notable. Hundreds of "thumb-nail sketches," diagrammatic representations of geologic phenomena and humorous drawings adorn the pages of text. These are from the facile and ofttimes facetious pen of Chichi Lasley and help greatly to clarify ideas and maintain interest. Photographic illustrations have been chosen with great care. Almost all are new pictures not previously used in text-books of geology. Many are photographs taken from the air, a point of view which is particularly illuminating in any study of the face of the earth.

The photographs are all assembled on 64 rotogravure plates, four to six pictures per plate, grouped in four fascicles of 16 pages each, distributed at roughly equal intervals throughout the book. This grouping of the pictures was, of course, dictated by the mechanics of press room and bindery, but the authors have done more than bravely bow to the inevitable. "Each group contains the plates that illustrate the chapters immediately preceding or following it. The individual plates illustrate or elaborate on

certain concepts presented in the chapters to which they are referred, but they have been designed to tell their own stories. They may be studied, therefore, as a group when the rotogravure sections are reached in the text, or they may be consulted in connection with each chapter. . . . It is hoped that this type of organization will enhance the usefulness of the book by making available for comparative study, in appropriate groups, the photographs on related subjects that commonly are widely scattered throughout a text."

This is a book which will arouse strong sentiments of approval or disapproval. It will either be greatly liked or energetically disliked; no one who appraises it can remain lukewarm. Its success as a teaching tool can only be ascertained by practical experience with it. Certainly, the experiment is well worth trying and in the opinion of the reviewer the chances are excellent that it will prove to be an unusually satisfactory piece of equipment for the modern classroom.

KIRTLEY F. MATHER

HARVARD UNIVERSITY

SPECIAL ARTICLES

PROPAGATION OF RABIES VIRUS IN TISSUE CULTURE AND THE SUCCESSFUL USE OF CULTURE VIRUS AS AN ANTIRABIC VACCINE

Rabies virus is being propagated in tissue culture. The culture virus, when used as a vaccine, protects mice adequately against "street rabies" virus.

The cultivation of rabies virus is carried out in the following manner. Using aseptic technique throughout, 50 cc Erlenmeyer flasks are prepared with 4 cc of Tyrode solution containing 10 per cent. normal monkey serum plus 0.02 cc of a thick suspension of minced mouse embryo brain. This flask culture medium is then inoculated with 1 cc of a 1 to 100 dilution of the brain of a mouse prostrate on the 7th or 8th day following an intracerebral injection of rabies virus. At 3 to 4 day intervals, the contents of the flask is withdrawn to a centrifuge tube, allowed to settle, and 1 cc of the relatively clear supernatant is transferred to a second culture flask. This passage technique is repeated routinely and the virulence of the culture virus is titrated by inoculating the material intracerebrally in tenfold dilutions in Swiss mice.

Skunk strain 3, following 7 mouse passages, has now been carried through 16 serial subcultures, and when inoculated into mice has been uniformly fatal through the 10⁻² and for the most part, the 10⁻³ dilutions. If the virus were merely surviving, the repeated dilution would have eliminated it at the transfer to the 6th subculture. Its persistence and titre indicate that it is

actually multiplying in the tissue medium. Dog strain 1, following 88 mouse passages, has likewise been successfully cultivated.

The culture virus produces typical dumb rabies in mice, following intracerebral, lingual and muscular inoculation, and is neutralized by sera from persons given Semple antirabic vaccine.

The possibilities of using tissue culture virus as an antirabic vaccine are being investigated. Rabies vaccines in current use are composed largely of animal brain or cord tissue containing virus in either a virulent or an inactive form. Nervous tissue is not only a superfluous, but a potentially dangerous vehicle which may produce paralysis following vaccination, hypersensitive reactions or secondary infections of animal origin. Until now, however, no other source of virus has been available and the brain tissue has remained inseparable from the virus.

The disadvantages accompanying the use of animal tissue as a source of virus are largely overcome by the use of culture media. The culture virus protects mice effectively against a direct brain inoculation of rabies "street" virus. A single peritoneal injection of the undiluted culture virus is innocuous and within 10 days makes the animal resistant to 100 intracerebral fatal doses of "street" virus of homologous or heterologous strains. The subcutaneous route of vaccination is not effective. The amount of active virus necessary for immunization is the same for both tissue culture

and mouse brain strains, namely, about 10,000 intracerebral fatal doses.¹

Dogs given a single peritoneal injection of culture virus remain healthy and show neutralizing antibodies in their sera against the homologous "street" virus strain within 14 days. Their ability to resist subsequent exposure to "street" virus is now being tested.

