S.24) on the Isc and PD of the rat jejunum.

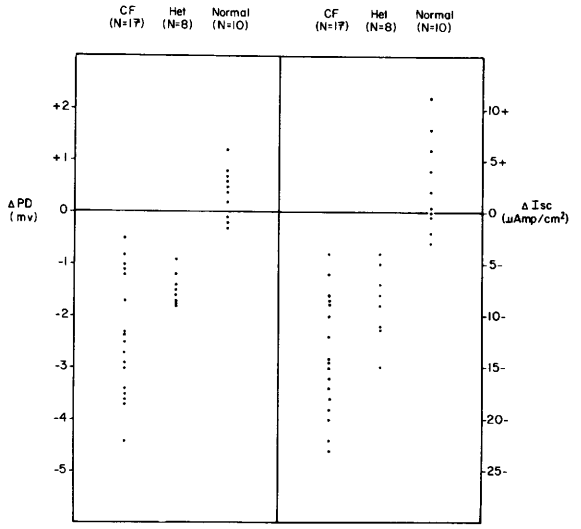

FIG. 5. Individual experiments showing the influence of the various sera on the PD and Isc of the rat jejunum.

and associates (4) that both CF and heterozygous sera decrease the PD and Isc of rat jejunum, while normal serum has no consistent effect. Our failure to show false positives or negatives may be due to the fact that the number of samples analyzed in our study was less than in that of Araki and associates. However, even with our small sample number we found a clear separation between normal serum and CF and heterozygous sera.

The mechanism whereby CF and heterozygous sera inhibit PD and Isc in the rat jejunum is not known. However, it would appear that it in some way decreases the entry of sodium into the cell rather than having a direct effect on a sodium-pumping mechanism. This idea is consistent with the finding of Kaiser and associates (7) that the factor in cystic fibrosis acts on the luminal side of the sweat gland but not on the contraluminal side and with our preliminary finding that CF serum has no effect on PD or Isc of the rat jejunum when placed on the serosal side. In both the sweat gland and jejunum the passage of Na^+ into the mucosal cell can be assumed to be with, and the extrusion out of the serosal or contraluminal surface against, its electrochemical gradient via a "pump" mechanism. If the factor in serum of either CF patients or homozygotes acted by inhibiting a sodium pump one would expect an effect when applied to the serosal or contraluminal surface.

The factor involved in CF does not affect Na transport in all tissues. Its inhibitory effect on the eccrine sweat and salivary gland is not found in other tissues. In addition, in preliminary experiments we found no effect of CF serum on the PD or Isc of frog skin whether it was applied to the mucosal or serosal surface.

Summary. The rat jejunum bioassay has been shown to be capable of identifying both the cystic fibrotic homozygote and heterozygote and therefore would appear to have great potential as a screening test for the latter. It is suggested that the cystic fibrosis factor does not influence sodium reabsorption by inhibiting an active sodium reabsorption mechanism but rather by inhibiting passive sodium entry into the cell.

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