When the plate is removed and developed, and a photometric curve is made across the "spectrum" thus obtained, it is possible to calculate from it which temperatures occurred during the observation period, and the part of the observation period during which the temperature was higher than a given value. To obtain such results certain points must be taken into consideration:

1. A suitable photographic plate must be chosen. It should be fine-grained and should not change its proper-

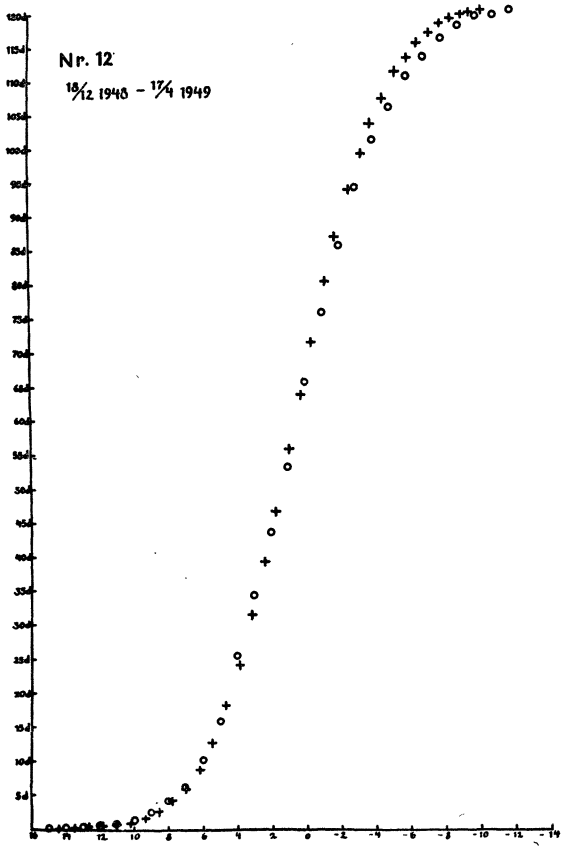

FIG. 2.

ties even during long observation periods. The Agfa Normal Diapositive Plate serves this purpose.

2. A suitable balance between the observation period, the activity of the radioactive paper, and the sensitivity of the plate must be found. For recordings of over one year an amount of approximately ½ γ radium sulfate/cm² was found suitable.

3. The relation between the time of exposure and the degree of darkening of the photographic plate must be found. The relation is very closely linear when a fine-grained plate is subjected to radiation by α- and β-particles even at low intensities.

4. The sensitivity of the plate as a function of temperature must be found. An experiment gave a sensitivity increase of 8% when the temperature was raised 23° C. This is an error for which correction must be made.

5. A standard exposure at definite temperatures must be made and photometric curves must be taken in the same way as in the original plates, to know which sectors of the plate correspond to which temperatures.

6. Because the slit has a certain width, the photometric curves give only an approximate representation of the relationship between the temperatures and the frequency of occurrence of each temperature interval, an error for which a correction must be made.

As a test, some of the apparatus was left for a certain period in the observation hut of the Norwegian Meteorological Institute at Blindern, Oslo, where continuous recordings with thermographs are made. From the thermograph curves the same factors may be calculated as from the photometric curves.

One of the results is given in Fig. 2. The ordinate shows number of days with temperatures higher than that given on the abscissa. Circles represent values calculated from the thermograph curves; crosses, values calculated from the photometric curves. The correspondence is good.

It will be evident that the same principle, although only with approximation, may be employed in all factors where the records can be transferred to the movement of a pointer, e.g., humidity, barometric pressure, etc. However, one does not know at which temperatures the exposures were made, and errors due to differences in the sensitivity of the plate with temperature cannot be corrected for. This difficulty may be overcome by making the width of the slit variable. If one side of the slit is fastened to a bimetal strip, this may be arranged so that the width of the slit decreases at higher temperature to compensate for the differences in sensitivity of the plate. Thus the same principle may be applied over a wide range of ecologic, climatologic, and other types of instruments.

