abscisic acid, and that the hormone does not act as a trigger in stomatal development.

1) Decreased water loss was observed in both old leaves (which completed their development before the treatment with ABA, and young leaves (which developed during the hormonal treatment).

2) Water loss in detached leaves from untreated mutant plants decreased proportionately with the hormone concentration when leaves were inserted in ABA solutions.

3) The treated *flc* plants regained their mutant phenotype very soon after termination of treatment. Wilting symptoms appeared several days after ABA spraying had ceased even in those leaves which had developed during the treatment. This fact might also suggest a rapid turnover of the hormone.

Abscisic acid is a natural inhibitor that interacts with kinetin, auxin and gibberellin in several hormonally regulated plant responses (*12*). The hormone antagonized the kinetin-stimulated transpiration of wheat and tobacco leaves when both were applied externally (*5*, *7*). An excessive kinetin-like activity has been found in the leaves of *flc* plants (*13*). There is also evidence of a lower concentration of substances that inhibit the growth of wheat coleoptiles in the leaves of the mutant, as compared with the leaves of the normal plant. The concentration of endogenous abscisic acid-like substances was ten times lower in *flc* than in normal plants (*8*). These facts and the reversing effect of abscisic acid suggest that the excessive opening of the stomata is caused by an insufficient amount of an internal inhibitor, which is presumably abscisic acid. The closing effect of this substance could be manifested either by affecting the closing mechanism directly, or by antagonizing the opening effect of the internal cytokinins.

DOROT IMBER
MOSHE TAL

Negev Institute for Arid Zone Research, P.O. Box 1025, Beer-Sheva, Israel

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Serum Hepatitis Antigen (SH): Rapid Detection by High Voltage Immunoelectroosmophoresis

Abstract. *An immunoelectroosmophoretic technique for rapid detection of the antigen (SH) associated with the serum hepatitis virus has been devised. The technique maintains the specificity characteristic of the Ouchterlony gel-diffusion method, yet detects in 1 to 2 hours one-tenth the amount of antigen required for gel diffusion. The test has immediate application to blood-banking practice since it permits the screening of such labile products as platelets and fresh whole blood, and the detection of antigen in additional serums negative by the Ouchterlony technique.*

A serum hepatitis related antigen (SH), which is identical to the Australia antigen Au(1) (*1*, *2*) and to the "hepatitis antigen" (*3*), has been detected in the serums of patients during the incubation period and early clinical course of hepatitis that follows some transfusions, but not in the serums of patients with the short-incubation type of infectious hepatitis (*2*, *4*). When blood containing the SH antigen is transfused, more than half of the recipients may be expected to develop either clinical or subclinical hepatitis (*5*, *6*), and it therefore is of considerable importance to identify SH antigen carriers in blood donor populations.

The Ouchterlony gel-diffusion technique, most commonly used now for detection of the SH antigen, permits determination of specificity with the use of known standards to obtain fusion of adjacent precipitin lines. This is important since antiserum is derived from multiply transfused patients who have numerous other antibodies. The Ouchterlony technique, however, has two disadvantages in the present application. It is relatively insensitive, detecting only 20 to 30 percent of serum hepatitis carriers in blood donor populations (*7*), and it is a relatively slow test, requiring as long as 1 to 7 days for definitive interpretation. It cannot therefore be used to screen blood products which must be transfused within 1 day of preparation, such as platelets or fresh whole blood.

A complement fixation technique for antigen detection, which has the advantage of a 20- to 100-fold increased sensitivity, has been described (*8*, *9*). This technique also has several disadvantages. Overnight incubation is required to attain a high degree of sensitivity; identification of antigen, particularly when present in small quantities, is difficult and in some cases impossible. Furthermore, since low-titer results must be interpreted with caution, the increased sensitivity afforded by this technique does not appear to be of great practical usefulness for screening.

