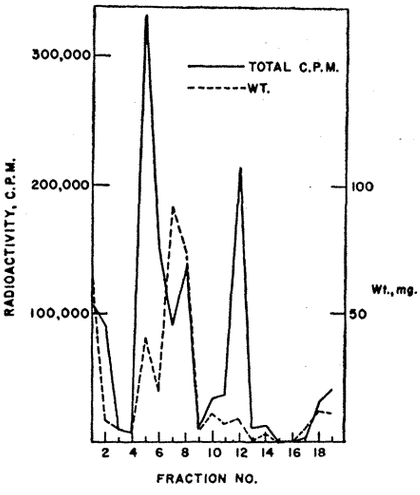


Fig. 1. Distribution of radioactivity in chromatographic fractions.

ingly, in all further work red tomatoes were used.

Thirty-one red tomatoes, weighing 4714 g, were injected with 2 μc each of mevalonic acid-2-C^{14}. After 24 hours, they were homogenized in a Waring blendor with an equal weight of water, hydrolyzed by refluxing with concentrated hydrochloric acid (150 ml/lit.), and extracted with dichloromethane. The extracts were washed with 2*N* sodium hydroxide and evaporated. The residue was refluxed with phthalic anhydride in pyridine and the hemiphthalates of the alcoholic material were isolated by extraction with base. Hydrolysis of the hemiphthalates by refluxing with sodium methoxide solution gave 865 mg of a crude alcoholic fraction. This material was chromatographed on 30 g of alumina (*11*), grade III, taking the following 100-ml fractions: 1 to 2, 10-percent, 3 to 4, 25-percent, and 5 to 6, 50-percent benzene in petroleum ether; 7 to 9, benzene; 10 to 11, 10-percent, 12 to 13, 25-percent, and 14 to 15, 50-percent ether in benzene; 16, ether; 17, 1-percent, 18, 5-percent, and 19, 20-percent methanol in ether. A 0.1-mg aliquot of each fraction was plated on a copper planchet and the radioactivity was determined under a micromil window tube in an atmosphere of Q gas. The distribution of radioactivity in these fractions is shown in Fig. 1. Fractions 7 and 8 contained the sterol mixture, which proved much more difficult to purify than in the case of the white tomatoes. After preliminary chromatographing and crystallization, the material that melted above 140°C was combined and acetylated, and the acetates (42 mg) were diluted with 42 mg of pure stigmasterol acetate. This material was purified by chromatography, crystallization from acetic acid, two more chromatographic separations, and recrystallization from methanol. At this point it weighed 8.2 mg, melted at 139° to 141°C, and had a specific activity of 17.8 count/min per milligram. After dilution with 9.6 mg of pure stigmasterol acetate, it was subjected to the following operations, as illustrated in Table 1. After each treatment, 2-mg aliquots were counted in duplicate to a 0.9-level of confidence. Treatment A was recrystallization from acetic acid. Treatments B, C, D, and E consisted of successive recrystallizations from methanol. The material from E was hydrolyzed with sodium methoxide in methanol, and the product (F) was recrystallized from methanol (G), and again from ethanol (H). These treatments gave stigmasterol having a constant specific activity of 770 count/min per millimole.

In another experiment, tomato fruits were injected with sodium acetate-2-C^{14} and worked up as before. In this case the sterol fraction was much less radioactive, and the pure stigmasterol finally obtained showed no significant radioactivity.

The presence of sterols in tomato fruits has not been reported heretofore and much remains to be learned about the biosynthesis of sterols in fruits. Unfortunately, the level of radioactivity of the stigmasterol obtained in these experiments was too low to permit degradation for establishing the pattern of labeling. In contrast to our previous findings on the biosynthesis of diosgenin in a tuber (*7*), it is now evident that mevalonic acid is a precursor of stigmasterol in tomato fruit. Acetate is apparently utilized so extensively for other biochemical reactions that only insignificant quantities are incorporated into stigmasterol under the conditions of our experiment.

