centrated HCl, H_2O, and acetone, 1:1:6). A major portion of the water-soluble phase could be recovered by this cleaning method. These metabolites did not move from the origin (spot A) on the same thin-layer chromatographic assay as in Fig. 1, which proves that this portion of the radioactivity is not due to absorbed dieldrin.

None of the dieldrin metabolites found in this work have been identified, except through matching R_f values of various candidate compounds with the use of the same thin-layer chromatographic method. Spot A consisted of materials that did not move by this chromatographic method (that is, very polar compounds) and could contain some amount of acidic metabolites; spot E matched with an authentic reference compound, 6,7-*trans*-dihydroxy-dihydro-aldrin; and spot H matched with dieldrin. The precise mechanisms of the degradation of dieldrin are therefore uncertain at this time. Neither has the actual role of these microorganisms been determined in eliminating this insecticide from the soil.

As shown in Table 1, two out of ten microbial cultures were identified to the species level (cultures 12 and 41 were *Trichoderma viride*). Six of them were found to belong to the genus *Pseudomonas*, and two to the genus *Bacillus*. It is interesting that culture 12 is the same isolate of *T. viride* that was previously reported to have malathion-degradation capacities (*12*). Contrary to this, Chacko *et al.* (*6*) reported this species to be inactive against both DDT and dieldrin. This difference could be due either to differences in testing methods or to differences, at the subspecies level, in the microorganisms themselves. Since the *T. viride* cultures were isolated from two completely different, but dieldrin-treated, soils and since this species is quite versatile in producing numerous variants, one might think that the variants isolated from contaminated soils are indeed very different from those tested by the above workers.

None of the six *Pseudomonas* cultures appeared to belong to the same species. The fact that this type of random screening resulted in the selection of six species of a single genus may indicate the flexibility of the pseudomonads to adapt to situations of high insecticidal pressure.

The major object of this report is to demonstrate, for the first time, the presence in soil of microbes capable of degrading dieldrin. The implications of these findings in the actual ecosystems in our environment may be very interesting. Problems of such an ecological nature, however, are quite complex and will require very careful study.

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Field Ion Microscopical Imaging of Biomolecules

Abstract. *Specimen molecules are protected from field desorption by embedding them in a platinum matrix, with the use of electrolytic codeposition on tungsten tips. High-resolution helium ion images are obtained when the biomolecules are exposed during gradual removal of surface layers by controlled field evaporation. Structured images of individual molecules of coenzyme I and vitamin B_{12} are seen.*

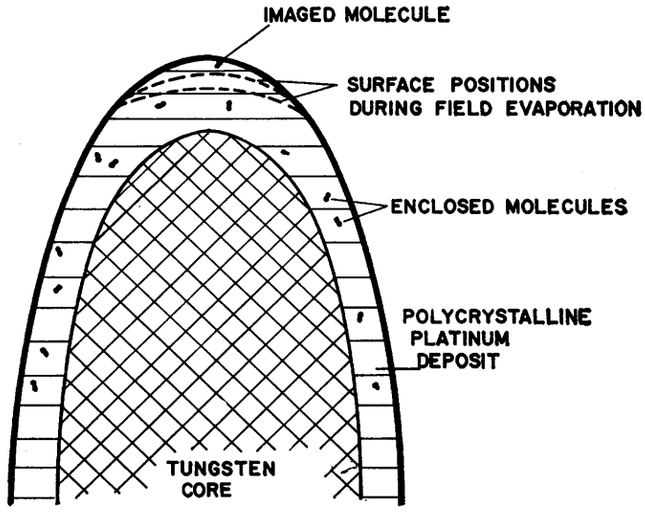
The attainment of atomic resolution in images of metal surfaces made with the field ion microscope (*1*) has been an incentive for trying to view organic molecules of biological interest. Unfortunately, phthalocyanine and similar molecules superficially attached to the surface of the specimen tip were found to be removed when a field of about 100 to 180 megavolts per centimeter is applied (*2*), while high-resolution imaging with helium ions requires about 400 Mv/cm. Similar low desorption fields were also found for transfer-RNA and light meromyosin (*3*). An attempt at shadow-casting organic molecules with a refractory metal that would form a complete layer on the surface of the tip and would display the circumference of the removed molecular specimen also failed because the condensed metal layers have a structure too random to be useful (*4*).

