

tively simple conjugate. It is common knowledge that many of the B vitamins occur in a "bound" form, that is, bound to macro-molecular substances, but the occurrence of a simple non-protein conjugate of vitamin B_C has not been previously recognized. It seemed desirable to call attention to these results at this time since they bear on the interpretation of nutritional data involving growth of the chick, especially with respect to the chemical identification of other chick factors, such as Factor U,¹⁰ the alcohol precipitate factor¹¹ and vitamins B₁₀ and B₁₁.¹² Any one or all four of these factors may be identical with vitamin B_C conjugate.¹³ Work is in progress on the chemical

nature of the non-vitamin B_C portion of the conjugate molecule.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A FIVE-MINUTE METHOD FOR STAINING FECAL SMEARS

Using the following technique, it is possible to prepare permanent hematoxylin stained slides of fecal smears in five minutes or less.

Solution I (mordant-fixative)

formalin, 10 per cent. by volume 3 parts
glacial acetic acid 1 part
ferric ammonium sulfate 3 per cent.
(3 grams of the iron alum per 100 cc formol-acid mix)

Solution II (stain)

0.5 per cent. hematoxylin (aqueous)

Solution III (dehydrating and clearing)

dioxane

Solution IV (dehydrating and clearing)

dioxane

Solution V (clearing)

dioxane and toluol, half and half.

PROCEDURE

(1) With a brush, make a very thin smear of the fecal material to be examined.

(2) Cover smear immediately with a few drops of solution I. Pass slide through a bunsen or alcohol flame a few times or until the fixative begins to steam. Be ready to add more solution to the slide if necessary to prevent drying. Quickly pour off the mordant-fixative.

(3) Immediately add several drops of solution II. Again pass the slide through the flame one or two times, tilting it back and forth. Be sure that enough stain is put on to keep the smear covered. The fecal material should become a dark purple color in three or four seconds.

¹⁰ E. L. R. Stokstad and P. D. V. Manning, *Jour. Biol. Chem.*, 125: 687, 1938; E. L. R. Stokstad, P. D. V. Manning and R. E. Rogers, *Jour. Biol. Chem.*, 132: 463, 1940.

¹¹ A. E. Schumacher, C. F. Heuser and L. C. Norris, *Jour. Biol. Chem.*, 135: 313, 1940.

¹² G. M. Briggs, Jr., T. D. Luckey, C. A. Elvehjem and E. B. Hart, *Jour. Biol. Chem.*, 148: 163, 1943; Abst. of 107th meeting, Am. Chem. Soc., Cleveland, Ohio, April 3-7, p. 15B (1944).

(4) Place the slide at once into a coplin jar of water and wash under running tap water for one minute.

(5) Lay the slide on blotting paper and remove excess water on the smear side with filter paper or cleansing tissue. Quickly transfer the slide to solution III.

(6) Put the slide into the second jar of dioxane for at least one minute.

(7) Transfer the slide to solution V for a minimum of thirty seconds.

(8) Mount in clarite.

COMMENTS

The results obtained are much superior to those from the iodine technique for rapid fecal examination and compare favorably with the usual speed-up process using warm solutions, which takes about an hour and a half. The material is not as satisfactory as that prepared by the standard longer methods for critical cytological work. Even so, this technique is well adapted for a rapid survey of intestinal protozoa of animals or of man. Leaving the stain on for an extra second will overstain the cells but will make cilia, undulating membranes and flagella stand out clearly. Careful staining will adequately show all chromatin particles. The length of time of staining depends on the size of the flame used and the temperature of the slide. In some cases, the slide is hot enough after the mordant-fixative treatment to require no additional heating with the stain. Care must be taken, however, not to overstain the smear. If it does become overstained, it can be rapidly destained by rinsing in water and then adding a few drops of cold solution I. Stop the destaining process and blue the smear by washing in tap water. Dehydration and clearing are aided by slight agitation of the slide while

¹³ We have demonstrated the occurrence of vitamin B_C conjugate in certain liver extracts.

it is in the dioxanes. A slide holder or clothespin should be used to hold the slide during the first two steps. Insufficient time has elapsed to determine whether or not the stain will fade after only one minute of washing. The dioxane solutions should be kept in tightly stoppered bottles when not in use and should be renewed often if used frequently.