A more detailed description and discussion is found in *Physiologia Plantarum*, 1949, 2, 272.

Cup Assay with Vitamin B_{12} as a Standard

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The cup assay method proposed by Heatley (*1*) for the estimation of penicillin has several advantages over standard turbidimetric or titrimetric procedures. Chief among these is the adaptability of the method to routine handling of large numbers of samples from a variety of sources. Evaluation of reproducibility of the method and discussion of factors influencing it have been presented by Heatley (*6*). The requirements for satisfactory microbiological assay methods have been listed by Foster and Woodruff (*4*).

Assays for biotin, thiamine, and riboflavin (*2, 5*) by the cup assay technique have been described. The factors crucial for antibiotic cup assays apply equally to the determination of growth-promoting factors by the cup method.

The microbiological estimation of LLD (*L. lactis* Dorner) type activity by means of *Lactobacillus lactis*,

or by means of *L. leichmannii*, using vitamin B_{12} (*10*) as a standard, is influenced by degree of aeration, by oxidation-reduction potential, and by accumulation of peroxides. These factors are difficult to control in the titrimetric assay procedure (*8, 9*). The cup technique has allowed more rigid control of influential factors, with a resulting assay of much greater precision. Also, by use of abnormal salt concentrations, it has been possible to eliminate the diffuse growth response of the test culture to desoxyribonucleic acid or to its constituent nucleosides.

The constituents of the medium described for the Merck modification (*3*) of the Shorb titrimetric estimation of LLD activity (Table 1) are combined as a dry mix and milled in a hammer mill or ball mill, in an area of low humidity. This milled ingredient dry mix is incorporated in the assay medium to give the final composition as shown in Table 1.

TABLE 1

Item	Amount
Milled ingredient dry mix (See Table 2)	22.9 g
Spray-dried HCl-hydrolyzed casein*	1.0 g
Adenine, guanine, and uracil solution†	10.0 ml
Sodium chloride‡	20.0 g
Agar	20.0 g
Distilled water	To 1 l

* Prepared by refluxing Sheffield high nitrogen casein with 20% HCl for 20 hr, then passing the hydrolyzate through a bed of an anion-exchange resin to neutralize the excess acid, and finally spray-drying it.

† Solution contains 1 mg/ml each of adenine sulfate, guanine hydrochloride, and uracil in distilled water, acidulated with just enough H_2SO_4 to maintain the ingredients in solution.

‡ Added in order to eliminate growth response to desoxyribonucleic acid, thymidine, or reducing substances.

This assay medium is sterilized by autoclaving 20 min at 120° C, in 1-liter quantities, which may be combined to yield sufficient medium for assay of all the samples accumulated on a single day. The medium, without adjustment, is at pH 6.2 after sterilization.

An alternate medium, which is less expensive for routine assays, may be prepared by incorporating 20 g L-cystine, 20 g DL-tryptophane, 10 g DL-aspartic acid, 20 g DL-α-alanine, and 20 g ammonium acetate in the dry mix, in place of the 18 amino acids listed in Table 2. For each liter of final medium, 20.1 g of alternate mix and 2 g of spray-dried HCl-hydrolyzed casein are required. Additional supplements are added, as in the previously described medium. Although growth response is adequate from the alternate medium, it is preferable to use the complete mix whenever possible, rather than to rely on the variable composition of casein preparations as a source of essential amino acids.

Inoculum is prepared by 18-hr incubation of *L. lactis* Dorner A.T.T.C. No. 10,697[1] in the assay medium without agar and NaCl, but supplemented with 0.0001 μg of vitamin B_{12} per ml. Five ml of the inoculum culture,

[1] Kindly supplied by Dr. Shorb, University of Maryland, College Park.