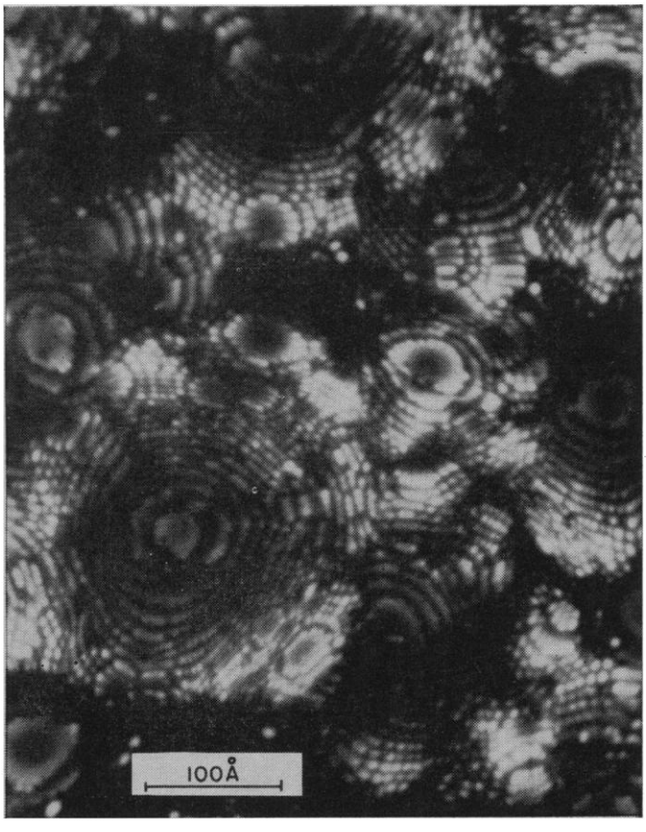
A new method was conceived and initially tried out in cooperation with I. R. Miller of the Weizmann Institute. We embed the molecules in an electrolytic metal deposit. During the field evaporation process the embedded molecules appear at the surface and can be imaged. Since the molecule is anchored in the surface and is surrounded by metal atoms on the sides, it will be desorbed at a much higher electric field than if it were simply adsorbed on the surface.

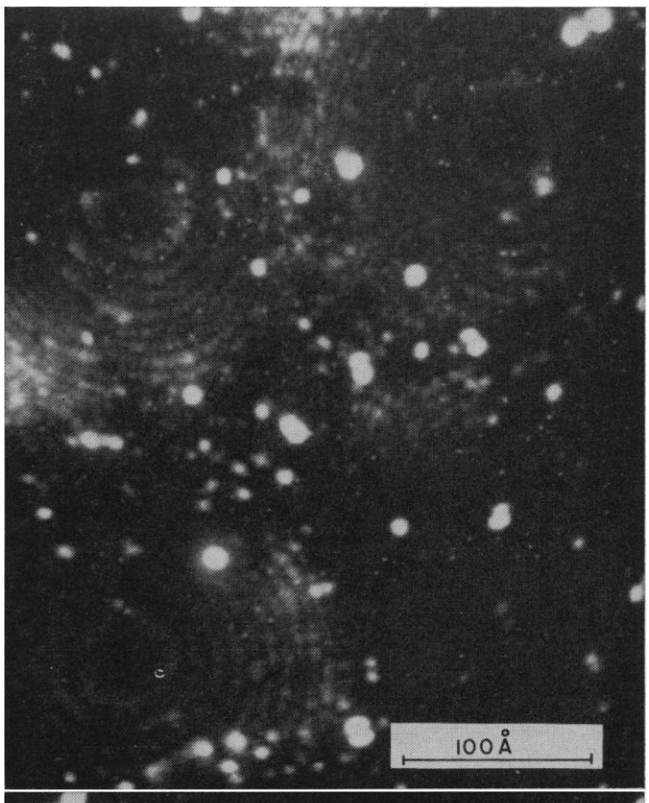
There are two possible ways to include the molecules into the metal matrix. In the first method we tried to adsorb molecules on an interface and subsequently cover the interface with the electrodeposit. If this process is repeated several times, the result is a sandwich-type specimen with layers of adsorbed molecules in the metal. This procedure has the advantage of permitting the use of separate solutions for the organic material and the plating bath, each with a suitable *p*H and the proper polarity of the tip, but it also has some disadvantages. The sample has a tendency to rupture at the intermediate layers during imaging. Irregularities in these layers make image interpretation difficult. The first exploratory investigations (with the plating done by Miller) were made mostly with electrophoretically attached DNA, and subsequent plating with platinum; but no conclusive images could be obtained when the specimens were examined at the Penn State Field Emission Laboratory. In later experiments, the molecules were introduced during the plating process so that, in the end, the molecules were distributed throughout the metal deposit (Fig. 1).