Most of the immunologic techniques applicable to this problem, such as passive agglutination and radioimmune assays, require either purified monospecific antibody or highly purified antigen if specificity is to be maintained together with an increase in sensitivity.

Table 1. Comparative sensitivity of Ouchterlony gel diffusion (OT) and immunoelectroosmophoresis (IEOP) for detection of serum hepatitis virus specific antigen (SH). Sixteen separate specimens were examined; parentheses indicate the number of assays on each.

Reciprocal of geometric mean titer		Sensitivity increase (No. of times)
OT	IEOP	
26 (4)	256 (8)	10
16 (1)	256 (1)	16
8 (1)	256 (1)	32
4 (1)	64 (1)	16
2 (1)	64 (1)	32
4 (4)	52 (8)	13
4 (1)	32 (1)	8
4 (1)	32 (1)	8
2 (4)	26 (4)	13
4 (1)	16 (1)	4
4 (1)	16 (1)	4
2 (4)	16 (4)	8
2 (1)	16 (1)	8
1.4 (4)	16 (8)	10
<1 (4)	5.6 (8)	>6
Weighted geometric mean:		>9.9

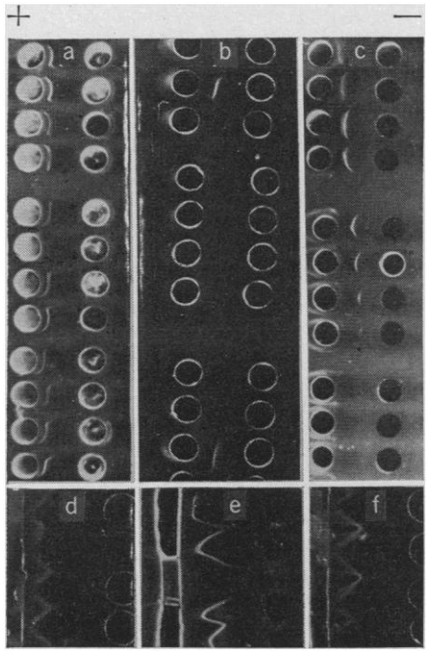

Fig. 1. (a) Nonspecific precipitin lines. Immunoelectroosmophoresis tests carried out for 2 hours at 4°C and 9 volt/cm. Antibody wells are on anodal side. (b) Specific precipitin lines and absence of nonspecific reaction. Immunoelectroosmophoresis tests carried out for 1½ hours at 23°C and 12 volt/cm. Antibody wells are on the anodal side. (c) Serial twofold dilution titration of antigen by immunoelectroosmophoresis. Undiluted specimen is in top well. Test carried out for 2 hours at 23°C and 9 volt/cm. Antibody wells are on anodal side. (d–f) Immunoelectroosmophoretic identity test. In d and f, the antibody trough is filled with 1 percent agarose containing 2× concentrated antiserum S; in e the antibody trough is filled with 4× concentrated antiserum S.

For this reason, we have reexamined the rarely used technique of high voltage immunoelectroosmophoresis (IEOP) (*10*). In this procedure antigen is caused to migrate in an electric field through a suitable medium of diffusion against a stream of antibody migrating in the opposite direction as a result of endosmotic flow.

We now describe an improved technique (*6*) that specifically identifies serum hepatitis carriers in 1 to 2 hours. This technique achieves a rapid combination of reactants, hence its speed; it brings the major fraction of the immunoreactants into the reaction zone, thus increasing sensitivty.

The antiserum used for these studies was fourfold concentrated serum "S," derived from a patient with hemophilia (*2*). Lantern slides (3.5 by 4 inches; 1 inch = 2.54 cm) were covered with 10 ml of molten 1.0 percent agarose suspended in barbital buffer having an ionic strength of 0.05 and a *p*H of 8.6 to 8.8. A special stainless steel punch was used to create wells 3 mm in diameter and 1 cm apart, arranged as shown in Fig. 1, a–c. [Our device punches eight wells at a time (four groups of two); the agar plugs are removed with a glass pipette attached to a vacuum pump.] Opposing wells were filled with antiserum and antigen in the form of serum, plasma, tissue extracts, and the like. Serum is preferable to plasma since coagulation has been found to release additional antigen detectable by immunodiffusion techniques (*11*).

