

however, too many units are required,<sup>7</sup> thus giving the impression that large doses of the material are needed for effective chemotherapeutic purposes.

The problem of defining a streptomycin unit becomes especially complicated when attempts are made to compare the *in vivo* activity of streptomycin with that of penicillin, especially when the measurement of the latter is based on the Oxford unit.<sup>8</sup> The fact that the basis for the standardization of the two materials is different, and, further, the use of two different test organisms can only lead to much confusion. This becomes clear when one compares the above figures given for the Oxford unit with the unit of streptomycin which is based upon inhibition of growth in 1 ml of medium; the Oxford unit is thus found to represent a far greater *in vitro* activity than the streptomycin unit.

Because of these considerations, it is proposed here to establish the following three units for designating streptomycin:

(1) An S unit, or that amount of material which will inhibit the growth of a standard strain of *E. coli* in 1 ml of nutrient broth or other suitable medium. This unit will thus correspond to the original *E. coli* unit.

(2) An L unit, or that amount of material which will inhibit the growth of a standard strain of *E. coli* in 1 liter of medium. An L unit is thus equivalent to 1,000 S units.

(3) When crystalline material becomes available, a weight unit will become possible. One can even now prepare for it by recognizing a G unit, comparable to one gram of the crystalline material. Should this material show an activity of 1,000 *E. coli* units per 1 mg, it will be equivalent to 1,000,000 S units, to 1,000 L units and to 1 G unit, per gram of material.

For the purpose of studying the production of streptomycin and its concentration in the medium as well as for the isolation of the material, and especially for measuring its concentration in blood, urine and other body fluids, the *E. coli* or the new S unit may still be used. For the purpose of utilizing the material for clinical treatments, however, the new L or even the G unit would no doubt prove to be far preferable. Thus, a good culture broth will have 100 to 200 S units of streptomycin per 1 ml. If a patient is treated with streptomycin, instead of using 1,000,000 to 5,000,000 units daily, on the basis of the old unit, the equivalent in terms of the new units will be 1,000 to 5,000 L units of streptomycin or 1 to 5 G units. The latter will thus be roughly equivalent

to about 5 gm of the purified material. The concentration of streptomycin in the blood of the patient may be 0.5 to 50 S units, depending on dosage used and rate of excretion.

The units of measurement of streptomycin are thus based upon the inhibition of growth of a standard strain of *E. coli*, as determined by the dilution method, using either a series of dilutions in liquid media or the agar plate streak method. The actual determinations are carried out by the agar diffusion or so-called cup method, using either a standard strain of *Bacillus subtilis* or *S. aureus*. Other convenient procedures, such as the turbidimetric method, can also be employed. A given preparation of streptomycin, preferably the crystalline product when it becomes available, is used as a standard for determining the potency of unknown lots of broth or of the isolated product.

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#### DARKFIELD ILLUMINATORS IN MICROSCOPY

It is usually recommended that a microscope be equipped with a special darkfield condenser and a funnel stop solely for darkfield work. An improvised darkfield stop has been found to be a satisfactory substitute for the darkfield condenser in the experience of the writer.

A special stop is generally supplied with the microscope by the manufacturer. An improvised darkfield stop can be made by having a piece of thin metal cut in the form shown in the illustration (Fig. 1), and of such a size that the outer narrowing ring fits snugly into the slot provided in most substage Abbe condensers.

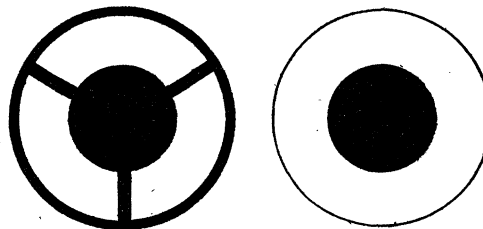


FIG. 1. Form and size of metallic stop used in conjunction with the Abbe condenser to produce darkfield illumination. FIG. 2. Form and size of stop made by pasting a circular disc of black paper on a glass disc for use in conjunction with the Abbe condenser to produce darkfield illumination.

<sup>7</sup> H. A. Reimann, W. F. Elias and A. H. Price, *Jour. Am. Med. Assn.*, 128: 175-180, 1945.

<sup>8</sup> F. R. Heilman, *Proc. Staff Meet. Mayo Clinic*, 20: 169-176, 1945.