RAYMOND D. BENNETT
ERICH HEFTMANN
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland
ALBERT E. PURCELL*
JAMES BONNER
Division of Biology, California Institute of Technology, Pasadena, California

References and Notes

1. E. Heftmann and E. Mosettig, *Biochemistry of Steroids* (Reinhold, New York, 1960).
2. H. Sander and H. Grisebach, *Z. Naturforsch.* **13b**, 755 (1958).
3. A. R. Guseva, M. G. Borikhina, V. A. Paseschnichenko, *Biokhimiya* **25**, 282 (1960); *Doklady Akad. Nauk S.S.S.R.* **133**, 228 (1960).
4. E. Ramstad and J. L. Beal, *J. Pharm. and Pharmacol.* **12**, 552 (1960).
5. H. Gregory and E. Leete, *Chem. & Ind. (London)* **1960**, 1242 (1960).
6. H. J. Nicholas, *Nature* **189**, 143 (1961).
7. E. Heftmann, R. D. Bennett, J. Bonner, *Arch. Biochem. Biophys.* **92**, 13 (1961).
8. A. E. Purcell, G. A. Thompson, Jr., J. Bonner, *J. Biol. Chem.* **234**, 1081 (1959).
9. Grown from seeds generously supplied by A. E. Thompson, Department of Horticulture, University of Illinois, Urbana, Illinois, at the Plant Industry Station of the U.S. Department of Agriculture, Beltsville, Maryland, through the courtesy of Joseph R. Haun, New Crops Research Branch.
10. E. Heftmann, B. E. Wright, G. U. Liddel, *Arch. Biochem. Biophys.* **91**, 266 (1960).
11. Nonalkaline, from M. Woelm, Eschwege, Germany.

* Present address: U.S. Fruit and Vegetable Products Laboratory, U.S. Department of Agriculture, Weslaco, Texas.

12 July 1961

Chemical Analysis of Surfaces by Use of Large-Angle Scattering of Heavy Charged Particles

Abstract. The Rutherford scattering of charged particles from the heavier elements and nuclear scattering and (α,p) reactions from the light elements result in energy spectra that are characteristic of the nucleus being bombarded. A simple apparatus for analyzing surfaces based on these ideas can be made by using an alpha source such as Cm^{244}, a solid state detector, and an electronic pulse height analyzer.

New methods of chemical analysis are always of interest, especially if they can be made more automatic than conventional techniques. This report calls attention to a nondestructive method, best used in vacuum, that is particularly applicable to the study of surfaces (the top 1 to 100 μ). The apparatus is very simple and the information is obtained in electronic form, which recommends the method especially for application at distances where the transmission of data is a problem.

It is proposed that scattering of a monochromatic, collimated beam of charged particles (for example, alpha particles from a thin radioactive source) be utilized for analysis of a solid. This method of analysis has been mentioned before (*1*), but the application of the newly developed solid state detectors makes it particularly attractive to both solid and particulate analysis.

For relatively low energy particles, such as alpha particles from the usual radioactive sources, and for those elements that are heavier than aluminum, the large-angle scattering of heavy charged particles is primarily Rutherford scattering. The energy of such scattered particles from a target thin enough so that the energy of the par-

ticles is not appreciably changed on traversing the target (less than 1 μ in the case of the usual alpha particles on solids) is given by

$$T = T_0 \frac{[X\cos\theta + (1 - X^2\sin^2\theta)^{\frac{1}{2}}]^2}{(1+X)^2} \qquad (1)$$

where T_0 is the initial energy of the particle; $X = a/A$; a is the mass number of the beam particle (that is, 4 for alpha particles); A is the mass number of the scattering nucleus; and θ is the angle of scattering ($\theta = 180° =$ backward scattering). The maximum separation between neighboring mass numbers is obtained when the scattering is backward. At this angle

$$T = \left(\frac{A-a}{A+a}\right)^2 T_0 \qquad (2)$$

Thus the method has good resolving power for light elements, including the possibility of isotopic analysis.

The intensity of scattering at a given angle (see, for example, *2*, p. 243) is determined primarily by the square of the nuclear charge (Z^2). It is a minimum backward, but the resolution advantage in that direction would appear to be the more important in many applications.

