The author wishes to express his gratitude to Dr. E. Anderson for the preparation of the l-glucose and to Professor W. L. Holman, of the department of bacteriology of this university, for carrying out the bacterial tests.

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TULAREMIA ENDOTOXIN

I OBTAINED a strong tularemia endotoxin from Hamzabey and Ceylon strains which I had isolated from a brook during the investigations[1] of the tularemia epidemic in Thrace in 1937.

These strains were cultivated in plates containing blood and cystin media. These cultures were then emulsified in sterile distilled water, placed in flasks containing beads. These flasks were then put in tin boxes lined with cotton and incubated in 37° C. incubators for autolysis. The boxes were shaken thoroughly twice daily. The incubation lasted 45 days, during which time all the tularemia bacteria died. After the product was found completely sterile it was centrifugalized. The clear, sterile autolysite was then desiccated in vacuum in the presence of sulfuric acid. The dry endotoxin thus obtained was dissolved in sterile distilled water, having 50 mg of dry toxin in 1 cc. It was recentrifugalized. The tests made with the broth-colored solution gave the following results:

(1) White mice weighing 20 grams, injected intravenously with 0.3 cc of this solution (15 millig. of dry toxin) = (M. L. D.), died instantly with severe convulsions.

(2) The intraperitoneal injections of the same amount killed the mice within 24 hours.

(3) Guinea-pigs weighing 250–300 grams were injected 15 centigrams of dry toxin intraperitoneally, and they died within 24 hours with typical cries.

(4) The intracutaneous injections of 10–15 millig. of dry endotoxin caused a passing vasoconstriction at the site of injection.

(5) Repeated intravenous injections to rabbits showed the antigenic value of the endotoxin by the presence of a marked flocculation reaction. Moreover, it was found that the endotoxin produced titratable flocculation and precipitation reactions with the sera of persons who had previously had tularemia infection, as well as with those of guinea-pigs with chronic tularemia infection. These sera also neutralized the cutaneous vasoconstriction.

(6) The endotoxin does not contain hemolysins.

Finding it possible that this endotoxin can be used as a standard toxin in the standardization and control of tularemia antisera, and that the detoxicated toxin can be used for the prevention of the disease, I thought the publication of this work might be of some use to other workers in this field.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE THROMBOPLASTIN REAGENT FOR THE DETERMINATION OF PROTHROMBIN

FOR the satisfactory quantitative determination of prothrombin by my method,[1] a thromboplastin is required which is stable and has a high and uniform activity. Such an agent can readily be prepared as follows: a rabbit brain, from which the superficial blood vessels have been removed by stripping off the pia, is mascerated in a glass mortar under acetone. After several replacements of fresh acetone, a granular non-adhesive product is obtained which is dried on a small suction filter. The dehydrating process should not require more than 10 minutes and the acetone must not be acid (Eastman's Practical is satisfactory). As soon as the thromboplastin is free of acetone, it is put in a glass ampule which is evacuated by means of an oil vacuum pump for 3 minutes and then sealed in a gas flame. For convenience 0.3 gm material is put in each ampule. To prepare the emulsion, this amount of dry material is mixed with 5 cc of a freshly prepared 0.85 per cent. sodium chloride solution, incubated for 15 minutes at 50° C., and then allowed to stand until the coarse material is settled and a milky supernatant liquid is obtained. The presence of suspended material does not interfere with the test. Centrifugation often reduces the activity of the emulsion. The thromboplastin solution prepared in this manner will, according to the technique of my test, clot human plasma in 11 to 12½ seconds and rabbit plasma in 6 seconds. For research purposes no preparation which does not bring about coagulation in these periods should be employed.

Curiously, the dehydration of rabbit brain with acetone or with dioxan markedly increases the thromboplastic activity. There is at present no explanation for this finding. Whereas an emulsion of fresh rabbit brain clots human plasma in 16 to 25 seconds, the thromboplastin made by the acetone method brings about clotting in 11 to 12½ seconds. Dehydration with acetone, moreover, makes the product much more constant in potency. It seems, therefore, advisable to

[1] *Zentr. f. Bak.* (*Referate*) *B.C.* xxix, No. 5–6, 1938; *Népegészségugy*, No. 21, 1938.

