forms are diploid, however, and are not known to produce other than haploid eggs. The peculiar meiotic process of these hybrids is probably delicately balanced and more susceptible to temperature shock than nonhybrid fish are. Suppression of a cleavage stage and the production of diploid eggs followed by fertilization with *P. lucida* sperm is the most likely origin of the triploid Cy. Once the triploid chromosome complement is acquired, meiosis is probably preceded by endomitosis, the population henceforth being sustained by gynogenesis from triploid eggs. This explanation is similar to that offered for the origin of the triploid *Ambystoma* (*16*), except that in *Poeciliopsis* the intermediate hybrid stage is still available in the form of diploid all-female strains.

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Delayed Hypersensitivity in Man: A Correlate in vitro and Transfer by an RNA Extract

Abstract. *The migration of sensitized human monocytic-phagocytic cells from lymph-node tissue-culture suspensions was regularly and specifically inhibited by purified protein derivative or histoplasmin. Moreover, when nonsensitive cells were incubated with an RNA extract from lymph nodes of donors sensitive to purified protein derivative, histoplasmin, or both, the migration of these cells was specifically inhibited by these antigens. Ribonuclease inactivated the RNA extract.*

Tests in vitro of delayed hypersensitivity have thus far depended on the inhibition of migration of sensitive cells (*1*) or on the appearance of transformed cells (mitogenesis) (*2*) by specific antigen. Although the correlation with skin reactions is high, there is no direct evidence that these phenomena in vitro are manifestations of delayed hypersensitivity. The technique of inhibition of migration in capillary tubes (*3*, *4*) offers advantages in experimental animals, but it has not been successfully used for man.

Using an inoculum of living cells, Landsteiner and Chase, and Chase (*5*) described the successful passive transfer of tuberculin hypersensitivity from sensitive donor animals to normal recipients. Lawrence, using a cell-free extract of disrupted leukocytes (*6*), has demonstrated that delayed hypersensitivity can be transferred in vivo in man. This "transfer factor" apparently has the following properties: (i) it is unaffected by ribonuclease, deoxyribonuclease, or trypsin; (ii) it is stable at −20°C for 5 months or at 25° to 37°C for 6 hours; and (iii) it is dialyzable and is separated by Sephadex G-25 (its molecular weight being less than 10,000). Recently a factor isolated in the same manner as "transfer factor" has been reported to transfer sensitivity in vitro, as judged by the mitogenesis assay for delayed hypersensitivity (see *7*).

I now report that the capillary-tube technique can be used with human lymph-node cells in tissue culture to devise an in vitro correlate of delayed hypersensitivity in man. I also report that a cell-free extract containing RNA can transfer delayed hypersensitivity in vitro as judged by this technique. The extract containing RNA reported here differs from "transfer factor" described by Lawrence (*6*) and by Fireman (*7*); but it is similar to that used by Fishman (*8*) and Cohen (*9*) in experimental animals to convert nonimmune cells into antibody-forming cells.

My study included (i) patients with active tuberculosis, (ii) patients with positive skin tests for purified protein derivative (PPD) or histoplasmin, and (iii) normal controls with negative skin tests. Human lymph nodes were obtained by biopsy or at the time of thoracic surgery and were placed in iced Earle's solution, until they were used for tissue culture. The lymph nodes were minced with a scalpel, forced through an aluminum-nickel No. 40 screen, and washed three times in Earle's solution. The separated cells were resuspended in TC 199 (Difco Laboratories), a tissue-culture fluid, containing 10-percent patient's serum and 10-percent fetal calf serum; they were then placed in siliconized tissue-culture bottles for 72 hours. The bottles were gently agitated, and the cell suspensions thus obtained contained both a monocytic-phagocytic "macrophage" type cell population (approximately 25 percent; ability to engulf Fe particles) and a lymphocyte-like population with early transformation (approximately 65 percent), which is similar to cell populations used by other investigators (see *10*).