In the case of a thick target scatterer, even a monoisotopic target will give a continuous reflected spectrum starting close to zero energy. The high energy cutoff at a given angle, however, will be sharp and will occur at the same energy as with a thin target (Eq. 1). The spectrum calculated on the basis of well-known formulas (*2*, p. 101) for the stopping of charged particles has an essentially rectangular shape from about 0.1 T_0 to the end point. The shape (except for the position of the end point) is practically independent of the target material. With a thick target, the intensity of Rutherford scattering will depend, in addition to the factors mentioned in the case of a thin target, primarily on the inverse of the atomic stopping powers (s) for charged particles of the target material.

These theoretical relations are valid for low-energy alpha particles or heavy elements. For light elements such as carbon and oxygen, and particularly with rather high-energy alpha particles such as those from Cm^{244} (5.80 Mev), nuclear effects enhance the scattering above that predicted from pure Rutherford scattering. This is accompanied by some distortion in the shape of the energy distribution. For other light elements, such as nitrogen and fluorine, protons from (α,p) reactions can also be expected.

The existence of a continuous spectrum from a single mass scatterer lowers the sensitivity of the method for analysis, since an observed spectrum from a surface containing several elements must be decomposed into components. The high-energy end points characteristic of each mass number are, however, unaffected by the chemical or physical state of the scatterer.

Some simple experiments have been performed to check these ideas, particularly as applied to thick targets. Two alpha sources in the range 10^9 to 10^{10} alphas per minute (*3*) were used together with surface depletion solid state detectors (*4*) and pulse height analyzers. The distance from source to scatterer and scatterer to detector was about 3 cm, and the average scattering angle was about 160°.

Figure 1 shows the energy spectrum of Pu^{238} alpha particles scattered from thick targets of $_{50}$Sn, $_{29}$Cu, and $_{13}$Al. The energy of the original Pu^{238} is indicated by the slight amount of contamination producing a peak in approximately channel 305. The energy zero is at a negative channel No. 20.

Figure 1 shows that the shape of the energy distribution is similar to that expected. The observed high energy cutoffs as well as the relative intensities from the different scatterers agree satisfactorily with theoretical expectations.

Figure 2 shows data obtained with another alpha source (Cm^{244}). Although more intense, the alpha particles of this source were not very monochromatic, as indicated by the source spectrum shown at the right of Fig. 2. This made the end points less sharp than in Fig. 1. Figure 2 shows spectra obtained with polyethylene (CH_2), quartz, calcium carbonate, and copper reflectors. Again, the positions of the end points correspond satisfactorily with those predicted. The shapes of the spectra are as expected from simple considerations, except in the case of carbon. For this element, the Cm^{244} alpha particles excite nuclear scattering levels of the C^{12} + He^4 system, and this results in a scattering some 40 times greater than Rutherford scattering. The scattering by O^{16} is also increased by similar effects by a factor of about 5 over that expected from Rutherford scattering.

The experimental work thus supports the theoretical expectations set forth in

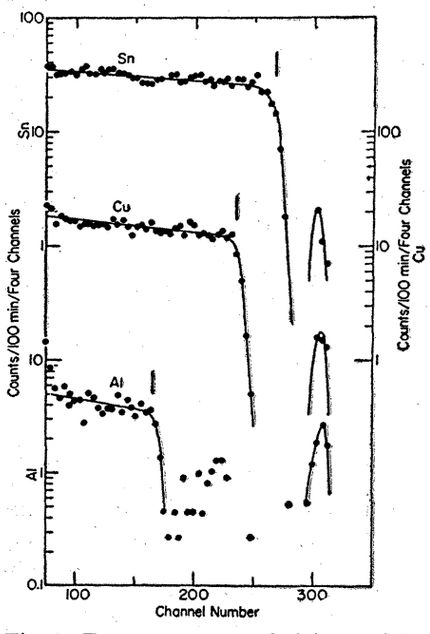


Fig. 1. Energy spectrum of alpha particles reflected from thick samples of $_{13}$Al, $_{29}$Cu, and $_{50}$Sn. The ordinates are in counts per four channels per 100 min. The scales for Sn and Al are on the left; that for Cu is on the right. The abscissae are the channel numbers which are linearly related to the energy of the alpha particles. The vertical lines indicate the predicted high energy cutoffs for the elements in question.