TABLE 2

INGREDIENTS FOR DRY MIX FOR CUP ASSAY*

20 g DL-Isoleucine	1000 g Dextrose
20 g DL-α-Alanine	600 g Sodium acetate
20 g L-Cystine	50 g Fumaric acid
20 g DL-Aspartic acid	50 g Sodium ethyloxalacetate
20 g DL-Norleucine	20 g $MgSO_4 \cdot 7H_2O$
20 g L-Tyrosine	1 g NaCl
20 g DL-Valine	129 g $Na_3PO_4 \cdot 12H_2O$
20 g DL-Methionine	67 g K_3PO_4
20 g DL-Glutamic acid	1 g $FeSO_4 \cdot 7H_2O$
20 g DL-Threonine	1 g $MnSO_4 \cdot 4H_2O$
20 g DL-Serine	20 mg Riboflavin
20 g DL-Phenylalanine	20 mg Calcium pantothenate
20 g DL-Leucine	20 mg Thiamine HCl
20 g L-Histidine	20 mg Nicotinic acid
40 g DL-Tryptophane	40 mg Pyridoxamine
20 g L-Arginine	4 mg *p*-Aminobenzoic acid
10 g L-Lysine	0.04 mg Biotin†
20 g Aminoacetic acid	

* Total mix contains 2289 g, sufficient for 100 l of assay medium.

† This small amount of biotin may be conveniently incorporated in dry form in the mix by mixing 0.4 ml of a stock solution of biotin containing 0.1 mg of biotin per ml with 5 ml of a water solution containing 1 g of the 20 g of DL α-alanine required in the final mix, and evaporating to dryness on a water bath.

which should give a transmission of 25 on an Evelyn photoelectrometer with 520 mμ filter, is transferred to each liter of liquefied assay medium, precooled to 52° C.

Inoculated medium is distributed into flat-bottom Petri dishes in 25-ml quantities and allowed to harden. Assay cylinders are then placed on the agar surface and the dishes stored at 4° C until needed. Solutions containing 0.03, 0.05, 0.1, 0.2, 0.4, and 0.6 μg B_{12} per ml, M/50 pH 5.2 Na_2HPO_4-KH_2PO_4 buffer are prepared for daily determination of a standard curve. Check standard solution containing 0.2 μg B_{12} is included on assay plates, in addition to unknowns diluted to approximately this level with M/50 buffer. Three assay cups are filled with standard solution and three alternate cups with unknown solution in each Petri dish.

After 18-hr incubation at 37° C, diameters of growth zones are measured and calculations made in the same manner used for antibiotic assays. A typical assay yields 1.8-mm growth zone diameter increase for each twofold increase of B_{12} concentration and approximately 17-mm growth zone for the 0.2-μg check standard.

Standard deviation (66% confidence limit) for the cup assay is ±10% as compared with ±21% for the titrimetric assay, with similar replication and the same assay organism.[2]

[2] One cup assay is calculated from the average diameter of three zones of solution under test and three zones of standard solution on a single Petri dish. One titrimetric assay is the averaged value from five assay tubes at different concentration gradients, compared with 20 tubes containing standard solution at concentration gradients.

By the cup technique, *L. lactis* shows no response to thymidine, desoxyribonucleic acid, or 0.5% ascorbic acid. Response is obtained with crystalline vitamin B_{12}, vitamin B_{12a} (*7*), liver concentrates, and certain microbiological fermentation products. The fermentation materials frequently require autoclaving to destroy associated antibiotics, or acidification for release of LLD growth-promoting factors from the cells. Insoluble adsorbates, and fermentation residues, often yield full activity following suspension in water and apportioning the suspension directly into assay cups, although supernatant liquors from such suspensions may contain little growth factor.

In order to obtain full LLD activity with some adsorbates, such as APF adsorbates, it is necessary to dilute suspensions to 0.05 μg B_{12} equivalent per ml. For these samples, the check standard solution should also contain 0.05 μg B_{12} per ml.

