viability of variants, and is, therefore, affected by environmental or inherent changes which affect these factors. Some experimental proof for this concept has been obtained. For example, an increase in the density of the population appears to increase the dissociation rate. In such altered environmental conditions the absolute degree of dissociation is changed, but the relative differences between two clones, such as a high dissociating one and a low dissociating one, are retained. Another example is provided by the results obtained when the effect of the pH of the environment was studied. Thus, a high dissociating strain showed 50 per cent. dissociation at pH 6.6 and 2 per cent. at pH 7.4, while a low dissociating strain showed 1 per cent. dissociation at pH 6.6 and none at pH 7.4. Tests with other strains, as well as observations with buffered and unbuffered broth, provided further proof that the pH of the environment affects the relative degree of dissociation (by affecting growth rates?), however, always within the limits of the inherent factors which determine dissociation rate; *i.e.*, environmental influences which lower the dissociation rate will decrease the dissociation rate of a high and a low dissociating strain proportionally. It should now be possible to test the relationship between dissociation rate and rate of multiplication, ability of variants to establish themselves within a population, etc., by starting broth cultures with a known number of low dissociating Smooth cells and low dissociating Rough cells and subsequent daily counts of the number of each type present.

Work with strains started from single cells has also indicated (1) that all formerly described types of variants as well as several so far undescribed types can arise among the progeny of a single cell; (2) that the type of dissociation, *i.e.*, whether primarily *S* to *R* or *S* to *Br*, etc., differs between clones, and (3) that the ability to withstand toxic effects (urea solutions of high concentration) differs between clones and can be subjected to systematic selection. These apparently inherent characteristics are now being studied and special attempts are being made to establish the feasibility of selection for immunizing power. Also, an analysis of actual chemical differences between variants has been started.

A detailed account of the work on dissociation rates will be published in the near future.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW REAGENT FOR VITAMIN A

VITAMIN A can be estimated biologically, optically or chemically. Sometimes it is desirable to make determinations in the field and for such purposes only chemical methods are suitable. Antimony trichloride is the only reagent commonly used in this way. It has, however, disadvantages not found in the reagent to be described.

This material is "Super-Filtrol,"[1] a commercial adsorbent made from the aluminum silicate mineral, Montmorillonite. It is cheap, easily handled and requires no precautions in its use except that both it and the oils to be tested be anhydrous.

When even small amounts of vitamin A in solution in petroleum ether, chloroform, benzene or carbon tetrachloride are brought into contact with the reagent it immediately becomes bright blue. All polar solvents prevent or destroy the color which can not be eluted as such from the particles of the solid. This color may be utilized as a measure of vitamin A potency in fish liver oils, and presumably for the analysis of food extracts if they are anhydrous. Materials other than fish oils have not been tested.

The color may be estimated visually by comparison with a series of standards prepared by mixing varying proportions of powdered cobalt glass with the reagent. These are standardized against oils of known potency and may be conveniently prepared in steps of about 15,000 I.U. The color may be measured more accurately by a reflection type photoelectric colorimeter which may be made very simply by utilizing a diverging and variable slit between the photocell and the colored surface.

The color may best be developed by placing about 10 gm of "Super-Filtrol" in a small flask and covering it with petroleum ether. To this add a known weight or volume of vitamin A-containing material either as solution or just as oil, and shake. The blue color forms at once. For field use it has been found convenient to deliver the oil from the blunt end of a 20-gauge, hypodermic needle on a 1 cc syringe. Drops of the order of 7 mg are delivered with an accuracy of about 3 per cent. Two such drops, containing as little as 28 I.U. (2,000 I.U. per gm) will make a color on 10 gm of the reagent.

Shantz, Cawley and Embree[2] have shown that in the case of the antimony trichloride reaction a dehydration takes place, splitting off the terminal alcohol group and forming "anhydro vitamin A." This reaction is probably common to most or all of the materials

[1] Manufactured by the Filtrol Corporation, Vernon, Calif.