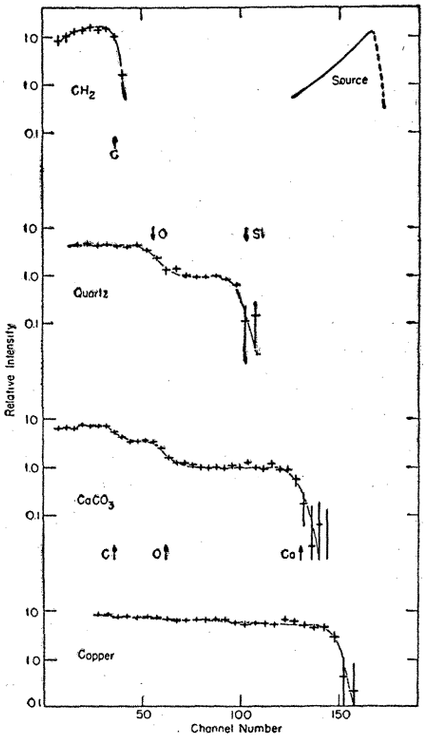


Fig. 2. Energy spectra of Cm^{244} alpha particles scattered at ~160° from various materials. The ordinates are in relative intensity units; the abscissae are channel numbers, linear in energy. The energy spectrum of the source is indicated at the top right. The theoretical end points for the constituent elements are shown by vertical arrows. The amplification was not exactly the same in the different experiments.

the first part of this paper. The shapes of the thick target spectra are approximately rectangular, with sharp high-energy end points. The positions of these end points is characteristic of the mass number of the scatterer. The positions of the end points are independent of the chemical state of the scatterer. Decomposition of scattering curves obtained by this technique can clearly give analytical information on the kinds and amounts of elements present in the scattering body (*5*).

ANTHONY TURKEVICH
Enrico Fermi Institute for Nuclear Studies, University of Chicago, Chicago, Illinois

References and Notes

1. S. K. Allison, private communication; see also C. W. Snyder, S. Rubin, W. A. Fowler, C. C. Lauritsen, *Rev. Sci. Instr.* **21**, 852 (1950), and S. K. Allison and S. D. Warshaw, *Revs. Modern Phys.* **25**, 779 (1953).
2. E. Rutherford, J. Chadwick C. D. Ellis, *Radiations from Radioactive Substances* (Cambridge Univ. Press, New York, 1930).
3. Kindly made available by members of the Argonne National Laboratory.
4. Furnished by Mr. A. Tuzzolino of the Chicago Laboratories for Applied Sciences.
5. This paper is based primarily on a proposal made on 13 September 1960 to the National Aeronautics and Space Administration for the chemical analysis of the moon and other planets. I gratefully acknowledge the experimental help provided by Dr. H. L. Anderson and Dr. George Reed, and the very helpful discussions with Professors S. K. Allison, J. A. Simpson and N. Sugarman. Mr. A. Van Ginneken performed useful calculations connected with this problem.

5 June 1961

10-Methoxyharmalan, a Potent Serotonin Antagonist Which Affects Conditioned Behavior

Abstract. 10-Methoxyharmalan, an alkaloid obtained by the cyclodehydration of melatonin, itself a derivative of serotonin, is a more potent serotonin antagonist than harmaline and is only slightly less active than lysergic acid diethylamide. It has a similar, yet slightly greater, effect on behavior as that of harmaline and is the most potent serotonin derivative, so far tested, that affects the avoidance-escape behavioral reflex.

The psychotomimetic activity of lysergic acid diethylamide was originally postulated as being due to its ability to antagonize the action of serotonin (*1*). It has been further suggested that an endogenously produced serotonin antagonist might be responsible for some psychotic states (*2*). Melatonin has been isolated from pineal tissue (*3*), and its biogenesis from serotonin has been established (*4*). The removal of a molecule of water from melatonin results in its conversion to 10-methoxyharmalan (1-methyl-6-methoxy 3,4 dihydro-2-carboline), an analogue of harmaline (Fig. 1), which has been shown to be a serotonin antagonist (*5*). Harmine, a closely related compound, has been reported to be hallucinogenic (*6*).

Therefore, the effect of 10-methoxyharmalan as a serotonin antagonist and on the behavior of trained rats was studied.

