tained at 37.5° C. when the response was negative at room temperature.

The need for standardization of the conditions under which the cephalin flocculation procedure is carried out is obvious. This is especially important in connection with the use of this test in various geographical locations, in which widely differing conditions, in respect to light and temperature, may be encountered. The most satisfactory results have been obtained when the reagents and sera were protected from prolonged exposure to bright light, and when the antigen was added soon after the serum was diluted with saline. It is not yet possible to define the ideal temperature conditions for this reaction, but it appears that more reliable results are obtained at 20 to 25° C. than at 37.5 degrees. Variable results due to differences in sensitivity of the antigen can be partially eliminated by the frequent inclusion of normal control samples. Work is continuing in an attempt to define more exactly the conditions that will yield the most dependable results. However, the procedure is even now capable of providing useful information when performed under the conditions described above.

SUMMARY

(1) Flocculation of cephalin-cholesterol emulsions by blood serum is markedly influenced by the amount of light to which the serum-saline-antigen suspensions are exposed. Protection from bright light, natural and artificial, has eliminated many falsely positive reactions. (2) Other factors that appear to influence the cephalin flocculation procedure have been briefly mentioned. Misses Dorothy Feinberg, Arvilla Howley and Mary Lanning contributed helpful technical assistance.

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A RHODOTORULA DEFICIENT FOR PARA-AMINO-BENZOIC ACID

IN November, 1943, Mr. Manfred Wahl of Philadelphia furnished us with a pink yeast which he had isolated and cultivated from a development in an old culture of beer yeast which Mr. Wahl had attempted to rejuvenate with other dormant cultures. Through the courtesy of Dr. Lynferd J. Wickerham, associate zymologist, Fermentation Division, Northern Regional Research Laboratory, Peoria, Ill., it was identified as a strain of *Rhodotorula aurantiaca* (Saito) Lodder. Preliminary experiments showed that this yeast grew well at 25° C on a basal medium composed of KH_2PO_4, $MgSO_4$, asparagine and dextrose solidified with purified agar and supplemented with thiamine and peptone. When peptone was omitted the growth in the first transfer was scanty, and subcultures on the same medium failed completely. It appeared probable that this strain of *R. aurantiaca* suffered from growth-substance deficiencies which were not corrected by the addition of thiamine to the basal medium but were satisfied by the substances supplied by peptone.

Yeasts with complete or partial deficiencies for thiamine, biotin, *i*-inositol, pyridoxine and pantothenic acid have been described. However, we were unable to induce the growth of *R. aurantiaca* by the addition of a mixture of these 5 substances to our basal medium. Excellent growth was obtained when the basal agar medium was supplemented with a mixture of para-amino-benzoic acid, calcium pantothenate, guanine, hypoxanthine, *i*-inositol, nicotinamide, pyridoxine, pimelic acid, riboflavin, thiamine, biotin methyl-ester and 2-methyl-1, 4-napthohydroquinone diacetate. In fact, the growth was more vigorous and the color a deeper red on the medium supplemented with the pure growth substances than on the peptone medium (containing 8 mg of neopeptone per tube). Good growth was obtained also on a medium prepared by adding 5 per cent. of desiccated malt extract to 1.5 per cent. Difco agar, but the color was a deep dull red instead of the bright red observed on the medium supplemented with the mixture of growth substances.

In order to determine the effective substances the basal agar medium was supplemented with 11 of the growth substances mentioned above, one being omitted. Growth was scant when thiamine or para-amino-benzoic acid was omitted; it was unaffected by the omission of any one of the other ten. It appeared, therefore, that *R. aurantiaca* suffered from a complete deficiency for these two growth substances. Further experiments confirmed this finding. The yeast grew well on the basal medium prepared with purified agar supplemented with thiamine and para-amino-benzoic acid. It did not grow on the same medium to which thiamine alone or PAB alone was added. It required molecular thiamine, as no growth was obtained when thiamine was replaced by the thiazole or pyrimidine intermediates of thiamine singly or together. The intensity of the pink color was related to the supply of PAB. In media with less than an optimum quantity of PAB the color was paler and tended toward orange as compared to the deeper pink which developed when more PAB was supplied.

