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.

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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*).

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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