The monocytic-phagocytic cell suspension, containing approximately 10^5 cells per milliliter, was placed in a sterile capillary tube and centrifuged at 500 rev/min for 5 minutes to obtain a cell pellet. The capillary tube was broken at the cell-fluid interface, placed in a Sikes-Moore tissue-culture closed system chamber and fastened with sterile silicone. To each chamber was added approximately 1 ml of tissue culture medium TC 199 modified to contain lymph-node donor's serum (10 percent), fetal calf serum (10 percent), and fresh-frozen glutamine (0.5 mg/ml). The chambers and contents were incubated for 24 hours at 37°C. The chambers were then placed in a Zeiss projection microscope, and the area of cell migration from the capillary tube was measured by planimetry. The percentage of migration in the presence of antigen is defined as the migration index (M.I.) (*1*), or the ratio of average area of migration with antigen to the average area of migration without antigen times 100.

Figure 1 shows the area of migration and migration index obtained when cell

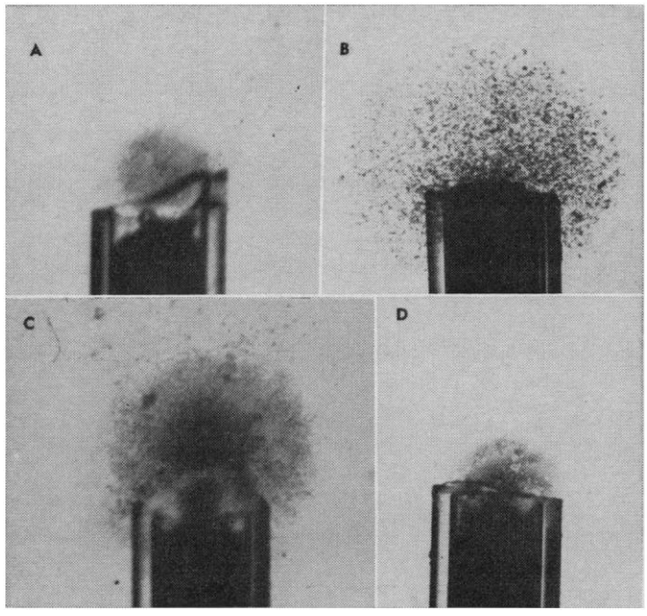

Fig. 1. Antigen-specific migration inhibition. Cells from PPD-sensitive donors challenged (A) with PPD, and (B) with histoplasmin; cells from histoplasmin sensitive donors challenged (C) with PPD, and (D) with histoplasmin.

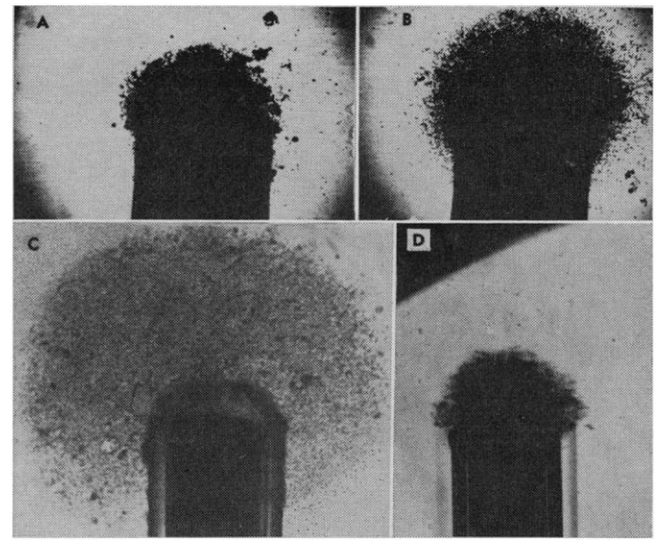

Fig. 2. Cells obtained from individuals who are not skin-sensitive to PPD or histoplasmin. These nonsensitive cells were incubated with an RNA extract from cells sensitive to PPD or histoplasmin. Cells from donors passively sensitized to PPD challenged (A) with PPD, and (B) with histoplasmin. Cells passively sensitized to histoplasmin challenged (C) with PPD, and (D) with histoplasmin.

suspensions from donors sensitive to PPD but not to histoplasmin are challenged with PPD (10 μg/ml) or histoplasmin (30 μg/ml) (*11*). The histoplasmin antigen did not inhibit migration (Fig. 1B; M.I. = 104); the area of migration was virtually identical to that of the control (not shown) in which neither antigen was present. However, when the specific antigen, PPD, was in the medium, significant inhibition did occur (Fig. 1A; M.I. = 12).

