powdered sample with CuO at 750°C for 4 hours in a carefully evacuated and outgassed Vycor flask that was connected to the vacuum system. The evolved gases were passed through a trap at liquid nitrogen temperature and treated with hot CuO–Cu. The standard atmospheric nitrogen was isolated from air by passing air repeatedly over cleaned copper turnings in a quartz tube at 700°C and then through a liquid nitrogen trap.

The mass spectrometer is of the design described by several workers for the measurement of small differences in isotope ratios (*5*). Mass spectrometry of nitrogen is troubled by the presence of background peaks in the instrument at mass 28 and 29. The background peaks were less than 0.1 percent the size of the signal peak at mass 28. The procedure of rapidly shifting from the standard nitrogen sample to the unknown sample minimized the effect of this background. It is imperative that N_2 samples be free of carbon monoxide, which gives an interfering mass spectrum. This problem was solved by passing the gas over hot CuO and through a liquid nitrogen trap. Several samples were prepared and their N^{15} content was measured both before and after repeated treatments in this manner. There was no detectable change in the N^{15} content.

In order to avoid isotopic fractionation in sample preparation, all operations were checked to insure quantitative yields. The estimated precision on each of the results listed in Table 1 is ± 0.5 parts per 1000.

THOMAS HOERING

Department of Chemistry, University of Arkansas, Fayetteville

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21 July 1955

Differentiation and Separation of the Tetracycline Antibiotics by Countercurrent Distribution

Recently, the discovery of tetracycline (*1*) in *Streptomyces* elaboration products has increased interest in methods designed to differentiate among the members of this family of compounds.

In the past, various procedures (*2–6*) have been reported for carrying out such a differentiation; these methods are for detection only and have as yet had no extension into the field of separation.

The countercurrent distribution system consisting of McIlvaine's phosphate-citrate buffer at *p*H 4.5 versus chloroform has been used in these laboratories for some time for the analytic separation of mixtures of the known tetracycline antibiotics. In a 50-tube distribution with this system, the peak tubes observed are as follows: chlortetracycline 26, tetracycline 39, and oxytetracycline 44. These values correspond to K aqueous/solvent values of 1.13, 3.90, and 8.80, respectively.

Pigments with a strong absorptive capacity in the ultraviolet, such as anhydrotetracycline (*7*), usually localize in the low-numbered tubes because of their excellent solubility in chloroform; thus their presence does not interfere with the ultraviolet determination of the peak tubes, which is carried out on the upper phase at a wavelength of 265 mμ after suitable dilution.

After the theoretical curves for each component have been calculated, the percentage composition of a mixture such as one containing tetracycline and chlortetracycline can be calculated within a few percent of the bioassay, via the peak heights as determined spectrophotometrically.

The method has been used not only on purified preparations but also on crude preparations, such as those obtained from butanol extractions of fermentation beers. Samples assaying 300 to 500 μg/mg have given satisfactory results in the identification of major components (not traces) when as little as 3 to 5 mg was placed in the first tube of a 50-tube apparatus containing 10 ml of each phase. If larger samples are used, as little as 1 percent chlortetracycline in tetracycline can be detected.

A change in *p*H from 3.5 to 5.5 seems to have little effect on the position of the peak tubes. Since the buffer can be made up in the range of *p*H 2.2 to 8.0 and since the solubility of the tetracyclines increases sharply at lower *p*H values, higher charges can be accommodated at a slightly lower *p*H (for example, 3.5). By the use of such higher concentrations in a larger apparatus, sufficient material can be handled to give a useful separation.

This method will be described in more detail in a forthcoming publication (*8*).

P. PAUL MINIERI*
A. G. MISTRETTA†

American Cyanamid Company, Princeton, New Jersey

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* Present address: Heyden Chemical Company, Garfield, N.J.
† Present address: Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y.

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Localized Electroretinograms from Isolated Poikilothermic Retinas with Macroelectrodes

What effect does the stimulation of some retinal elements have on the effect of stimulating others? Is there a real effect, or only an apparent one caused by stray light, chiefly from scattering in the dioptric media of the eye?

The interchangeable effects of stimulus area, duration, and intensity on the electroretinogram (ERG) of the intact frog eye were considered by Granit (*1*) to be evidence for interaction of retinal elements. Fry and Bartley (*2*), using the intact rabbit eye, showed that stray light could explain the apparent interaction and that, indeed, the ERG was mainly a response to stray light within the eye. Granit, Rubinstein, and Therman (*3*) obtained results apparently supporting interaction when they minimized stray light by using small stimuli of low intensity in an excised and opened frog eye. Recently, the stray light theory of Fry and Bartley has been revived and confirmed for the human ERG (*4*). This, together with the inconclusive character of the evidence for retinal interaction, raises several questions regarding the effects that stimulation of one retinal locus may have on the response from another locus.

We have recorded ERGs from isolated frog (*Rana pipiens* and *R. catesbeiana*) and terrapin (*Pseudemys elegans*) retinas. We removed the retina under subdued illumination and placed it flat on a black felt pad that had been soaked in Ringer's solution. The preparation was placed in a black box to minimize further any effects from stray light. Thread wicks from silver-silver chloride electrodes were led to any desired points on the surface of the retina, and another to the supporting pad. Two channels of alternating-current amplification led to a dual-beam cathode-ray oscilloscope and permitted simultaneous registration of potential changes that occurred between two pairs of electrodes, if desired, or between one pair of electrodes if the second beam was used for a time scale and stimulus marker.

Each stimulus was a 1-mm spot of light of adjustable intensity and duration. Two such spots can be presented at any

desired retinal locations and with any desired timing.