Serotonin antagonism was measured (i) on the isolated estrus rat uterus, (ii) on the isolated guinea pig ileum, and (iii) on the blood pressure of rats previously treated with ganglioplegic agents, atropine, and bilateral vagotomy.

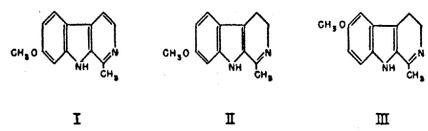
The standard oxytocic response to 0.2 μg of serotonin was completely blocked by the addition of 0.5 μg of lysergic acid diethylamide, 2.0 μg of 10-methoxyharmalan (Fig. 2), 50 μg of harmaline (Fig. 3), or 50 μg of harmine to a 10-ml muscle bath 5 min before addition of the serotonin. At higher dose levels, the harmala alkaloids frequently caused contractions, and it was noted that if a contraction was elicited the subsequent serotonin antagonism was decreased.

Similar results were obtained with the isolated guinea pig ileum. The dose response curve of the ileum to serotonin showed a plateau at 10 μg of serotonin, the dose level at which the muscle strip contracted maximally. In the presence of 5 μg of 10-methoxyharmalan, the dose response curve to serotonin was depressed, and the maximum contraction was less. Since the two curves were parallel, competitive antagonism could be postulated, and thus the action of serotonin and 10-methoxyharmalan on the same receptor site could be considered possible.

Preliminary studies of the effect of 10-methoxyharmalan on the blood pressure of ganglion-blocked rats indicate that it is a more potent vasodepressor than harmaline and that it antagonizes to some extent the pressor effect of serotonin.

The effect on behavior was assayed by using rats conditioned to an avoidance-escape schedule in a conventional shuttlebox. The number of mistakes was plotted against the intraperitoneal dose level of the compound used, ten animals being subjected to ten trials in each assay.

Melatonin caused no behavioral disturbance at dose levels of 0.2 mmole/kg. 10-Methoxyharmalan caused conditioned rats to make mistakes at doses as low as 0.008 mmole/kg with a linear dose response relationship up to 0.25 mmole/kg, at which level animals made ten mistakes out of ten trials. Harmaline exhibited a linear dose-response relationship parallel to, but slightly less active than, that of 10-methoxyharmalan, the dose level at which ten mistakes out of ten trials occurred being 0.28 mmole/kg. 10-Methoxyharmalan was thus approximately twice as potent as 5-methoxy-*N*,*N*-dimethyltryptamine and six times as active as bufotenine, both of which were previously tested in a similar fashion (*7*).



I II III

Fig. 1. Chemical structure of harmine (I), harmaline (II), and 10-methoxyharmalan (III).

Rats given 10-methoxyharmalan at doses greater than 2 mg/kg exhibited tremor which lasted for approximately 1 hour, but were well able to walk at dose levels as high as 10 mg/kg.

Although lysergic acid diethylamide

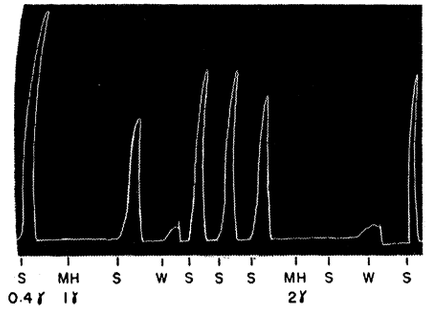


Fig. 2. 10-Methoxyharmalan (MH), 2 μg, caused complete inhibition of oxytocic activity of serotonin (S), 0.4 μg. Oxytocic response returned after washing (W).

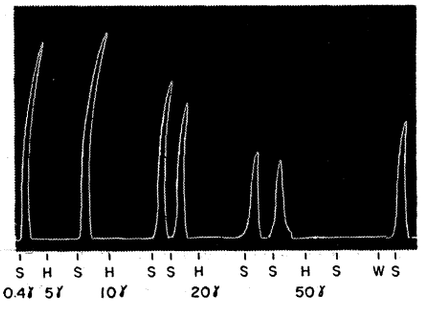


Fig. 3. Harmaline (H), 50 μg, caused complete inhibition of oxytocic activity of serotonin (S), 0.4 μg. Oxytocic response returned after washing (W).