tests are carried out with a plate having a continuous antiserum-containing trough (Fig. 1, d–f). To assure electrical continuity, it was desirable to suspend 5 × concentrated antiserum in one-fifth its volume of 5 percent agar.

The specificity of the test was established by carrying out tests of identity on the newly detected positives. Most additional positives could be detected by the IEOP identity test described above. Most of these specimens could also be identified by the standard Ouchterlony technique if the unknown serum received a preliminary fivefold concentration by the acrylamide-gel procedure, and if the antigen wells were then subsequently filled three times in order to achieve a net 15-fold increase in sensitivity of the Ouchterlony technique. This modification of the Ouchterlony technique results in approximately the same sensitivity as the IEOP test, yet lacks the speed and simplicity of the IEOP method.

Most of the additional positives detected by the IEOP technique showed precipitin lines located closer to the antibody well, in a location similar to that of most of the positives detected by the standard Ouchterlony technique. Since low concentrations of antigen give rise to precipitin reactions closer to the *antigen* well, this finding suggests that the concentration of the antigen in all of the serum hepatitis virus carriers tested does not vary greatly. The fact that some specimens were not detected by the standard Ouchterlony technique may reflect the presence of antigen-antibody complexes (*8, 14*).

The standard Ouchterlony technique used in our laboratory detects at least 20 percent of the carrier population among blood donors. This estimate was made by testing donors suspected of being serum hepatitis carriers on the basis of association with multiple post-transfusion hepatitis episodes. A larger series of similar donors tested by Taswell yielded a 30 to 40 percent detection rate with the Ouchterlony technique (*7*). When the IEOP test was used on blood donor populations, it detected from 1.0 to 3.0 times as many carriers when unconcentrated serum was used. Additional sensitivity was achieved when concentrated serum was used (Table 2.)

Since this paper was submitted, others have reported similar but lower voltage procedures (*15*). Serum samples drawn from ten cases of primary biliary cirrhosis were examined by (i) lower voltage immunoelectroosmophoresis, (ii) the Ouchterlony gel-diffusion assay, and (iii) high voltage IEOP. The SH antigen was detected in nine of ten serums when the high voltage IEOP technique was used; antigen was not detected in any of these serums when lower voltage immunoelectrophoresis or gel diffusion was used (*16*). In addition, both antigen and antibody were detected in four of these serums with the IEOP technique. These results again suggest that the higher voltage technique has a greater sensitivity for detection of antigen present in the form of immune complexes. Until simpler techniques are solved, the IEOP test offers a practical, rapid, sensitive, and highly specific approach to screening blood donors for carriers of the serum hepatitis virus.

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Cystinosis: Selective Induction of Vacuolation in Fibroblasts by L-Cysteine-D-Penicillamine Disulfide

Abstract. *In cultured fibroblasts from individuals with cystinosis vacuolation is induced by exposure to* L-*cysteine*-D-*penicillamine disulfide. Normal fibroblasts do not show vacuolation on such exposure. These observations provide direct evidence that cystinotic cells have deficient activity of a lysosomal system for disulfide metabolism or transport. Induction of vacuolation by the mixed disulfide in cystinotic but not in normal cells furnishes a histological marker for cystinotic fibroblasts.*

Cystinosis (cystine storage disease) is a recessively inherited disorder characteristically manifested by the Fanconi syndrome and lethal, progressive glomerular insufficiency during childhood (*1*). Variants of cystinosis without kidney disease (*2*) or with onset of renal failure beyond childhood (*3*) have been identified. By electron microscopy, presumed cystine crystals have been demonstrated within lysosomes (*4*) or mitochondria (*5*) in biopsies of cystinotic tissues. Accretions of cystine in cystinotic leukocytes migrate in sucrose density gradients together with lysosomal particles (*6*).

