

dynamic lag characteristic of the first coil should approximately equal the dynamic lag characteristic of the second coil. Any existing lag difference will not vitiate accurate timing provided that the extent of such lag difference is determined, since the existing difference itself is a constant when the parallel circuit E.M.F. is constant.

In actual experimentation, the director first presents to the subject a "get ready" signal by depressing a special circuit key which may either illuminate a small tungsten lamp or activate a high frequency buzzer. This preliminary signal is afforded for the purpose of informing the subject just when to place his finger on the concave cushion knob of the reaction key. After an appropriate interval, the time value of which may be controlled by a seconds pendulum and varied at the discretion of the director, the experimenter then depresses the duo-circuit stimulus key. Depression of the reaction key followed by depression of the stimulus key closes circuit B (Fig. 2), thereby forcing the plunger rod through the finger knob aperture of the reaction key simultaneously with the initial movement of the pointer on the time dial of the chronoscope. The subject has been previously instructed to release his finger from the reaction key the instant that he perceives the plunger rod touch his skin. Release of the reaction key by the subject provides a circuit transfer from B to A which magnetizes the external coil X of the chronoscope clutch and thus attracts the dial pointer outward to a state of rest. Since a valve rectifier (VR) is inserted between the synchronous motor of the chronoscope and the alternating circuit main, the conventional 60-cycle input is converted into a 30-cycle input (sixty impulses per second) which provides a dial measuring unit of one six-hundredth of a second. According to Dunlap, a measuring unit of two sigma is small enough for practical purposes. The investigator should never utilize alternating current that is not centrally synchronized.

If the experimenter wishes to investigate reaction time to auditory and visual stimuli in addition to reaction time to touch stimuli, the necessary apparatus adequate to provide such sensory stimulation may be inserted in the same chronoscope circuit without difficulty. A three-point switch shifts the stimulus control current from the plunger coil (circuit E) either to a pair of 2,000 ohm headphones (circuit C) or to an induction coil which is directly connected with a neon lamp (circuit D). The same reaction key may be used for each of the three types of presented stimuli.

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## A METHOD TO SOFTEN TISSUE ALREADY IMBEDDED IN PARAFFIN<sup>1</sup>

THE method is believed to be generally applicable. It was first tried by the writer on lily ovary tissue in Professor W. C. Coker's laboratory at the University of North Carolina. The most convincing results, however, have just this summer been obtained while doing histological work on the pineapple leaf.

The pineapple leaf in a fresh condition, though rigid, is not particularly tough. In paraffin it is definitely brittle, and without softening treatment the tissue crumbles on the knife instead of cutting.

The following method has been used very successfully in obtaining smooth, even and straight paraffin ribbons of sections of pineapple leaf.

1. Rectangular pieces of leaf not over 4 by 10 mm, preferably smaller, are fixed in FAA. The pineapple leaf varies in thickness from less than 1 mm to about 2 mm.

2. Dehydrate and clear in the usual way with ethyl alcohol and xylol.

3. Infiltrate and imbed in paraffin. Infiltration must be as nearly perfect as possible. Paraffin of melting-point 52°-54° C. has been used on account of lack of that of 56°-58° C., which it is believed would be better suited for our laboratory temperature range of 27°-29° C. during the daytime. At this temperature sections could not be cut continuously successfully at less than 12  $\mu$ .

4. Shape the imbedded tissue ready for cutting. Trim the two edges and one end so that the leaf tissue will be directly exposed. This is very necessary to facilitate the subsequent treatment. If the piece is long, both ends may be exposed. Drawing the edge of a sharp, thin razor blade across the surface of the paraffin block to which the leaf tissue is nearest will also help, but is not necessary unless large pieces are used.

5. Store in 95 per cent. alcohol (at a temperature of about 30° C.) containing enough carbol fuchsin to make it pink. If material turns red throughout when transferred to water containing a little carbol fuchsin, infiltration was not complete. Such material will not cut satisfactorily. Paraffin is very slightly soluble in 95 per cent. alcohol at 28°-30° C.

Two to 4 days is sufficient to make the younger leaf tissue cut satisfactorily. Two to 3 weeks improves it and is necessary for the older leaf tissue.