With a single spot of 50 msec duration and intensity as high as 1×10^6 ft-lam, we have found the ERG to be extremely localized. When the supporting pad is the indifferent electrode, an ERG is registered only when the thread wick is at the locus illuminated. The response (*a*-wave plus *b*- or *x*-wave) may then be more than 100 μv but generally is less, depending on the retinal locus and the age of the preparation. Moving either the light spot or the wick as little as 1 mm extinguishes the response to at least below the noise level of about 3 μv.

By systematically moving the light spot together with the thread electrode to various retinal loci, we have been able to map the retina electroretinographically. Such a map reveals a functional outline of the optic disk, within which no response can be obtained. It also outlines the retinal margin where the response again falls to zero. Curiously, although we have found a definite increase in sensitivity from the periphery toward the center of the retina, it is not a smooth gradient. Instead, "peaks" and "valleys" of high and low sensitivity appear to exist. It is possible that these are artifacts caused by trauma of preparation, although no other evidence of physical injury can be found. Actual tears in the retina completely eliminate the ERG at the site of the injury.

Early investigators (*5*) cited by Granit reported rapid disappearance of the *b*-wave when the frog retina was removed from the bulb (although, to be sure, they were using less responsive apparatus). Our preparations have yielded apparently normal ERGs for more than 5 hours of experimentation, at times with little evidence of any deterioration or significantly decreased responses. Responses at a given locus are repeatable from one time to another within about 20 percent when the total height of the ERG is measured from the trough of the initial negative wave (*a*-wave) to the crest of the first positive wave (*b*- or *x*-wave). During an experimental session, the preparation was moistened occasionally with Ringer's solution to prevent drying. The use of isotonic glucose does not appear to enhance the response or to prolong the usefulness of the preparation.

Despite the differences in technique, especially the localized recording described here, we have been able, although not consistently, to confirm the inhibition of Granit, Rubinstein, and Therman.

Using two stimulus spots spaced 2 to 3 mm apart on the retina, we did not find any effect of one stimulus on the ERG registered from the retinal locus of the other spot, regardless of the time relationship of the two flashes. With the spots very close together (½ to 1 mm), however, evidence of interaction has been noted. Stimulating one spot alone produces no recordable response at the retinal locus of the other spot, but may *inhibit* the ERG response to stimulation of the second locus for many seconds afterwards. The recovery of the inhibited locus may be observed by repeatedly stimulating that locus and noting the progressive increase in potential throughout the ERG. Thus it appears that interaction of the ERG takes place over small distances on the retina but not over large ones.

Investigation of these and similar phenomena is continuing and a more complete report will be submitted for publication elsewhere. However, we wish to invite attention at this time to this relatively simple technique of registering localized ERGs without the use of microelectrodes.

ELWIN MARG
GORDON G. HEATH
School of Optometry, University of California, Berkeley

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Vegetative Changes at Pinacate, Sonora, Mexico

Since 1931, a considerable area at Pinacate, Sonora, Mexico (Fig. 1)—which has certainly been barren of vegetation since 1907–09 (*1*), almost certainly barren of vegetation since 1774–76 (*2*), probably barren since 1697–1701 (*3*), and possibly barren since 1541 (*4*)—has acquired a surface cover of grasses and has developed a soil profile up to 2 inches thick in some places.

Surface material at Pinacate formerly consisted of basaltic lava flows, volcanic cinders, volcanic ash, and saline playa deposits. All areas except lava flows now show some soil profile development, most of the soil consisting of roughly equal parts of local materials (cinders, ash, or playa deposit), blow sand (calcareous), and organic material of recent local origin.

Known factors contributing to the growth of surface cover are (i) slight increase in annual precipitation; (ii) decline in intensity of precipitation (more days with rain, less rain per day); (iii) an increase in winter (gentle) rainfall; and (iv) virtual extinction of mountain sheep and wild burros in the area.

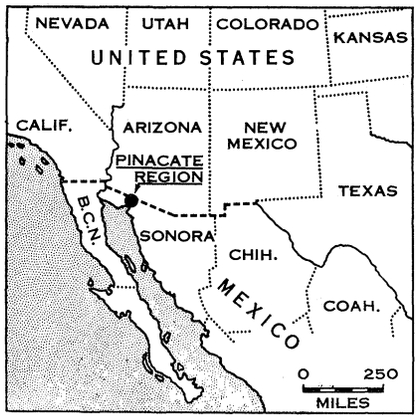

Fig. 1. Summary outline map of southwestern North America showing the location of the Pinacate region.

During the last decade, many of the plunge-pool water holes in the area ("tinajas") have been dry repeatedly, indicating a decline in the cloudburst type of precipitation that refills them; but dry farming in areas just south of Pinacate has been occasionally profitable, suggesting greater soak-in of precipitation.

Many of the larger cinder cones adjacent to the main Pinacate Peaks now support a new growth of cholla cactus (*Opuntia Bigelovii*), all of the cacti being of uniform height and having an estimated age of about 10 years. As a result of these vegetative changes, the Pinacate lava region, when seen from a distance on the ground or viewed obliquely from the air, now has a distinctly green tinge, in place of the dark grays, dull reds, and blacks of former decades.

The cause of this somewhat localized vegetative change is not surely known, although increase in rainfall and alteration of its seasonal distribution and intensity are certainly important factors. It is possible that some of this climatic change is "carryover" from cloud-seeding operations in the mountains east of San Diego.

RONALD L. IVES
5415 Main Street, Williamsville, New York

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