[1] A. J. Quick, M. Stanley-Brown and F. W. Bancroft, *Am. Jour. Med. Sci.*, 190: 501, 1935; A. J. Quick, *Jour. Am. Med. Assn.*, 110: 1658, 1938; 111: 1775, 1938.

abandon the use of fresh brain, even though Souter, Kark and Taylor[2] have recently described a satisfactory way for preserving thromboplastin extract by means of the lyophile procedure of Flosdorf and Mudd.

Thromboplastic activity is destroyed by oxidation. If dried rabbit brain be allowed to remain in contact with air, it slowly turns yellow and a concomitant loss of potency occurs. By storing the reagent in an evacuated tube, oxidation is prevented and the activity is retained apparently indefinitely. As an example, a preparation made on March 3, 1938, was tested on June 6, 1939, and found it clotted human plasma in 11½ seconds. The unused portion of the sample was resealed under vacuum and tested on June 24, 1940. Again clotting occurred in 11½ seconds.

Thromboplastin possesses a relative but not an absolute species specificity. Rabbit thromboplastin appears to be specific for the plasma of man, dog, cat, horse and cow, but not for the plasma of the guinea pig or for birds. It is interesting to note that repeated search has failed to find any better source for thromboplastin than rabbit brain.

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A STOPCOCK LUBRICANT FOR USE WITH SOLVENTS, ACIDS OR ALKALIES

DURING the past few years the writer has done considerable work with solvents such as carbon disulfide, petroleum ether, acetone and other similar solvents in separatory funnels and burettes.

Many different compounds have been used as stopcock lubricants. Some of these are good for certain solutions but unsatisfactory for others.

About two years ago while searching for a satisfactory stopcock lubricant for use with carbon disulfide the writer tried some powdered graphite which was on the shelf in the laboratory. It has proved to be a valuable substance for use as a lubricant where the ordinary compounds are dissolved away.

Graphite is insoluble in all ordinary solvents and is not affected by acids or alkalies. It does not allow the stopcock to bind and it gives a very satisfactory seal. Only a very small amount should be used. A stopcock once coated usually lasts a long time.

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SHADOWGRAPH RECORDING OF AVENA COLEOPTILE CURVATURES

GROWING oats in battery jars, in place of specialized seed holders, for the Went coleoptile assay method for auxin, seems to have a number of distinct advantages. When this technique is employed, shadowgraph records may be conveniently taken with the aid of an easily constructed device described below and figured in the accompanying drawing.

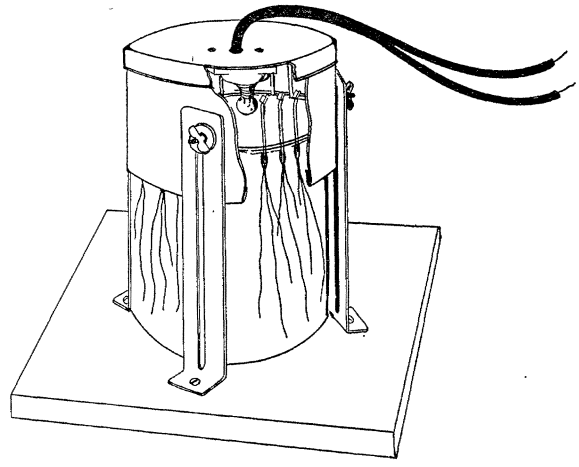

FIG. 1.

A copper cylinder 4″ in diameter and 3″ in height is made movable up and down along slots in three copper legs attached securely to a base board. The cylinder may be held in any position by means of winged nuts that tighten against the legs on screws attached to the cylinder. The lower rim of the cylinder is curled up on the inside to support a strip of photographic paper. Covering the cylinder is a copper lid, in the center of which is placed a small single filament flashlight bulb operating on two dry cells.

We have used this set-up with battery jars 3⅞″ in diameter and 5″ in height in which 20 to 25 oats are able to grow uniformly. Satisfactory results have been obtained with both single and double exposures of the reacting coleoptiles.

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