6. Transfer to water 2 to 24 hours before trying to cut.

7. If, after half a block has cut well, the ribbon

<sup>1</sup> Since this announcement went to press, more definite results have been obtained and will be published later. The work is being continued in the Botanical Laboratory of Johns Hopkins University.

begins to twist and fold, place the material in water again.

The method is not recommended for tissue which is tough in a fresh condition, nor will it take the place of a sharp knife. The writer prefers a regular microtome knife, stropped at frequent intervals, to any of the razor blade holding devices. Investigations are to be continued along this line to find a water or alcohol soluble substance which has greater softening qualities than pure water that can be used successfully in connection with the 95 per cent. alcohol treatment to soften tissue which is definitely tough in a fresh condition.

Chamberlain recommends a method used by Dr. Land of storing the paraffin cakes in water. The writer is not in a position to make comparisons. Obviously, however, the 95 per cent. alcohol treatment upon the partially exposed material would facilitate the infiltration of water later. It is hoped that this modification of Dr. Land's method may meet with some favor among paraffin workers.

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## SPECIAL ARTICLES

### IMMUNIZATION WITH ALUMINUM HYDROXIDE MIXTURES OF POLIOMYELITIS VIRUS

THE recrudescence of poliomyelitis in the United States and Europe during the past two or three years has led to a restudy of the disease from many points of view. This brief report deals with the experimental evidence that the virus of poliomyelitis, inactivated by adsorption on particles of aluminum hydroxide, is still capable of producing immunity when inoculated into *Macacus rhesus* monkeys. Previously several investigators had shown that a variety of viruses could be adsorbed and rendered ineffective by a number of colloidal and particulate chemical substances. No one seems, however, to have tested the inactivated materials for the production of artificial, active immunity.

The aluminum hydroxide employed was the type C suspension of Willstätter containing 22.5 grams of aluminum per liter. The virus was either Berkeley N filtrate of fresh monkey pooled virus,<sup>1</sup> or suspension of glycerolated material of the same strain. Mixtures of virus and suspension were allowed to stand 30 minutes at room temperature. The experiments carried out were of three types: simple observations on the inactivation of poliomyelitis virus by aluminum hydroxide; studies of the effect of the pH of the mixture on the inactivating power; and determinations of the value of the inactivated virus in the production of immunity. Intracerebral inoculations of the aluminum suspension alone were without pathological effect.

In respect to these three tests it was found first, that the filtrate and aluminum hydroxide mixed in equal volumes became inactive; second, that inactivation was promoted by acid (5.5) and prevented by alkaline (8.8) reactions; and third, that repeated subcutaneous injections of the inactivated virus led to active immunity.

<sup>1</sup> Rhoads, C. P., *Jour. Exper. Med.*, 49: 701, 1929.

The immunity thus induced was tested in three ways. First, glycerolated virus was repeatedly instilled into the nostrils. All the previously treated animals resisted infection, although the control developed typical poliomyelitis. The second test, carried out 28 days after the first, consisted of intracerebral inoculation of fresh virus. Of three treated animals so tested, one developed poliomyelitis, as did the control, and two resisted infection. The third test was made with the blood serum of the treated monkeys. Each of the three sera was tested separately and each neutralized the virus.

It may, therefore, be concluded that the virus, when adsorbed on aluminum hydroxide, is incapable of producing poliomyelitis, but still capable of inducing active immunity in *Macacus rhesus*. In a small series of animals thus immunized, no symptoms of experimental poliomyelitis arose, and in one only was the degree of immunity, although adequate to protect against nasal instillation, insufficient to protect against intracerebral injection of virus. That all three treated monkeys developed immunity is shown by the serum neutralization tests.

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### THE EFFECT OF TESTICLE EXTRACT AND NORMAL SERUM ON THE GROWTH OF A TRANSPLANTABLE EPITHELIAL TUMOR OF THE RABBIT<sup>1</sup>

EARLIER investigations in this laboratory<sup>2,3</sup> have shown that extracts of the testes considerably enhance

<sup>1</sup> From the laboratories of the Rockefeller Institute for Medical Research.

<sup>2</sup> F. Duran-Reynals, *Soc. Biol.*, 1928, 99, 6; *J. Exp. Med.*, 1929, 50, 327.

<sup>3</sup> F. Duran-Reynals and J. Suñer Pi, *Soc. Biol.*, 1928, 99, 1908.