Cystinotic fibroblasts cultured in vitro have an intracellular cystine content nearly 100 times the normal amount (*7*); smaller amounts of cystine accumulate in the fibroblasts of individuals with the late onset or benign variants of cystinosis (*2, 3*). The cystine stores in fibroblasts are compartmentalized within a subcellular organelle (*7*). Cystine crystals in cystinotic fibroblasts have not been observed with phase contrast or electron microscopy, although amorphous inclusions presumed to represent accretions of cystine have been found in the lysosomes of these cells (*8*). Our studies provide evidence that cystinosis is a derangement of lysosomal disulfide metabolism or transport; we also describe a method for identifying cystinotic fibroblasts by light microscopy.

Table 1. Fibroblasts were cultivated from patients with the three variants of cystinosis and from normal individuals, exposed to L-cysteine-D-penicillamine disulfide, and examined as described in the text. The degree of vacuolation was determined by one observer (J.S.) who was unaware of the identity of the cells being examined. The cultures were rated as follows: negative, only rare or no cells with vacuoles; intermediate, 10 to 50 percent of cells with generally small vacuoles; positive, nearly all cells with abundant and relatively large phase-lucent vacuoles. Ratings were consistent on duplicate or triplicate observations except as indicated for adult nephropathic cystinotic cells. The difference between the number of positive and negative scores for childhood nephropathic cystinotic and normal cell lines is highly significant (χ^2, $P = .002$). Vacuolation has not so far been observed in fibroblasts from obligate heterozygotes for cystinosis.

Cell lines	Negative	Intermediate	Positive
Childhood nephropathic cystinosis	0	0	8
Adult nephropathic cystinosis	0	(1)*	(1)*
Benign cystinosis	0	1	0
Normal	6	1†	0

* Cells were intermediate or positive in different trials. † These cells also showed intermediate vacuolation in the absence of the mixed disulfide, illustrating that cell lines should be examined after cultivation in the presence and absence of disulfide.

Mouse peritoneal macrophages maintained in vitro demonstrate cytoplasmic vacuolation after exposure to relatively high concentrations of substances that seem to have difficulty penetrating the lysosomal membrane (*9*). Compounds ingested by cellular endocytosis are sequestered within lysosomes; if a compound cannot be metabolized within or transported from the lysosomes it accumulates therein, and the organelles undergo osmotic expansion and become visible under the phase microscope as lucent vacuoles. We have adapted the methods employed with mouse macrophages to permit study of human lysosomal metabolism.

Skin fibroblasts from cystinotic and normal individuals were cultured by standard methods (*7*). Three days before exposure to each of the test compounds the cells were dissociated with trypsin. They were then subcultured at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 in 35-mm petri dishes (Falcon) containing several small cover slips and Eagle's medium No. 2 (NIH media division), with added nonessential amino acids, glutamine, neomycin (50 μg/ml), and 20 percent fetal calf serum. On the 4th day the test compound was dissolved in identical culture medium at a concentration of 0.04 mole/liter. The *p*H was adjusted to 7.4, and the solution was sterilized by Millipore filtration. The original culture medium was removed from the petri dishes and the cells were incubated in the freshly prepared mixture for 20 to 24 hours. Cover slips bearing the fibroblasts were fixed for 10 minutes at 4°C in 1.2 percent glutaraldehyde in Sorenson's phosphate buffer (*10*), rinsed twice with water, and examined as unstained wet preparations at × 400 on a Nikon model MS phase-contrast microscope with a Polaroid photographic attachment.

Cystinotic and normal fibroblasts did not vacuolize in the presence of amino acids or D-peptides with molecular weights of less than 220. D-Trialanine, with a molecular weight of 230, consistently produced dramatic vacuolation in all cell lines. These observations indicate that human lysosomes, like those of the mouse (*9*), are relatively impermeable to substances with molecular weights greater than 220 to 230. Cystinotic and normal fibroblasts behave similarly in this regard.

Cystine, which has a molecular weight of 240, is too insoluble to be directly tested in this system. The more soluble mixed disulfide of L-cysteine and D-penicillamine, a very close structural analog of cystine, was synthesized by the method of Crawhall (*11*), and its purity was confirmed by quantitative amino acid analysis. This compound induced vacuolation in cystinotic but not in normal cells (Fig. 1, Table 1). The mixed disulfide has a molecular weight of 268 and would have been expected to produce vacuolation in both types of cells. Its failure to cause vacuolation in normal cells which vacuolize on exposure to the smaller molecule, D-trialanine, indicates that normal lysosomes possess either an enzymatic mechanism for converting the disulfide to a smaller and more permeable molecule (perhaps by reductive cleavage), or a transport system for facilitating efflux of the disulfide from the lysosomes. The presumed natural substrate for this enzyme or transport system would be cystine itself, a molecule also larger than trialanine and hence potentially susceptible to intralysosomal accumulation.