With cell suspensions from donors sensitive to histoplasmin but not to PPD, no inhibition was obtained with PPD antigen (Fig. 1C; M.I. = 99); whereas again the specific antigen, histoplasmin, significantly inhibited the cell migration (Fig. 1D; M.I. = 17).

Similar results were obtained in migration-inhibition experiments with nine other PPD-sensitive donors and nine other histoplasmin-sensitive donors. Suspensions of cells from three donors sensitive to both PPD and histoplasmin were also tested. The M.I. with PPD was 16, 21, and 25; but with histoplasmin the M.I. was 35, 23, and 20, respectively. In these six experiments (not shown) either antigen, PPD or histoplasmin, produced migration-inhibition of the sensitive cells.

For the experiments on the transfer of sensitivity by cell-free extracts containing RNA, lymph nodes collected from sensitive donors were immediately frozen in dry ice. To minimize RNA breakdown by nucleases, the frozen lymph nodes were disrupted and homogenized in the cold (0°C) while the cells were immersed in 5 ml of freshly distilled phenol (Mallinckrodt chromatographic grade) saturated with 0.01*M* acetate buffer, *p*H 5, containing polyvinyl sulfate (2 μg/ml). After about 10 minutes, when practically no intact cells remained, an equal volume of acetate buffer containing 0.5 percent sodium dodecyl sulfate and 2 mg of bentonite was added. The RNA was then extracted (*12*). The pellet obtained after the final alcohol wash was dissolved in 0.1*M* NaCl containing 0.0001*M* Mg^{++} and polyvinyl sulfate (2 μg/ml) at 0°C. A portion of this solution was centrifuged in a sucrose-density gradient (7 to 20 percent) at 25,000 rev/min for 15 hours at 28°C to detect RNA breakdown. Those samples with more than 50-percent 4*S* RNA were not used. To transfer sensitivity, 0.1 to 0.3 ml of RNA extract containing approximately 500 μg of RNA (as determined by absorbancy at 260 mμ) was added to 1-ml portions of the suspension of mononuclear cells (72 hours in tissue culture) and incubated for 15 minutes at 37°C; the suspension contained 10^9 cell/ml, more than 90 percent viable, as judged by trypan blue staining.

Cells from donors who did not show skin sensitivity to PPD or histoplasmin showed no migration inhibition with either antigen; with PPD the M.I. was 98; with histoplasmin the M.I. was 105. Figure 2 shows typical examples of the area of migration and migration index when nonsensitive cell populations are incubated with cell-free extracts obtained from PPD or histoplasmin-sensitive donors (*11*). Nonsensitive cells, thus passively sensitized to PPD, when challenged with PPD (10 μg/ml) were significantly inhibited in their migration, the M.I. being 15. When these cells were challenged with histoplasmin as a control of specificity, the M.I. was 95.

Similarly in Fig. 2, nonsensitive cells were passively sensitized to histoplasmin, and, when challenged with the specific antigen (30 μg of histoplasmin per milliliter), substantial inhibition of migration occurred, the M.I. being 14. When these cells passively sensitized to histoplasmin were challenged with PPD as a control, no inhibition occurred, the M.I. being 113.

The extent of specific inhibition of migration was essentially the same for passively sensitized cells (Fig. 2) as for cells from skin-sensitive donors (Fig. 1); for PPD, these indices are 15 and 12, respectively; for histoplasmin, these indices are 14 and 17, respectively. Essentially no inhibition of migration due to cross reaction was observed; PPD did not inhibit histoplasmin-sensitive cells; histoplasmin did not inhibit PPD-sensitive cells (Figs. 1 and 2). Thus, an RNA extract from lymph-node cells that were sensitive to PPD

or to histoplasmin confers upon nonsensitive cells specific sensitivity for PPD or histoplasmin, respectively.

The conversion of nonsensitive cells to antigen-specific sensitive cells has been accomplished in 18 separate experiments: eight from PPD-sensitive donors (Fig. 2), eight from histoplasmin-sensitive donors (Fig. 2), and two from donors sensitive to both. In the last two experiments, either PPD or histoplasmin produced inhibition of migration.

The same amount of RNA extract used for sensitizing cells (500 μg of RNA) was incubated for 2 minutes at 26°C with 0.002 μg of ribonuclease. The ribonuclease-treated extract did not confer sensitivity on nonsensitive cells; migration indices (greater than 85) not significantly different from the controls were obtained. When a portion of the RNA extract treated with ribonuclease was analyzed by density centrifugation at 260 mμ, some degradation of the 8*S* and 12*S* peaks was found, but this was not complete. This experiment suggests that intact RNA has some role in the transfer in vitro of sensitivity to migration inhibition by specific antigen.