In the cup assay described, quantitative values are assigned to the growth response of *L. lactis* Dorner (A.T.T.C. No. 10,697) to unknown preparations on the basis of an assumed value of 11×10^6 activity units per mg of vitamin B_{12}. To distinguish from the Shorb LLD unit (*11–13*), the unit of *L. lactis* Dorner activity as determined by the herein-described cup method is designated as the LLDCC unit of LLD type activity. The cup assay shows no LLD response to desoxyribonucleic acid or its constituent nucleosides, whereas the Shorb titrimetric assay shows LLD activity for these substances. Cup and titrimetric assays show somewhat different responses to modified substances, such as vitamin B_{12a}, when assayed against a vitamin B_{12} standard. Impure materials, containing various LLD-active substances, do not necessarily yield equal results by both methods. Shorb's liver concentrate standard, assigned a value of 1,000 LLD units per mg, from which the above-noted LLD activity of B_{12} was determined by titrimetric assay, was found experimentally to contain 1,120 units/mg by the cup assay with crystalline vitamin B_{12} standard.

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Note: Description of a cup assay for the antipernicious anemia factor, in which thymidine and related compounds caused interference, was presented orally by Dr. W. F. J. Cuthbertson before the Biochemistry Society in London, January 22, 1949.

Aureomycin in the Cultivation of *Endamoeba histolytica*[1]

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Three attempts have been made to establish a single strain of *Endamoeba histolytica* in Shaffer-Frye medium (*3*) containing aureomycin in place of other antibiotics. The aureomycin (Duomycin) was obtained from Lederle Laboratories in vials each containing 50 mg of powdered aureomycin. This was diluted in a phosphate buffer, pH 6.9, and used within 2 hr. Final concentrations of aureomycin in the culture tubes ranged from a dilution of 1:1,000 to 1:5,000,000.

In each experiment the inoculum used consisted of 48-hr cultures of the amebae collected in large numbers by centrifuging the buffered saline overlay containing rice starch from flasks of coagulated egg base (*2*). These flasks were inoculated with amebae from Balamuth's culture medium (*1*). Counts were made on the material inoculated into the aureomycin cultures, and a volume of 0.5 ml was inoculated into each tube. Growth of the amebae in culture was estimated by examining them through the wall of the test tube placed under the 16-mm objective of the microscope and recording the relative numbers in terms of 0, ±, +, ++, +++, or ++++.

In the first attempt to establish our human strain XXII in the aureomycin cultures, five dilutions of aureomycin were used with two tubes for each dilution, and four control tubes were set up, two having 10,000 units of streptomycin and 5,000 units of penicillin in 0.5 ml of buffered saline, and two having the same amounts of streptomycin and penicillin in 0.2 ml of physiological saline. The aureomycin dilutions used were 1:1,000, 1:2,000, 1:10,000, 1:100,000, and 1:1,000,000. Transfers of the cultures were made at 48- or 72-hr intervals.

In this first experiment, positive cultures were obtained only in the tubes containing aureomycin diluted 1:100,000 and 1:1,000,000, and in the controls. The two 48-hr cultures of 1:1,000,000 dilution (++++ growth) were pooled and from them tubes containing aureomycin diluted 1:33,000, 1:50,000, 1:1,000,000, and 1:2,000,000 were inoculated. Growth was obtained in all dilutions, but it was decidedly better in the 1:1,000,000 and 1:2,000,000 than in the lower dilutions. The two tubes containing the 1:33,333 dilution remained positive only 48 hr and those containing 1:50,000 remained positive 72–96 hr. One tube containing 1: 2,000,000 was positive 168 hr after inoculation. A third passage was made 72 hr after the previous transfer and positive cultures in dilutions of 1:50,000, 1:100,000 (inoculum 120 hr old), 1:1,000,000, and 1:2,000,000 were transferred. Only two tubes became positive for amebae and they had a ± rating 48 hr after inoculation. After the third transfer the

[1] Supported by a grant-in-aid from the National Institutes of Health, Bethesda, Md. Thanks are due Dr. Herald Cox, Lederle Laboratories, Pearl River, N. Y., for furnishing supplies of aureomycin and important data on its properties.