Vacuolation of cystinotic cells by the mixed disulfide suggests that a physiologically significant mechanism for the transport or metabolism of cystine is deficient in the lysosomes of patients with severe childhood cystinosis, and may be partially so in lysosomes from individuals with less severe variants of cystinosis. These studies provide direct evidence that cystinosis is due to a derangement of lysosomal disulfide transport or metabolism and that cystine accumulations within lysosomes are not a secondary manifestation of a disorder of cystine metabolism elsewhere in the cell.

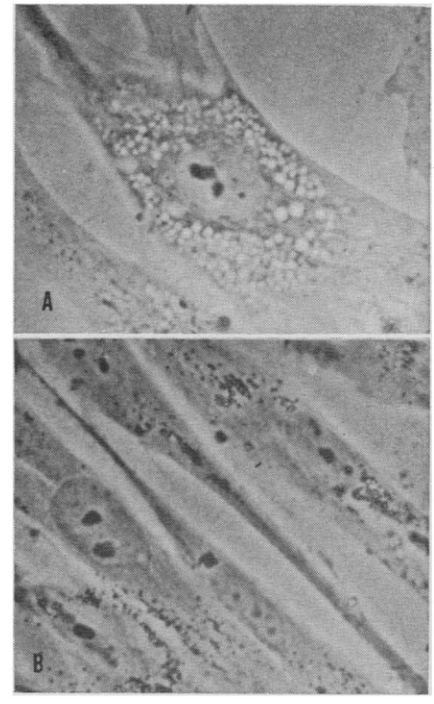

Fig. 1. The appearance of vacuolation in cystinotic fibroblasts exposed to the mixed disulfide (A) and the absence of vacuolation in normal cells (B). Field magnification, × 1000.

Induction of vacuolation by the mixed disulfide in cystinotic but not in normal cells furnishes a histological marker for cystinotic fibroblasts. In certain studies this marker may replace or supplement the more difficult methods of chemical analysis of intracellular cystine; it can be used to study the cystinotic defect within the individual cells. The vacuolation phenomenon may prove useful for establishing or confirming the clinical diagnosis of cystinosis, and could possibly facilitate prenatal diagnosis of the disease (*12*). However, the technique is a new method which has not yet been extensively tested for its specificity and reliability as a diagnostic tool. Therefore, great caution should be used if this technique is applied in attempts at prenatal, or postnatal, diagnosis of cystinosis.

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Thermosensitivity of Neurons in the Sensorimotor Cortex of the Cat

Abstract. *Extracellular action potentials were recorded from 80 neurons in the sensorimotor cortex of the cat as brain temperature was varied by 4° to 8°C. The discharge rate of 37 percent of the neurons studied increased with increasing brain temperature. The discharge rate varied inversely with temperature in 11 percent of the neurons.*

The anterior, preoptic hypothalamus has an important function in mammalian thermoregulation, and the discharge rate of neurons in this region has been correlated either directly or inversely with local changes in temperature (*1*). Recently, similar thermosensitive units have been found in the brainstem (*2*). We report here the results of experiments designed to test the thermosensitivity of neurons in the cat sensorimotor cortex, an area not known to have a thermoregulatory function (*3*).

Brain temperature was changed without interrupting cerebral circulation by perfusion of alcohol at various temperatures through a small metal coil placed next to the carotid rete, an extracranial arterial network interposed between the carotid artery and the Circle of Willis (*4*). By changing the temperature at the rete, we were able to alter the temperature of the area supplied by the carotid by as much as 8°C in 5 minutes, without significantly affecting body temperature over short periods of time.

