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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References and Notes

1. G. S. Christie and J. D. Judah, *Proc. Roy. Soc. London Ser. B* **141**, 420 (1953).
2. L. Siegel and S. Englard, *Biochim. Biophys. Acta* **54**, 67 (1961); S. Englard and H. H. Brieger, *ibid.* **56**, 571 (1962); E. Shrago, *Arch. Biochem. Biophys.* **109**, 57 (1965).
3. C. J. R. Thorne, L. Grossman, N. O. Kaplan, *Biochim. Biophys. Acta* **73**, 193 (1963).
4. R. G. Davidson and J. A. Cortner, *Nature* **215**, 761 (1967).
5. The MTT tetrazolium was obtained from Sigma Chemical Co.
6. D. J. L. Luck and E. Reich, *Proc. Nat. Acad. Sci. U.S.* **52**, 931 (1964); E. Reich and D. J. L. Luck, *ibid.* **55**, 1600 (1966).
7. W. E. Barnett and D. H. Brown, *ibid.* **57**, 452 (1967).
8. R. Sager, *New Engl. J. Med.* **271**, 352 (1964).
9. D. F. Tapley, D. V. Kimberg, J. L. Buchanan, *ibid.* **276**, 1124 and 1182 (1967).
10. G. B. Kitto, P. M. Wassarman, N. O. Kaplan, *Proc. Nat. Acad. Sci. U.S.* **56**, 578 (1966).
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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

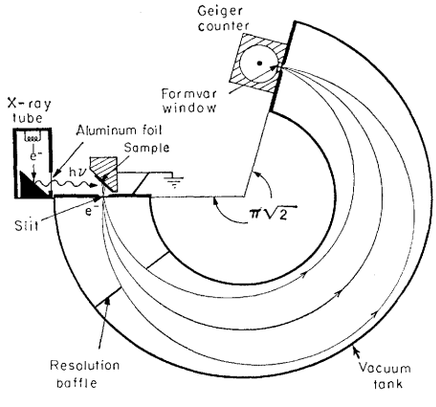

Fig. 1. Schematic illustration of the experimental apparatus.

a shiny surface. This surface oxidized immediately to a mixture of the 2+ state (EuO) and the 3+ state (Eu_2O_3), as can be seen by comparing spectra A and B. Upon prolonged exposure to air, the same sample oxidized completely to the 3+ state (Eu_2O_3), as can be seen from spectra C and D. The spectral intensity does not decrease to background level on the left side of the peaks, because of the close proximity of another line—the 2+ peak excited by a second characteristic x-ray line of copper. The interpretation in terms of chemical reaction is nevertheless straightforward.

The striking contrast between the magnitude of the observed chemical shift accompanying a unit change of oxidation state in europium and that in iodine has an obvious qualitative interpretation. Europium is highly ionic; oxidation from Eu^{2+} to Eu^{3+} corresponds rather closely to the actual removal of one electron from an atomic orbital (*5*). Free-ion wave functions indicate that this removal corresponds approximately to a 20-ev shift. Iodine compounds are not strongly ionic, and oxidation corresponds to removal of only a small fraction of one electronic charge from the vicinity of the iodine atom.

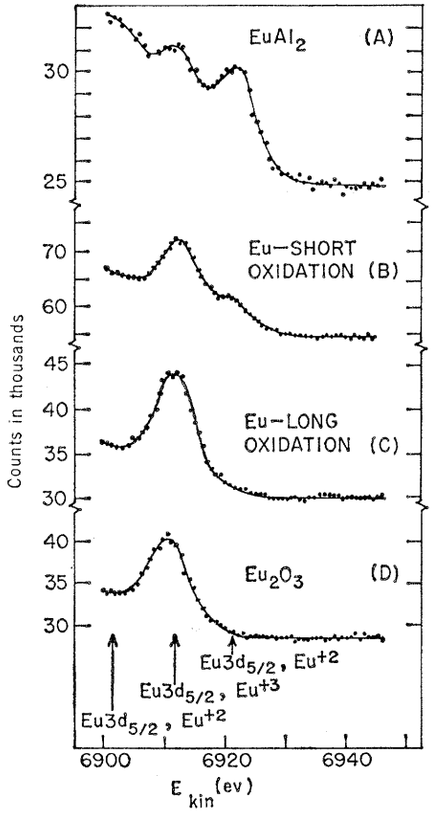

Fig. 2. Photoelectron peaks from various europium compounds. The two peaks for the 2+ state are due to excitation by two different x-ray lines. See text for details. The counts indicated on the ordinate were accumulated by counting for a total of from 2 to 4 minutes at each energy value.