The yeast was grown at 25° in test-tubes containing 5 ml of the basal solution and 10 mμ moles of thiamine per tube plus various amounts of PAB. Turbidity measurements were made after 48, 72, 96 and 149 hours incubation. Under these conditions a positive effect of 0.001 mμ mole of PAB (0.000137 μg) was observed after 72 hours. Growth increased with

the amount of PAB up to 0.1 mμ mole; it was approximately the same with 0.1, 0.5 and 1.0 mμ mole. Somewhat less growth was obtained with 10 mμ moles of PAB than with 1 mμ mole.

Schopfer[1] reported that *R. aurantiaca* grew poorly in a mineral-dextrose solution containing asparagine. The addition of thiamine doubled growth, but it was still poor. The addition of pyrimidine, thiazole or thiochrome was ineffective. Schopfer's results as far as they go agree substantially with ours.

We concluded that our strain of *R. aurantiaca* suffers from a complete deficiency for thiamine and for PAB. Its sensitivity to PAB appears to be of the same order of magnitude as that of some other organisms to biotin. Its growth on media supplemented with peptone or malt extract demonstrates the presence of PAB (or a substitute therefor) in those natural substances. *R. aurantiaca* might be useful for the microbiological assay of PAB or of molecular thiamine and for the study of the function of PAB and its relation to the sulfa drugs.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ATTACHING POINTERS TO MICROSCOPE SLIDES

IN preparing practical slide examinations in such subjects as vertebrate embryology or histology, it is frequently necessary to direct the student to a particular detail selected from many others present in a preparation. Various methods for solving this problem are in use in different teaching laboratories; *e.g.*, (1) accompanying the slide with a mimeographed sketch on which various selected details may be indicated by name or number; (2) covering all extraneous matter with gummed paper, leaving exposed only the detail to be observed by the student; (3) pasting paper pointers to cover slips; (4) using ocular pointers; (5) ringing cover slips with diamond point object markers fitting into the nosepiece of a microscope. It is unnecessary to point out decided disadvantages inherent in each of the methods mentioned above.

Since our difficulties with this problem must be paralleled in many other laboratories, it may be of general interest to describe here a technique which we find to be very satisfactory. Using very sharp scissors, small pointers, in the form of isosceles triangles, are cut from thin, tinted Cellophane. Pointers cut from a good quality of bond paper are often good enough, but under a magnification of 300 or 400 diameters such paper pointers look quite ragged.

The pointers are glued to clean cover slips with thin clarite, balsam or damar, and the cover slips dried on a warming stage. Sections fresh from xylene are mounted in clarite (60 per cent. by weight in toluene) under such cover slips, with the pointer between the section and the cover. By gentle manipulation of the cover slip under a dissecting or compound microscope, it is easy to place the tip of the pointer in any position desired. We have not been troubled by having pointers move during the drying process. After the preparation has been thoroughly dried (*e.g.*, one month at 50° C.), there is no further danger of moving or blunting the pointer. The slide then constitutes a permanent item in a practical examination set.

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[1] W. H. Schopfer, *Protoplasma*, 31: 105–135, 1938.

LOAN TEACHING SETS ON BACILLARY DYSENTERY

THERE are now available for teaching purposes six sets, each consisting of eighty-six 28 × 40 mm slides, thirty-five in Kodachrome, and a condensed lecture brochure. These slides cover the subject of acute and chronic bacillary dysentery, including the newer aspects of the epidemiology, pathology, bacteriology, serology, clinical phases, prophylactic and curative therapy. They are available on loan to Army, Navy, public health and university teachers without cost except that of mailing. The project is part of a long-range plan of the Dysentery Registry for the dissemination of our ever-growing knowledge of the important subjects of bacillary dysentery, enteritis and colitis. It was deemed expedient to stress the military aspects at this time. Requests will be honored in order of their receipt. The date on which the slides will be used should be specified. The total time of presentation is approximately 90 minutes at the ordinary talking speed. The slides are so aranged that they may be presented in a single lecture, two lectures of 45 minutes each or three lectures of 30 minutes each.

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