Organic substances present in the plating solution are adsorbed on the

electrodes. During this process the surface of the cathode is renewed constantly, and the adsorbates are included in the electrodeposit. It is known from commercial electroplating that, with the introduction of organic "brightening agents" such as glue, gelatin, and albumin into the plating solution, the electrodeposit contains an appreciable amount of organic matter (*5*). For small molecules the amount included is controlled by a diffusion-adsorption mechanism. When larger molecules are used, the amount supplied to the metal surface will be enhanced by electrophoresis, while the amount actually incorporated is uncertain as metal ions may creep underneath the molecules, keep-







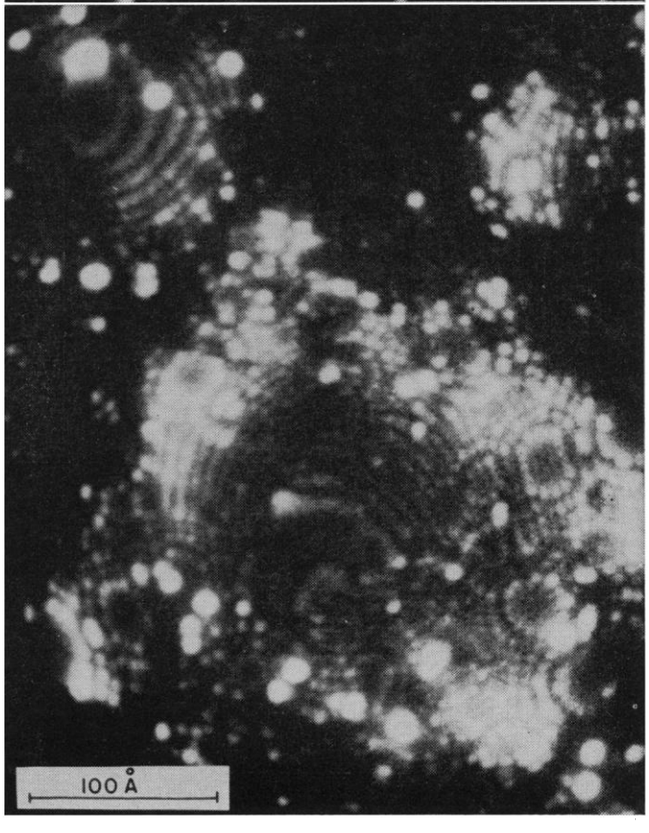


Fig. 1 (upper left). Schematic diagram of a tip covered with a platinum electrodeposit containing organic molecules. Fig. 2 (lower left). Field-ion micrograph of a pure platinum electrodeposit without organic additives. Fig. 3 (upper right). Electrodeposit containing molecules of vitamin B_{12}. Fig. 4 (lower right). Field-ion micrograph of an electrodeposit containing coenzyme I molecules.

ing them only superficially attached. Below the *p*H of the isoelectric point the molecules usually migrate towards the cathode. Platinum was chosen as metal for the electrodeposit for several reasons. First of all, the platinum field evaporates very regularly and gives a stable ion image. Second, platinum is a good catalyst for hydrogenation and dehydrogenation, and will readily effect chemisorption of organic molecules. On the other hand, a catalyst like platinum does not interact too strongly with the electronic structure of the molecules so that probably bonds are broken within a small percentage of the molecules only. This hopefully permits observation of whole molecules, not just dissociation products.