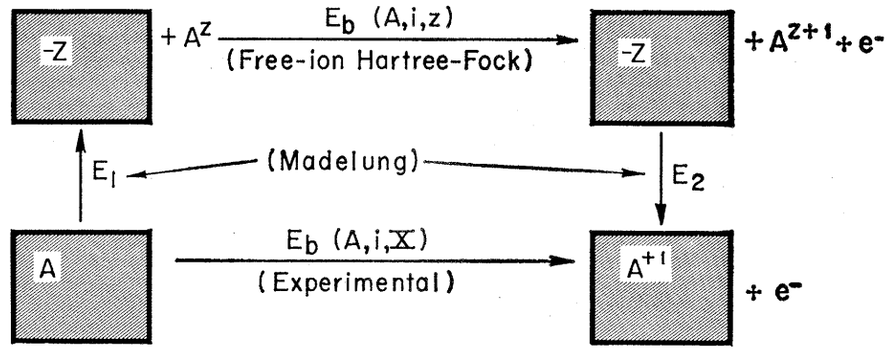

Fig. 3. Energy cycle for the calculation of binding energies.

To extract more quantitative information on chemical bonding from these data, a theoretical model must be considered. As a first approximation we used a semiclassical model that assumes that electronic charge participating in bonding is removed from the valence shell to a spherical charge shell of larger radius, r. The energy shift of a core level upon formation of a chemical bond is the shielding energy of the removed valence electrons minus the repulsive potential of this spherical charge shell. We have calculated the shielding energy with a free-ion Hartree-Fock computer program (*6*). The repulsive potential is obtained classically. This model was applied to our europium data. The experimental shifts yield $r \cong 1.3$ Å, which is approximately half the Eu-O interatomic distance in Eu_2O_3.

A more accurate model is illustrated in Fig. 3 (*7*). For example, we are interested in the binding energy of the ith core level of atom A in the solid compound X. Atom A is assumed to possess some net charge, z; self-consistent charges are assigned to all the other atoms in the lattice so that overall electroneutrality is maintained. The binding energy can then be calculated by means of the following energy cycle: the first step is to remove A from the lattice to form a free ion of charge z; next, an electron is removed from the ith level of this ion; and then the ion is inserted back into the lattice. Since the net result of this cycle is the same as that of a photoelectric process (subtracting any kinetic energy), the experimental binding energy in the solid is given by the sum of the three energies required in the cycle. The second energy in the cycle is a free-ion binding energy $[E_b(A,i,z)]$ and can be calculated by using the Hartree-Fock technique. The sum of the first and last energies ($E_1 + E_2$) represents the classical Coulomb interaction energy of an electron in the ion of interest with all the other ions in the lattice, which are treated as point charges. This classical Coulomb energy is related to the Madelung constant of the solid. The chemical shift between two compounds is simply the difference between binding energies computed in this fashion.

From this cycle it is clear why all the core levels in a given atom are shifted by very nearly the same amount. The Madelung energy depends only on the lattice structure; differences in the shifts of various core levels must arise in the free-ion contributions. In fact, our calculations show that for the halogens all inner-core levels shift by the same amount to within ± 2 percent.

The Madelung energy was calculated for the three iodine compounds to give the chemical shifts relative to KI in terms of the charges on the iodine atom in KIO_3 and KIO_4. Comparison with experiment yielded an iodine charge of 1.10 in KIO_3 and 1.24 in KIO_4. Mössbauer experiments (*8*) have given 0.83 (KIO_3) and 1.44 (KIO_4). Our values involve a somewhat uncertain correction for surface effects; on the other hand, they were obtained with a minimum of assumptions about the bonding orbitals in each compound, whereas the Mössbauer results rely heavily on such assumptions.