LESLIE T. WEBSTER ANNA D. CLOW

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

A CHEMICAL REAGENT FOR THE DETECTION AND ESTIMATION OF VITAMIN B.

THERE has not been reported as yet in scientific or patent literature any information concerning a specific chemical reagent capable of reacting with small quantities of vitamin B₁.^{1, 2} Such a reagent would provide a means of qualitative as well as quantitative estimation of the vitamin in foodstuffs or biological preparations.

Investigation carried out in our laboratory has shown that certain derivatives of aniline or the naphthyl amines have the property of producing characteristic colorations with solutions of the vitamin. After intensive research it was found that certain derivatives under certain conditions will react with vitamin \mathbf{B}_1 to produce a stable colored compound which is insoluble.

We have found that when a solution of either p-amino acetanilid or methyl-p-amino phenyl ketone (p-amino acetophenone) is treated with nitrous acid and the resultant product is treated under certain conditions with vitamin B₁, there is produced a characteristic purple red compound which is stable and highly insoluble in water. The solutions of the treated amines will not react under these conditions with any substances as yet tried to form the same characteristic insoluble compounds which are colored.

The respective reagents produced from the amines mentioned have been successfully tried on samples of wheat germ, rice polishings, Seidell's International Adsorbate, Anheuser-Busch yeast concentrate, Merck concentrate, Eli Lilly adsorbate, Merck crystals (natural) and synthetic crystals from the Winthrop Chemical Company (Windaus synthesis). In each case the same characteristic product previously mentioned was obtained.

The colored vitamin-reagent compound may be extracted by means of a suitable selective solvent. Such

¹ A short report on the immunization of mice against rabies will appear in the *Am. Jour. Pub. Health*, 1936 (December).

¹ This research was supported by a grant from Eli Lilly and Company, Indianapolis, Ind.

² Preliminary report. The authors wish to express their appreciation for the kind suggestions received from Dr. S. M. Weisberg, Sealtest System Laboratories, Inc., Baltimore, and Dr. Elsa R. Orent, of this laboratory. a method may afford means of concentrating vitamin $\mathbf{B_1}$. Since the coloration is of a permanent nature, it provides a method for the quantitative as well as qualitative estimation of the vitamin. This test is extremely sensitive; preliminary examinations show that determinations may be made within several millionths of a gram of the active material.

Work is now under way to permit accurate chemical determination of vitamin B_1 in natural products as well as commercial preparations.

Investigation on regeneration of the vitamin-reagent compound and feeding tests of such products is now in progress.

Further details on the progress of this problem will be published in the near future.

> H. J. PREBLUDA E. V. McCollum

BIOCHEMICAL LABORATORY, SCHOOL OF HYGIENE AND PUBLIC HEALTH, THE JOHNS HOPKINS UNIVERSITY

EFFECT OF COLORED CELLOPHANE ON THE PRODUCTION OF SUN-RED COLOR IN MAIZE

PRELIMINARY attempts have been made to determine, if possible, the wave-length of light responsible for producing the sun-red pigment in maize plants of the genetic composition A B pl.

It has been long known that such plants when exposed to light develop a red pigment in all parts of the plants, especially pronounced in the husks on the ears. The outer husks are deep red, while those underneath show very little if any of this color. Ears that develop under kraft paper bags fail to develop pigment.

In this experiment we used several hand-pollinated ears of a stock of Purdue $39 \times$ Connecticut 75 (A B pl), backcrossed twice to Purdue 39 and then selfed once. The plants were either homozygous B B or B b, otherwise were quite uniform. The ear shoots were bagged as soon as they appeared, and the developing ears were also covered with kraft paper bags. Hence the husks had not been exposed to sunlight and showed no red color.

On September 15, 1936, the paper bags were removed and the ears were covered with various colors of Cellophane purchased at one of the S. S. Kresge stores. Four ears were covered with each kind of Cellophane. The colors used were red, pink, Tango (yellow), amber, dark green, dark blue, light blue, violet and light violet. Also four ears were covered with clear Cellophane to serve as checks.

On October 1, the ears were harvested and the color of husks noted. By that time an intense red had developed under the clear wrapping and all but one of the