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

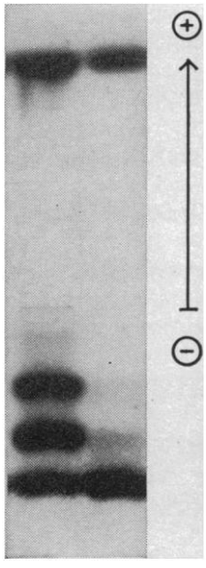
Five unrelated individuals were found with the same electrophoretic variant of M-MDH (Figs. 1 and 2). In the usual tissue MDH pattern, there appear to be five bands of M-MDH activity, one major band (band 5) and four minor bands of progressively decreasing intensity (*3*). The variant pattern consists of the usual major band 5 and two additional major bands of mitochondrial MDH activity which have identical electrophoretic mobility with minor bands 4 and 3 of the usual pattern. Bands 2 and 1 tend to be more prominent in extracts from individuals with the variant. Electrophoresis for as long as 48 hours failed to demonstrate any difference in mobility between bands 4 and 3 of the variant and the corresponding usual bands. From the pedigrees of three individuals with the variant M-MDH (in Fig. 3), simple Mendelian inheritance is apparent. Persons with the variant are apparently heterozygous for a relatively common autosomal mutant gene. Because the variant electrophoretic pattern is the same in males and females, it is unlikely that the genetic locus controlling M-MDH activity could be located on the X chromosome.

Our studies represent the first demonstration of a genetic variant of a mitochondrial protein in man, and the findings are different from those generally found in lower organisms. Luck and Reich (*6*) demonstrated conclusively the presence of intramitochondrial DNA in *Neurospora* and detected the presence of an intramitochondrial RNA polymerase that was dependent on DNA and was insensitive to the external addition of deoxyribonuclease or ribonuclease; this finding suggests that the polymerase was protected by the mitochondrial membrane. They also showed that mutations of mitochondrial DNA were transmitted by the maternal parent alone, that is, that all offspring of a mutant maternal organism were generally affected. In addition, the mitochondria appear to have a unique set of transfer RNA's and amino acid synthetases (see *7*). These data support the concept that mitochondria have a separate genetic system. Examples of nonchromosomal heredity are also known in plants, in other microorganisms, and in *Drosophila* and other insects (*8*). Maternal inheritance seems to be the general rule. However, Tapley *et al.* (*9*) point out that, based on the measured amount of DNA in the mitochondria, "it appears theoretically impossible for all the proteins associated with mitochondria in vivo to be coded by the limited available mitochondrial DNA." Thus, one might expect to find some mitochondrial proteins under nuclear or chromosomal genetic control; this appears to be the case with M-MDH in man.

This variant of M-MDH is apparently a new human genetic polymorphism in that the variant electrophoretic pattern was found in approximately 1 percent of the population studied. The family studies showed that the locus controlling M-MDH activity is probably autosomal and that individuals with the variant are heterozygotes. Thus far, a homozygous mutant individual has not been found, because such an individual would be extremely rare. The appearance of the usual soluble MDH pattern in all individuals with the mitochondrial variant provides further evidence that the two cellular forms of this enzyme are under separate genetic control.

The nature of the variant remains obscure. Kitto *et al.* (*10*) provided convincing evidence suggesting that the isozymes of M-MDH represent conformational changes of the same enzyme molecule rather than the random association of subunits as with lactate dehydrogenase. The most likely explanation of the electrophoretic patterns seen in the leukocyte or placental extracts of persons with the variant is that they are heterozygous for a structural mutation of the gene normally responsible for a dimeric molecule composed of two identical subunits. Thus, the usual allele (MDH^U) would be responsible for band 5 (UU) and its conformational manifestation, minor bands 4 to 1. The mutant allele (MDH^A) would code for a subunit whose dimeric form is represented by major band 3 (AA), and major band 4 would represent the association of one usual and one atypical subunit (UA). If this were the case, the identical migration of conformers 4 and 3 with the mutant bands 4 and 3 would be coincidental. Apparently, the mutant bands 4 and 3 also produce conformers, in that in all cases the enzyme from affected individuals shows more prominent bands 2 and 1. Investigation of the exact nature of this unusual variant will be greatly facilitated by the discovery of an individual homozygous for the atypical allele.

In addition to the immediate biochemical problems posed by our find-



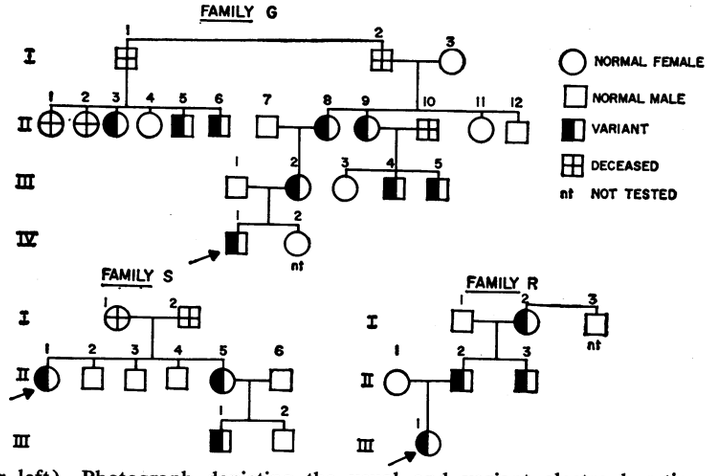


Fig. 1 (far left). Photograph depicting the usual and variant electrophoretic patterns of human MDH. The anode is toward the top. Fig. 2 (left). Diagram of Fig. 1. Fig. 3 (above). Pedigrees of the three families examined. In each family the proband is indicated by an arrow.

ings, this relatively common variant will be of interest for further genetic investigations. It should prove to be a useful marker in population and linkage investigations, although the pedigrees to date do not suggest linkage with any of the blood groups studied.