A detailed treatment of this work is in preparation (*9*).

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References and Notes

1. S. Hagström, C. Nordling, K. Siegbahn, *Alpha-, Beta-, and Gamma-Ray Spectroscopy*, K. Siegbahn, Ed. (North-Holland, Amsterdam, 1965), vol. 1, appendix 2, table on pp. 845–862.
2. C. Nordling, S. Hagström, K. Siegbahn, *Z. Phys.* **178**, 433 (1964).
3. G. Axelson *et al.*, *Nature* **213**, 70 (1967); R. Nordberg *et al.*, *ibid.* **214**, 481 (1967); S. Hagström, C. Nordling, K. Siegbahn, *Z. Phys.* **178**, 439 (1964); A. Fahlman, R. Carlsson, K. Siegbahn, *Arkiv Kemi* **25**, 301 (1966).
4. K. Siegbahn, C. Nordling, J. M. Hollander, *Rep. UCRL-10023* (Lawrence Radiation Lab., Univ. of California, 1962).
5. V. Jaccarino, *et al.*, *Phys. Rev. Letters* **5**, 251 (1960); A. L. Borovik-Romanov and N. M. Kreiss, *Soviet Phys. JETP (Engl. Trans.)* **2**, 657 (1956).
6. C. C. J. Roothaan and P. Bagus, *Methods in Computational Physics* (Academic Press, New York, 1963), vol. 2.
7. N. F. Mott and R. W. Gurney, *Electronic Processes in Ionic Crystals* (Clarendon Press, Oxford, 1948), p. 80; C. S. Fadley, S. B. M. Hagström, M. P. Klein, D. A. Shirley, *Bull. Amer. Phys. Soc.* **11**, 884 (1966).
8. D. W. Hafemeister, G. de Pasquali, H. de Waard, *Phys. Rev.* **135**, B1089 (1964).
9. C. S. Fadley, S. B. M. Hagström, M. P. Klein, D. A. Shirley, *Rep. UCRL-17005* (Lawrence Radiation Lab., Univ. of California, 1967).
10. Work done under the auspices of the AEC (contract No. W-7405-eng-48).

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Strain of Mycoplasma Associated with Human Reproductive Failure

Abstract. *A strain of mycoplasma not previously described has been isolated from the chorion, decidua, and amnion of a patient who sustained a spontaneous abortion during the middle trimester. The fetal membranes exhibited an inflammatory reaction, but no evidence of other infectious agents, bacterial or viral, was noted. The T strain identified is not a classical mycoplasma; it differs in growth and nutritional requirements from the T strains previously characterized.*

Inflammatory reactions observed in the fetal membranes associated with many spontaneous abortions and premature births have been unexplained bacteriologically (*1*). In a series of such specimens, mycoplasma species were sought with the use of two techniques. The standard Hayflick medium (*2*) was inoculated and incubated under microaerophilic and aerobic conditions in a search for the classical, large-colony mycoplasma strains. Shepard's low-*p*H medium (*3*) was inoculated for identifying small-colony T strains and incubated under microaerophilic conditions. Blood-agar cultures were inoculated and incubated under microaerophilic and aerobic conditions for bacterial flora. A microaerophilic atmosphere on all media was accomplished with the Fortner plate method (*4*). All cultures were incubated at 37°C.