[2] E. M. Shantz, J. R. Cawley and N. D. Embree, *Jour. Am. Chem. Soc.*, 65: 901, 1943.

making a color with the vitamin, since they are all dehydrating agents of various degrees. In the case of this reagent the dehydration takes place on the surface of the particles because they have an adsorbed layer of sulfuric acid as a result of the leaching process used in their manufacture. Attempts at elution with solvents such as acetone destroy the blue color but yield an orange oil which has a different absorption spectrum from the vitamin.

Complex formation between the reagent (adsorbed sulfuric acid) and the anhydro compound appears to be the most probable explanation of the phenomenon. That such a complex is possible is indicated by the fact that carotene also makes a color even though it is a hydrocarbon.

Interesting possibilities are suggested by the principle of this assay. Not infrequently it is desirable, either for synthetic or analytic purposes, to react two substances which can not be brought into solution in a common solvent. Carrying one substance into contact with another by means of an inert adsorbent might solve such problems.

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A MODIFIED PETRI DISH FOR CONTINUOUS TEMPERATURE OBSERVATION

IN investigations upon free-living and parasitic Protozoa, I have found that greater accuracy could be obtained in using a modified Pyrex Petri dish for

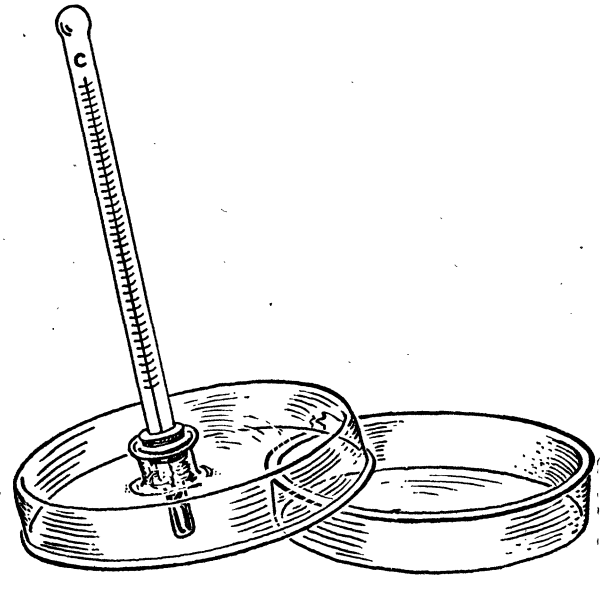

FIG. 1.

controlling and maintaining constant temperatures of culture fluids, stains and various fixing reagents.

The dish (Fig. 1) is a regulation stock four-inch Pyrex Petri dish (bottom and lid) in which a hole measuring approximately one centimeter in diameter is made close to the rim of the lid. A piece of glass tubing slightly larger than the diameter of the hole is then fused over it so that a collar is formed about one centimeter high. A piece of rubber tubing about six millimeters in length is inserted in the glass collar. Then a small clinical-type thermometer is inserted into the rubber-cushioned collar so that the bulb of the thermometer which should be immersed in the fluid comes to rest slightly above the bottom of the dish. Direct temperature readings may now be made of the contents of the dish without removing the lid.

Many investigators in protozoology and parasitology make smeared preparations directly on cover-slips which are then dropped into the heated fixing fluid. With this dish, it is possible to kill and fix the organisms at a definite temperature and still be able to maintain the correct temperature over a given period of time with the lid covered. In staining, it has proved to be extremely useful, especially in the Feulgen test for thymonucleic acid where a given temperature must be maintained for a definite period of time.

The dish should prove to be convenient and useful for protozoologists, parasitologists and those working with small animals where greater accuracy is desired in this phase of technical work. Entomologists may find the dish useful by simply using it as a cover with a stoppered opening through which fluids may be added to developing embryos without removing the lid thereby lessening the possibility of contamination.

It seems likely that with greater emphasis being placed upon research in parasitology and tropical medicine, there will be considerable usage for a dish of this nature.

The author wishes to express his thanks to Dr. James A. Harrison for his aid in construction of the dish.

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