time between the appearance of contraction in the two muscles is usually about twice the normal time. This is particularly true in the left arm, which was originally the more ataxic. The records of cerebellar rebound were apparently normal.

As a check upon the possible sources of these disturbances especially regarding our first case, we obtained records from a moderately advanced uncomplicated tabetic patient who had complete loss of tendon pain, a much reduced muscle pain, and absent patellar reflexes. On this subject we obtained records from the left arm and leg. These records were not different from normal in any of the above points. This seems to eliminate the possibility that proprioceptors are determinants of activity of the antagonist in a simple reaction.

Though 2 cases of cerebellar disease do not constitute a satisfactory series, it is striking that they deviate in a similar way from the normal in recordings of simple reactions. Thus, instead of the nearly simultaneous contraction of agonist and antagonist which we have observed in normals, the cerebellar cases show a suppression of potentials in the antagonist. A tendency to increased reaction time is also notable. A generalization from these observations as to their applicability for the diagnosis of cerebellar involvement must obviously await the accumulation of more data.

8793 P

Rapid Procedure for the Quantitative Estimation of Antitrypsin.

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Gates,[1] Gilman and Cowgill,[2] and Pickford and Dorris,[3] have found that photographic film furnishes a useful substratum for the titration of proteolytic enzymes, since the gelatin layer, which is readily digested by pepsin or trypsin, is sufficiently uniform in thickness and consistency to permit quantitative tests. The release

1 Gates, F. L., PROC. SOC. EXP. BIOL. AND MED., 1926-27, **24**, 936.

2 Gilman, A., and Cowgill, G. R., *J. Biol. Chem.*, 1930, **88**, 743.

3 Pickford, G. E., and Dorris, F., *Science*, 1934, **80**, 317.

of the silver from an exposed (opaque) film, through the progressive proteolysis of the gelatin, causes a proportionate decrease in density, and finally, when digestion is complete, the film becomes entirely transparent.

We have utilized this principle in devising a new technique for the quantitative titration of the antitryptic power of serum or other fluids. At the same time, we have modified and simplified the procedures followed by the authors mentioned. We have avoided the use of the colorimeter for estimating changes in the density of the film, as advocated by Gilman and Cowgill, and instead take for the end-point the film showing complete transparency to the naked eye, thus measuring digestion of the gelatin only, without reference to an arbitrary standard of initial density. After determining by experiment the influence of various factors on the reaction, we are able to outline a definite procedure, which permits the exact reproduction of results at will, and the expression of the findings in simple figures. Further, we have provided for the preservation of the films themselves as a permanent record.

The following is the procedure now recommended.

Eastman Process film is fully exposed to light, and developed for 5 minutes in D-72 developer. It is then cleared and fixed in freshly prepared hypo solution, containing the usual proportion of Fl—a hardener, for 15 minutes. After thorough washing and drying, the film is cut into discs ¼ inch in diameter with a hand punch.

A stock 2.0% solution of Fairchild's trypsin is made in a borax-boric acid, salt, buffer solution, pH 7.3, prepared according to the directions of Palitzsch, as given by Clark.[4] One gm. of the powdered trypsin is stirred into 50 cc. of the buffer solution, and the mixture allowed to stand at room temperature, with frequent shaking of the flask, for 15 minutes. It is then filtered into a sterile, screw-top bottle, and 0.1 cc. of a 1:1000 aqueous solution of merthiolate is added. Kept in the refrigerator, this stock solution loses potency gradually, but remains sufficiently active for at least 10 days.

Human serum to be tested for antitryptic power is diluted with 4 parts of the buffer solution just before use.

A series of dilutions of the stock trypsin solution is prepared by pipetting into test tubes amounts of this solution, beginning with 0.1 cc., and increasing each time by 0.1 cc., and then adding sufficient buffer solution to bring the total volume in each tube to 2.0 cc. A

[4] Clark, W. M., *The Determination of Hydrogen Ions*, Williams and Wilkins Co., Baltimore, 1923, pp. 115, 117.

range of 8 or 10 dilutions is usually enough to give convenient end-points.