Although DNA and protein are presumably removed in the preparation of the RNA extract (*12*), possible contamination of DNA or protein in amounts sufficient to induce biologic activity was evaluated. In some experiments, other portions of the active RNA extract were treated with trypsin (maximum conditions of 10 μg of trypsin per 500 μg of RNA at 37°C for 8 hours) (*13*) or deoxyribonuclease (maximum conditions of 50 μg of deoxyribonuclease per 500 μg of RNA at 37°C for 8 hours). Under these conditions, neither trypsin nor deoxyribonuclease significantly altered the density centrifugation patterns; and they did not have any appreciable effect on the transfer of sensitivity in vitro. For example, after treatment with trypsin, transfer of PPD sensitivity to nonsensitive cells yielded a migration index of 20 to 25; after treatment with deoxyribonuclease, the M.I. was 16 to 30. These experiments suggest that contamination with protein or with DNA is not the basis for the transfer of sensitivity in vitro by this RNA extract.

By its method of isolation and sensitivity to ribonuclease, this RNA extract differs from the cell-free extract described by Lawrence (*6*); his "transfer factor" is ribonuclease-insensitive. Fireman, using mitogenic activity as an assay system (*7*), has recently estimated the molecular weight of this "transfer factor" at 700 to 4000. The RNA extract here described as 8*S* to 12*S* RNA suggests a molecular weight greater than 80,000, but this extract might still contain components of lower molecular weight as the active principle. In control experiments, the RNA extract, without addition of histoplasmin or PPD, did not inhibit migration of sensitive cells. Also histoplasmin or PPD, without the RNA extract, did not confer sensitivity to nonsensitive cell populations after incubating for 24 hours at 37°C. The conversion of nonsensitive cells to sensitive cells by the RNA extract in this in vitro system does not appear to be the result of active immunization by antigen or antigen fragments.

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Mitochondrial Malate Dehydrogenase: A New Genetic Polymorphism in Man

Abstract. *Starch-gel electrophoresis patterns of malate dehydrogenase from human tissue indicate a new genetic polymorphism for the mitochondrial form of the enzyme. Studies of families showed simple Mendelian segregation rather than maternal inheritance, suggesting that not all mitochondrial proteins are coded by mitochondrial DNA.*

Malate dehydrogenase (MDH) is widely distributed in mammalian tissues and plays an important role in carbohydrate metabolism. It is one of several enzymes known to exist in two distinct forms: (i) in the cytoplasm as soluble or supernatant MDH (S-MDH) and (ii) tightly bound to the mitochondria (*1*). These two forms of the enzyme have different physical and chemical properties (*2*), and each has a characteristic electrophoretic pattern (*3*). Only one electrophoretic variant of the supernatant form was found in our study (*4*) of approximately 3000 individuals. We now report on a survey of 523 white individuals to determine the prevalence of variants of mitochondrial MDH (M-MDH). Because mature erythrocytes lack mitochondria, leukocyte extracts and extracts from placentas have been used. We studied patients and personnel selected at random from two Buffalo hospitals. Placentas were furnished by the obstetric service of Buffalo Children's Hospital.

Leukocyte extracts were prepared by removing the buffy coat from sedimented whole blood with a Pasteur pipette. The cells were washed briefly in distilled water to hemolyze most of the erythrocytes and then washed again in normal saline. The cells were then suspended again in an equal volume of distilled water and subjected to sonication for 1 minute. Approximately 1 g of placental tissue was ground in a hand homogenizer, and the homogenate was spun at 20,000*g* to obtain a clear extract. Vertical starch-gel electrophoresis was carried out in a phosphate-citric acid buffer at *p*H 7.0 (*3*). A voltage gradient of 4 to 6 volt/cm was applied for 16 hours at 4°C. The gels were sliced horizontally after electrophoresis, and the developing solution consisted of 725 mg of DL-malic acid, 5 mg of NAD, 5 mg of phenazine methosulfate, and 5 mg of MTT tetrazolium (*5*) in 50 ml of 0.5*M* tris hydrochloric acid at *p*H 8.6