Extracellular recordings were made with glass micropipettes (10 to 20 megohms) filled with 2.75*M* potassium citrate from neurons in 28 cats anesthetized with either chloralose or urethane. Brain temperature was measured by means of a 24-gauge thermistor needle placed 1 mm deep in the cortex within 2 mm of the electrode tract. Thermistor probes positioned in the aorta, inferior vena cava, and rectum continuously monitored blood and body temperatures. These factors, as well as blood pressure and expired CO_2, were recorded graphically. Brain temperature and unit discharge were recorded on magnetic tape and on a pen-writer recorder, where a one-shot multivibrator device gave a signal for each spike. Discharge frequency was determined by manual counting of the spikes.

A total of 80 neutrons in the sensorimotor cortex were studied for a sufficient period of time so that their sensitivity to one or more cyclic changes of 4° to 8°C in brain temperature (mean examination time, 25 minutes; range, 6 minutes to 2.5 hours) could be assessed. Characteristically, these units discharged at less than three per second and yielded extracellular potentials of 4 to 8 mv. Units whose spike configuraation changed significantly during the period of study were discarded.

The spontaneous discharge rate of 37 percent (30) of the neurons studied increased with increasing temperature. The average change in mean rate for an extrapolated 10°C temperature increase (Q_{10}) was 8.1 times (S.E.M., ± 0.7; range, 3 to 21.5). An example of this type of temperature-dependent discharge is shown in Fig. 1A. The activity of this cell closely paralleled changes in brain temperature. As the temperature was raised from 30° to 37°C, the frequency of discharge increased fivefold. Cooling to 29°C reduced activity by a similar order of magnitude. On rewarming, the discharge once again increased. During alterations in brain temperature aortic, caval, and rectal temperatures remained constant.

The discharge rate of nine neurons (11 percent) varied inversely with tem-

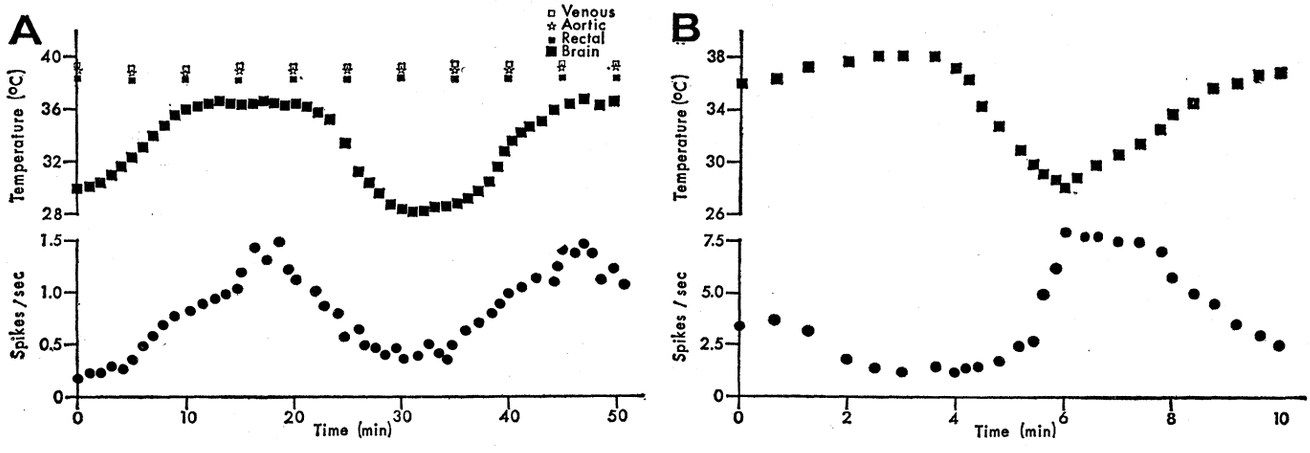

Fig. 1. Change in brain temperature and frequency of response of two neurons in the sensorimotor cortex. Discharge rate (1-minute averages) directly related to temperature is shown in (A), and (B) shows unit activity (25-second averages) inversely related to temperature. Aortic, venous, and rectal temperatures (A only) do not change in spite of the change in brain temperature.