The platinum deposits were prepared from a solution of sulfato-dinitrito platinous acid (*6*) at *p*H 1, 30° to 40°C, and an estimated current density of 1 amp/cm^2 a-c. Figure 2 shows an a-c electrodeposit without organic addition agents. There are only a few impurities, and these appear as bright dots. The properties of electrolytically deposited pure platinum layers have been described in detail (*7*). The pure platinum images are perfectly stable under the best imaging conditions of the field ion microscope. However, the molecular images appearing at the surface during controlled field evaporation are often of a more transient nature, and it is therefore practical to use photoelectronic image intensification (*8*) which permits the photographic recording with exposure times of a fraction of a second. With image stability being a problem no longer, we also found it preferable to work with liquid-nitrogen cooling of the tip. In spite of the somewhat lesser resolution, the contrast conditions seemed to be more favorable than with liquid-hydrogen cooling.

It appeared highly improbable that we would succeed in attaching a long strand of DNA or another large molecule right over the small visible cap section of the tip; therefore, we turned to much smaller molecules. In a series of experiments, vitamin B_{12} (molecular weight 1300), in a concentration of 1 g/liter, was added to the plating solution. In the helium-ion image of the electrodeposit a large number of bright dots is visible (Fig. 3). That means that molecules or parts of molecules are included in the metal and can be imaged. What ion image can we expect from a molecule of a particular shape?

The field ion microscope image displays the local distribution of the ionization probability about 4 Å above the surface, which is essentially a function of the local field. The field is determined by the charge distribution in the surface, that is, mostly by its topography and possibly by chemical (valency) contributions. As far as the geometrical interpretation goes, there is, for the case of pure metals, a good agreement of the experiments with a model by Moore (*9*) which assumes that only those atoms are imaged whose centers lie inside of a spherical shell with a thickness of about 0.2 Å put around the tip surface. Therefore, we have no view into the depth, and it is very improbable to find the fourfold platelet of a vitamin B_{12} molecule in a position to cause a fourfold image. In the micrograph of platinum containing vitamin B_{12} only a few twofold symmetric patterns are visible besides a large number of structureless bright dots. These twofold patterns could correspond to molecules anchored in the surface on one edge.

Another molecule chosen for its solubility and relative stability in acid solution is coenzyme I, diphosphopyridine nucleotide. It was added to the platinum electrolyte in a concentration of 1 g/liter. The molecule contains a phosphate chain between two nonidentical ring structures. In the ion image of the Pt deposit with coenzyme I added, a large number of double dots, which can be interpreted to represent the two sections of the molecule, are visible (Fig. 4). The width of the doublets is approximately 10 Å. In more than one-half of all doublets, one dot is brighter than the other one, while in the case of vitamin B_{12} the two dots are predominantly of equal brightness. These doublets appear on or near (111) faces where the undisturbed substrate structure only shows the net plane rings. In one of the experiments two of the doublets and one single dot were visible together at a grain boundary, indicating preferred adsorption at this site.

The method of embedding molecules in an electrodeposit seems to be a suitable technique for imaging organic molecules by field ion microscopy. The main advantage compared to the shadow-casting technique is that the electrodeposit of the pure matrix metal easily develops crystallographically perfect sections (Fig. 2) (*7*) which permit a reasonably well-founded interpretation of the effect of the molecular additives. Evaporated matrices are inherently random, and the statistical piling up of atoms makes the interpretation ambiguous. So far, image interpretation in field ion microscopy has been based almost exclusively upon a topographic explanation of the local field distribution. However, the fact that small individual nitrogen atoms (*10*) and interstitial oxygen atoms (*1*) appear much brighter than the larger metal atoms, and also some specific brightness effects in ordered alloys (*11*) suggest that the electronic pertubation of the surface as expressed in the occurrence of abnormal local charge densities is of major importance. Szent-Györgyi's hypothesis (*12*) that aggregates of biomolecules act as semi-conductors with side chains representing donors or acceptors [Brillouin (*13*)] points to the possibility that such electronically active regions of the molecules may be differentiated in the field ion image. Of course, our experimental conditions resemble anything but the natural biological ambient, and it remains to be seen how much of the electronic structure particularly of the somewhat larger biomolecules will be affected by our exceedingly strange conditions, beginning with the low *p*H of the plating solution and the conditions of the electrolytic embedding, and ending with the exposure to the highly polarizing effect of the strong electric imaging field.

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