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A PRESSURE-CONTROLLED ELECTRIC CIRCUIT

THIS circuit consists of two ordinary bell-ringing transformers and what may be called an electrolytic

switch actuated by pressure. It is connected to the ordinary 60-cycle lighting circuit, and the output power is sufficient to light a neon glow lamp or to actuate a sensitive relay.

The two transformers are connected together so that in effect they constitute a single one-to-one transformer. The power output is from the high-voltage winding of the second of the pair, the low-voltage windings of the two being simply connected together as shown in the diagram. The purpose of the transformers is to permit grounding of any selected part of the power output circuit, and to provide a safe limitation on the power that might accidentally be obtained. The power is so limited that no flash can be obtained, and no shock more than a nip of the finger, by inadvertence or accident of any degree.

The electrolytic switch may be of any of widely various forms. The diagram shows one designed to be actuated by the least fluid displacement. A small metallic rod extends down into a short glass tube. A wire is fixed centrally in the bottom of the rod, extending down to make contact with the electrolyte in the tube. This glass tube is cemented into a metallic sleeve or tube which connects below to the water vessel or source of pressure. One electric connection is made to this metallic tube, the other to the rod above. The action of the switch will be readily understood. What calls for remark is the fact that this arrangement is effective, that it works out advantageously in practice. Ordinary tap water is sufficiently conductive to afford a clear and definite signal even with a 3-watt neon glow lamp. It takes very little to increase the conductivity of the water to the point where a one-watt lamp is lighted substantially as it is when connected directly in the lighting circuit. There is no electrode trouble, because the current is alternating and small.

A striking sensitivity is obtained by connecting a length of rubber tubing to the switch, pinching off the lower end and carefully adjusting the water level and the upper electrode. The adjustment can readily be made so that the circuit is closed by a very slight movement of the rubber tubing or by a very slight pressure.

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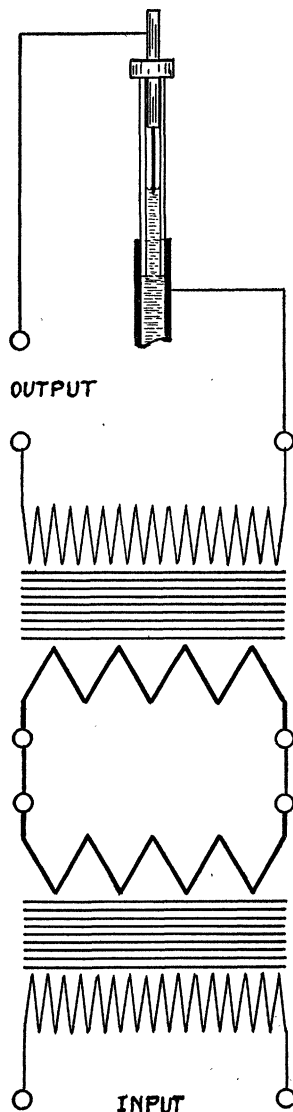


FIG. 1.

BOOKS RECEIVED

- GOLDBRING, WILLIAM and HERBERT CHASIS. *Hypertension and Hypertensive Disease*. Illustrated. Pp. xv + 253. The Commonwealth Fund. \$3.50.
GRAHAM, VERNE OVID. *Mushrooms of the Great Lakes Region*. Illustrated. Pp. vii + 390. The Chicago Academy of Sciences.
KROEBER, A. L. *Peruvian Archeology in 1942*. Illustrated. Pp. 152. Viking Fund, New York City. \$3.00.
MORRISON, A. CRESSY. *Man Does Not Stand Alone*. Pp. 107. Fleming H. Revell Company. \$1.25.