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Chemical Bonding Information from Photoelectron Spectroscopy

Abstract. *High-resolution measurements of photoelectrons produced by x-rays in compounds of iodine and europium have revealed chemical shifts in the core-level energies, from which chemical bonding information can be obtained. The observed shifts, 0.8 electron volt per unit change in oxidation number in iodine and 9.6 electron volts in europium, are discussed in terms of two theoretical models.*

A new spectroscopic method, recently developed by Siegbahn and co-workers in Uppsala (*1*), has found application in elementary chemical analysis (*2*) and in studies of chemical bonding (*3*). The principle of this method is to deduce the binding energies of the inner-core electrons of an atom from high-resolution measurements of spectra of photoelectrons produced by x-rays. Its utility in studies of chemical bonding arises from the observation that these binding energies are sensitive to the chemical environment of a given atom. Chemical shifts in the core-electron binding energies have been measured in several light elements (*3*), and we report here on shifts in two heavier elements, iodine and europium. Our experimental shifts can be understood in terms of two theoretical models, one using a charged-shell approximation for the molecular orbitals, the other making use of an energy cycle including free-ion and lattice interaction terms.

The experimental technique involves expelling an electron from a level, i, of an atom A with x-radiation greater in energy than the binding energy. Disregarding contact potential effects, the energy conservation equation is

$$h\nu = E_b(A,i,X) + E_{kin}$$

where $h\nu$ is the x-ray energy, $E_b(A,i,X)$ is the binding energy of the ith level of atom A in compound X, and E_{kin} is the photoelectron kinetic energy. Since x-ray energies previously have been determined to high accuracy, only the kinetic energy need be measured to obtain a binding energy. For this purpose a high-resolution magnetic spectrometer was used (*4*). The energy resolution of this spectrometer was adjusted to 0.06 percent full width at half maximum, thereby yielding instrumental line widths of 0.6 to 4.8 ev over the kinetic energy range of interest (1 to 8 kev). As the natural line widths are also a few electron volts, it is easily possible to be able to detect shifts in binding energy of the order of 1 ev with such a spectrometer.

The apparatus is shown schematically in Fig. 1. Radiation from the x-ray tube is filtered slightly with aluminum foil, and then impinges upon a flat rectangular (10 by 13 mm) powdered sample of the compound under study. Photoelectrons emitted from the sample pass through a defining slit into the spectrometer. For a given current in the spectrometer coils, electrons of a narrow range of energy are brought to a focus at the entrance to the Geiger counter. The current is scanned in a stepwise fashion over the region of interest and the resulting pulses from the Geiger counter are stored in a multiscalar (multichannel analyzer). Multiple scans were made in order to average out variations in x-ray flux.

The compounds studied and the oxidation numbers of iodine or europium in each were: KI(−), KIO_3(5+), KIO_4(7+), potassium salt of *p*-iodobenzoic acid (oxidation state uncertain), $EuAl_2$(2+), Eu_2O_3(3+), and europium metal (quickly oxidized to a 2+ or 3+ state). Typical photoelectron peaks are shown in Fig. 2. In the notation $Eu3d_{5/2}$, Eu^{2+} designates a peak due to photoelectrons expelled from the $3d_{5/2}$ level of a 2+ europium compound. A chemical shift of about 10 ev between the peaks from Eu^{2+} and Eu^{3+} is apparent.

Our experimental results may be summarized by the following: (i) The direction of the observed shifts is such that the higher oxidation state has the higher electron binding energy. (ii) The magnitudes of the shifts are essentially the same, for a given element, for all core levels investigated. (In iodine, levels from $2s_{1/2}$ to $4d_{5/2}$ were included, involving a hundred-fold change in the total binding energy.) Thus one can refer to an "average core shift" between compounds of different oxidation number. (iii) In iodine, the average shifts relative to KI are: K-salt of *p*-iodobenzoic acid, 0.0 ev; KIO_3, 5.3 ev; and KIO_4, 6.3 ev. These numbers correspond to an average core shift of about 0.8 ev per unit change in oxidation number. In europium the average shift is 9.6 ev.

Figure 2 shows the chemical shift that results from the oxidation of europium metal. Spectrum A, obtained from $EuAl_2$, shows clearly the peak arising from the 2+ state, although a small 3+ peak is visible because of surface oxidation. Spectra B and C were obtained from a piece of europium metal that was initially polished in air to give

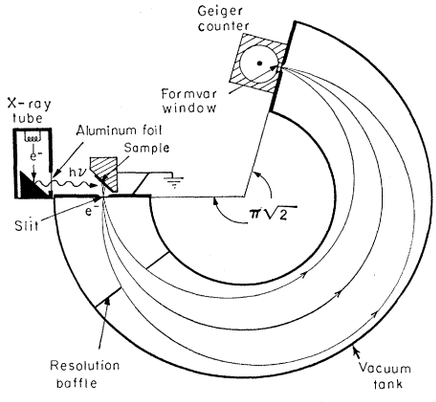

Fig. 1. Schematic illustration of the experimental apparatus.