Conditions of electrophoresis are critical. If any one of several variables, such as voltage, temperature, and ionic strength, is not optimum, nonspecific precipitin lines (Fig. 1a) may appear, especially if the serum being tested is not fresh. Because of the adverse effects of temperatures higher or lower than the optimum, the IEOP test is carried out in an electrophoresis chamber (*12*) with the lantern slides resting on a steel plate covered with Mylar film, which is cooled by water at a high rate of flow at 23°C. A second layer of Mylar film covers the agar and wicks to prevent evaporation. Under the conditions that we are now using—1½ hours of electrophoresis at 12 to 15 volt/cm and an agar temperature of 23°C—nonspecific precipitin reactions do not occur, whereas specific precipitin reactions are readily visible after 30 to 75 minutes of electrophoresis.

Precipitating antigens can be quantitated by this technique by testing serial dilutions. In titration of an SH-positive serum (Fig. 1c) the precipitin reaction is located progressively closer to the antigen well as antigen is serially diluted.

Comparative assays of 12 different serums containing antigen revealed that the immunoelectroosmophoretic (IEOP) technique is on the average ten times as sensitive for detection of antigen as the standard Ouchterlony gel-diffusion technique in our laboratory (Table 1).

To evaluate the sensitivity of the IEOP test for detection of SH-positive carriers, 1023 serums from different populations were tested by this technique and by the standard Ouchterlony technique (Table 2). Since our technique was developed as a means of screening blood donors, populations with a high frequency of carriers were selected. In all instances, it was found that serums positive for SH antigen by the Ouchterlony technique were also positive by the IEOP technique. The IEOP technique detected additional carriers in most of the populations tested.

The serums from paid donors were, in addition, examined by the IEOP test following a preliminary fivefold concentration of the serums during 5 hours at 4°C, after the addition to 0.5 ml of serum of 80 mg of desiccated acrylamide gel (*13*). This last procedure further increased the number of carriers detected (Table 2).

To maintain specificity, identification of antigens is required. Identity

Table 2. Comparison of Ouchterlony gel diffusion and immunoelectroosmophoresis for detection of serum hepatitis antigen in chronic carriers. Serums were concentrated prior to testing; those not concentrated are marked 1×, and those concentrated to one-fifth their volume are marked 5×. Only serums in which reaction was confirmed by identity testing were included. In all instances it was found that serums positive by the Ouchterlony technique were also positive by IEOP. The sensitivity ratio is the ratio of the percentage of positives detected by IEOP to the percentage of positives detected by the Ouchterlony method.

Serums		Ouchterlony		IEOP		Sensitivity ratio
Concentrate	No. tested	No. positive	Percent positive	No. positive	Percent positive	
Retarded children in large institutions						
1×	464	126	27.2	137	29.5	1.1
Paid plasma donors						
1×	133	1	0.75	3	2.3	3.0
5×	131			11	8.4	
*Highly implicated donors and controls**						
1×	150	11	19.5	11	19.5	1.0
Paid blood donors						
1×	75	0	0.0	0	0.0	0.0
5×	75	0	0.0	3	4.0	>4.0
Senegalese						
1×	201	8	4.0	18	9.0	2.3†

* Serums were made available by Dr. Howard Taswell. † Observed difference in percentages was more than three times greater than standard error of difference between proportions, therefore the result is significant ($P < .01$).

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.

ALFRED M. PRINCE
KATHLEEN BURKE
Laboratory of Virology, New York Blood Center and *Department of Pathology, New York Hospital–Cornell Medical Center, New York*

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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.