Using separate, cotton-plugged pipettes, equal quantities (0.3 cc.) of each dilution and of buffer solution (in titration of the trypsin alone), or of diluted serum (in titration of antitrypsin), are added to a 2nd series of small test tubes, and the fluids thoroughly mixed. A control tube is prepared, containing 0.6 cc. of buffer solution only.

These tubes are now placed in a water bath at 40°C. for 10 minutes, solely for the purpose of warming the test fluids. The contents of each tube are then poured quickly into separate concavities of a Coors glazed porcelain plate, previously warmed for at least 15 minutes in a 40°C. incubator. A single film disc, handled with forceps, is now dropped, gelatin side down, upon the surface of each mixture. The plate is returned at once to the 40°C. incubator, the atmosphere of which is kept saturated with moisture, and digestion is allowed to proceed for 15 minutes.

The plate is then removed, and, as rapidly as possible, each film disc is picked up with forceps, rinsed briefly in 4% formalin (to stop the reaction and fix the film), and deposited, gelatin side up, upon filter paper to drain. The discs are now mounted upon a strip of transparent, gummed cellulose tape stretched across an opening prepared in a 4 by 6 inch card. As soon as the films have dried, a 2nd strip of the tape is placed over them, sealing them between the gummed surfaces. The card, which may now be freely handled, bears all data necessary to describe the experiment and may conveniently be filed as a permanent record.

When viewed against the light the first of the film discs in the series to be fully cleared is apparent to the naked eye. This is taken as the end-point, and the titer of tryptic activity is expressed directly in terms of milligrams of trypsin per cc. in this mixture, containing the lowest concentration of trypsin, which has brought about complete digestion. Thus, a fresh 2.0% trypsin solution will almost invariably have a titer of 1.0, *i. e.,* complete digestion will first occur in the mixture made by diluting 0.2 cc. of this solution up to 2.0 cc. and further diluting, for the test, with an equal volume of buffer solution, so that the final concentration of trypsin per cc. in this mixture is 1.0 mg.

The difference between the titer in the presence of a diluted serum and the titer in the absence of serum represents the antitryptic power of that serum. This figure, multiplied by the serum dilution, is assumed to express the *antitryptic index* of the undiluted serum.

In preliminary titrations, the sera from 50 normal women showed

a mean antitryptic index of approximately 7.5, whereas the mean value for the sera of 15 pregnant women was found to be approximately 10.0. This is in accord with the findings of others, who have observed a rise in antitrypsin during pregnancy.[5]

8794 P

Pregnancy in Cholesterol Fed Rats.

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The disturbances in blood cholesterol level known to be associated with pregnancy, and the prevailing impression that pregnancy may increase the incidence of diseases associated with abnormal cholesterol deposition have indicated the desirability for study of the influence of cholesterol feeding during pregnancy.

There is strikingly little record of such a study in the literature. Schönheimer[1] reported that pregnant animals were more subject to the deposition of anisotropic fat than non-pregnant ones. He reports feeding cholesterol to a rabbit which became very sick 14 days after mating and showed resorption of 3 fetuses with one fetus dead at term and abnormal deposition of anisotropic fat in the placenta.

We have used rats for the present study. These were placed at weaning on a diet made up of 20 parts raw casein, 4 parts Osborne and Mendel salts, 4 parts agar, 15 parts Crisco, and 57 parts starch, with one part cholesterol dissolved in Crisco and incorporated in the diet. Vitamin supplements were given separately 3 times a week as yeast, tiki-tiki, or yeast extract for B, and tuna or sea bass liver oil standardized in this laboratory and diluted with corn oil for A and D. With tiki-tiki, raw casein supplied G. After successful mating was demonstrated by the finding of sperm, the protein in the diet was increased to 26% and the starch decreased to 51% and the vitamin B increased 2 to 4 times (in different groups). This diet was modeled on that shown by Morgan and Simson[2] in this laboratory to be adequate to meet the food requirements of the rat during pregnancy.

5 Flexner, L. B., Berkson, J., Winters, H., and Wolman, I., PROC. SOC. EXP. BIOL. AND MED., 1928-29, **26**, 592.

* Deceased, November 16, 1935.

1 Schönheimer, R., *Arch. Path. Anat.* (Virchow), 1924, **249**, 1.

2 Thesis: Catherine M. Cave Simson with A. F. Morgan, Department of Household Science, University of California, Berkeley, 1934.