Another type of stop can be made by pasting a circular disc of black paper in the center of a large

round cover glass or on the glass disc which is generally furnished with the microscope (Fig. 2).

A little experimentation will indicate the proper size of the central disc, which depends upon the aperture of the objective used. The relation of condenser, specimen and objective is the same as with the regular darkfield illuminator: *Limit of Resolution* =  $\frac{0.5 \lambda}{N.A.}$

where  $\lambda$  is the wave-length of light used and *N.A.* is the numerical aperture of the objective.

A satisfactory examination under oil immersion can be made if a funnel stop is inserted in the objective to reduce the aperture of the oil immersion lens.

The use of improvised darkfield illumination has the advantage of allowing a rapid shift from the light to the dark field without disturbing the relation of the objective, specimen or condenser. Results with this apparatus may be relied upon for the demonstration of *Treponema*.

#### DIRECTIONS FOR USE OF IMPROVISED DARKFIELD ILLUMINATION

Insert the stop in the special ring beneath the Abbe condenser. Lower the substage and place a drop of immersion oil, free of bubbles, on the upper surface of the condenser. Put the slide preparation on the stage and center the specimen. Raise the substage until the oil is spread by contact with the slide, filling the space between the slide and condenser. Examine under low and high power. If examination under oil immersion is desired, it may be accomplished by properly inserting a funnel stop in the oil immersion objective and placing a drop of immersion oil on the coverslip before lowering the objective over the specimen. It is important that a strong light source be employed.

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## DISCUSSION

### AN APPROACH TO THE NUTRITION PROBLEMS OF OTHER NATIONS

DURING the past several years, the Nutritional Biochemistry Laboratories of the Massachusetts Institute of Technology, in collaboration with the Mexican Institute of Nutrition and the Pan American Sanitary Bureau, have been working in the training of food chemists and nutritional clinicians; in the analysis of Mexican foods<sup>1</sup>; in the appraisal of the nutritional status of Mexican school children; and in the development of a school lunch program in Mexico City. As a result of this experience several observations have been made as to what appear to be the most practical approaches to the solution of the food and nutrition problems of countries other than our own. While it is realized that more extensive experience may modify some of these observations, they are presented at this time for the guidance of those actively concerned with the food problems of other countries, especially those in Latin America. No attempt is made to discuss here the adequacy or inadequacy of food production in the United States to meet the needs of other nations. The current controversy on that subject only serves to emphasize the desirability of examining carefully every practical approach to the problem of feeding mankind.

#### NUTRITIVE QUALITY OF FOODS

Because of the high nutritive quality of indigenous Mexican foods it was not difficult to formulate a school lunch containing one third of the daily allowance<sup>2</sup> of

protein, calories, minerals and vitamins. Most Mexican foods are unknown in the United States; similarly many American foods are not widely consumed in Mexico. For instance, milk, meat and eggs, while admittedly excellent foods, can not be used extensively there because of high cost, low production and inadequate transportation.

In our investigations, we have encountered several very nutritious food plants which are eaten by significant segments of the Mexican population. One of these is malva, an uncultivated plant which grows extensively on the Mexican plateau. Its leaves are eaten much as we eat spinach. A serving of malva (100 grams) can supply 40 per cent. of the calcium, 90 per cent. of the iron, 140 per cent. of the vitamin A (as carotene), 60 per cent. of the ascorbic acid and a significant portion of the thiamine and niacin allowances<sup>2</sup> for adult man. Charal, sesame seed, calabaza seed, pulque, piñón and corn tortilla are foods especially rich in various nutrients.

#### NUTRITIONAL STATUS OF PEOPLE

The effect of the high nutritive value of Mexican foods was reflected in the nutritional status of the people. Malnutrition appeared to be no more common<sup>3</sup> among a group of 1,000 children in a poverty-stricken area of Mexico City than in a group of 800 children from middle-class families in Michigan.<sup>4</sup>

<sup>2</sup> "Recommended Dietary Allowances," Reprint and Circular Series No. 115, National Research Council, 1943.

<sup>3</sup> E. E. Lockhart, *et al.*, unpublished data.

<sup>4</sup> R. S. Harris, E. Weeks and M. Kinde, *Jour. Am. Diet. Assoc.*, 19: 182, 1943.

<sup>1</sup> R. Cravito, E. E. Lockhart, R. K. Anderson, F. de P. Miranda and R. S. Harris, *Jour. Nutrition*, 29: 317, 1945.