A strain of mycoplasma which does not resemble any strains previously described was recovered from chorion, amnion, and decidua of a spontaneous abortion during the middle trimester. This strain grew only on Shepard's medium and not on Hayflick's and had the colonial morphology of a T strain 20 to 40 μ in diameter, but it differed from previously reported T strains (*5*) in its slower growth on primary isolation and its fastidious nutritional requirements. In primary cultures, colonies could be visualized only after 4 days of incubation, were irregular in shape, had a deeper-staining central core, and accepted the Dienes' stain up to 2 weeks after isolation on the original medium. Shepard's broth did not support subcultures. A new broth (developed by Shepard) supplemented with urea and containing a phenol red indicator did support subcultures of this strain (*6*). The membranes cultured on blood agar under both aerobic and microaerophilic atmospheres showed no bacterial growth, thus eliminating the consideration that L forms had been induced from bacterial forms by penicillin present in the medium on which the initial isolation was made. Colonies of the newly encountered mycoplasma also grew on ascitic agar (*7*) which contains no penicillin. Lung and liver cultures of the fetus were negative for mycoplasmas. The liver showed no growth on blood agar. A few colonies of diphtheroids grew from culture of the lung.

Histologically, the decidua showed extensive necrosis and subacute inflammation; the fetal membranes and umbilical cord vessels were severely inflamed (Figs. 1 to 3). The infection of the membrane appeared to be of long duration. Unusual sclerosis of placental villi was observed. Acute inflammatory exudate, apparently aspirated and swallowed by the fetus (Fig. 4), filled the lumens of the bronchi and stomach. There was no evidence of subjacent tissue reaction. No organisms were identified in sections stained with hematoxylin and eosin, Giemsa, periodic acid-Schiff, or Gram stains. The placentitis did not resemble that which accompanies such antenatal viral infections as rubella, cytomegalovirus disease, herpes, vaccinia, or varicella. No inclusion bodies were found.

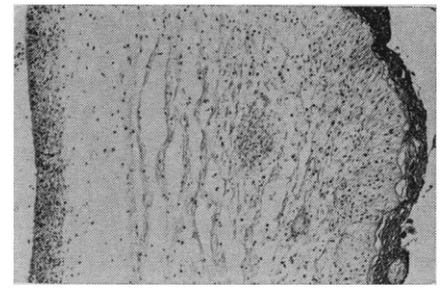

Fig. 1. Fetal membranes, amnion to the left. Necrosis and inflammatory infiltration are evident. (× 45)

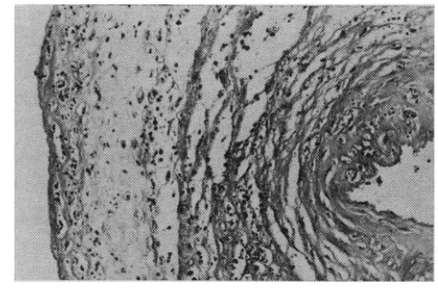

Fig. 2. Fetal membranes, amnion to the left. The amniotic epithelium is necrotic. Polymorphonuclear leukocytes are scattered in the chorionic membrane and amniotic connective tissue. Karyorrhexis of these cells is evident. (× 45)

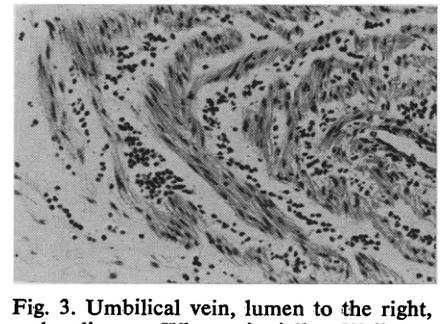

Fig. 3. Umbilical vein, lumen to the right, and adjacent Wharton's jelly. Well-preserved polymorphonuclear leukocytes lie between muscle bundles of the media and in the otherwise hypocellular contiguous stroma. (× 167)

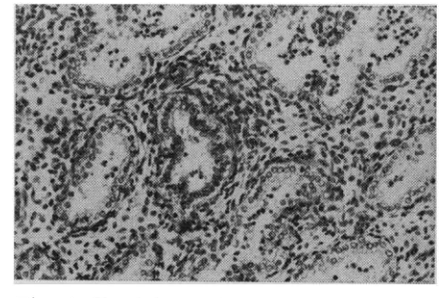

Fig. 4. Fetal lung. A suspension of polymorphonuclear leukocytes and proteinaceous debris lies within bronchiolar lumens. The immaturity of the fetus is indicated by the broad cellular septa. (× 167)