a distinct retarding effect on the growth of both sarcoma 180 and adenocarcinoma C3H-BA. This effect is apparently dependent upon the amount administered, little retardation being noted when the diets containing 0.015% was fed. Methyl testosterone, likewise, retarded the growth of these tumors when 100 mg/kg daily was given by the oral route. Growth of adenocarcinoma EO-771 and sarcoma 37 was not greatly retarded by methyl androstenediol given in concentrations which markedly impaired the growth of the other tumors.

The retarding effect of the compounds on the tumors is confined essentially to the first two weeks of treatment; after this time, growth proceeds at a rapid rate in spite of continued treatment. When implantations were allowed to grow for 5 days before oral treatment with methyl androstenediol was begun, little or no inhibition of growth was seen.

No retardation in growth of sarcoma 180 has occurred in repeated experiments in which either methyl androstenediol or methyl testosterone has been administered *subcutaneously* in 100 mg/kg daily doses for 13 days.

Summary. The growth of sarcoma 180 or adenocarcinoma C3H-BA is markedly retarded in mice by *oral* administration of methyl androstenediol or methyl testosterone in doses approximating 100 mg/kg daily, when treatment is started the day following implantation. Inhibition of sarcoma 37 and adenocarcinoma EO-771, under similar conditions, is slight and of short duration. No retardation of growth of sarcoma 180 was noted when 100 mg/kg doses of the compounds were administered daily by *subcutaneous* injection.

Received November 17, 1950. P.S.E.B.M., 1950, v75.

Use of Bromsulphalein for the Measurement of Proteolytic Activity. (18353)

ROGER L. GREIF. (Introduced by R. M. Archibald.)

From the Hospital of the Rockefeller Institute for Medical Research, New York City.

Disodium phenoltetrabromophthalein disulfonate (Bromsulphalein), widely used as a test of hepatic function(1), has been found to be an enzyme inhibitor(2). In the study of this property, it was noted that protein could be precipitated completely from an acid solution by addition of this indicator, and that the color intensity of an alkaline solution of the precipitate was proportional to the amount of protein present. Carroll(3) by reporting recently the effect of pepsin on the binding of bovine albumin with the dye orange I, suggested the present investigation.

Experimental. 1. *Relation of protein concentration to amount of Bromsulphalein in precipitate.* Casein prepared according to Hammarsten(4) (Kahlbaum) was dissolved in aqueous 0.1% Na_2CO_3 solution so that 1 ml contained the amount of protein shown on the abscissa of Fig. 1. In 9.8 x 1.2 cm test tubes, 1 ml of casein solution and 1 ml of distilled water were mixed with 2 ml of indicator solution made by adding 3 ml of 5% aqueous Bromsulphalein (Hynson, Westcott, and Dunning) to 197 ml pH 2.5 citrate-phosphate buffer (McIlvaine)(5). The tubes were centrifuged at 2500 rpm for 10 minutes, inverted, and allowed to drain for several minutes, after which the inner walls of the tubes were wiped dry with absorbent cotton on an applicator stick. The precipitates at

1. Rosenthal, S. M., and White, E. C., *J. Pharm. Exp. Therap.*, 1924-25, v24, 265.

2. Archibald, R. M., *J. Biol. Chem.*, 1944, v154, 657.

3. Carroll, B., *Science*, 1950, v111, 387.

4. Hammarsten, O., *Act. Reg. Soc. Scient., Upsaliensis*, 1877, vX.

5. McIlvaine, T. C., *J. Biol. Chem.*, 1921, v49, 183.

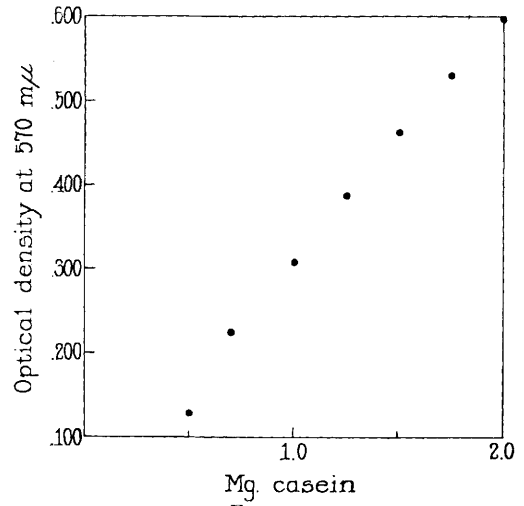

FIG. 1.
Relation of optical density of alkaline solution of precipitate to amount of casein.

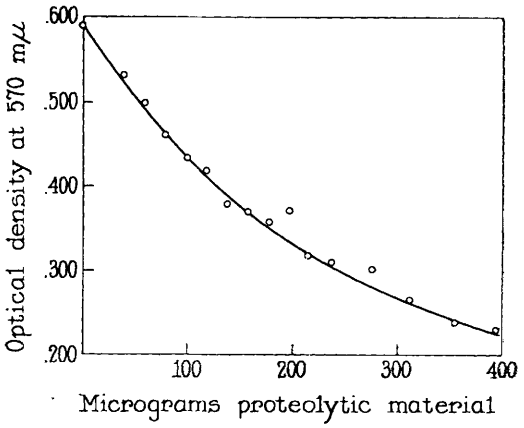

FIG. 2.
Relation of optical density of solution of precipitate from 2 mg of casein to amount of proteolytic material.

the bottoms of the tubes were dissolved in 2 ml, 10% NaOH, transferred quantitatively to a graduated cylinder, and diluted to 100 ml with distilled water. After thorough mixing, a portion of the solution was transferred to a 15 x 1.6 cm cuvette, and the optical density was measured at a wavelength of 570 mμ in the Coleman, Jr. Spectrophotometer (Model 6A). Fig. 1 shows the optical density obtained by following the above procedure with concentrations of casein between 0.50 and 2.0 mg.

2. *Measurement of proteolytic activity.* 50 mg of a commercially available proteolytic enzyme preparation (Trypsin "pure," Amend) were suspended in 100 ml of distilled water, and allowed to stand at room temperature (26-28° C) for 2 hours with occasional shaking. The resulting opalescent solution was filtered through washed cotton to remove particulate matter, and a portion evaporated, then dried at 120° C to obtain the dry weight. The filtrate was found to contain 394 μg per ml. Water dilutions of the remaining solution were made to contain in 1 ml, the weights indicated on the abscissa of Fig. 2. To 1 ml of each dilution was added 1 ml of solution containing 2 mg casein in 0.1% Na_2CO_3. Following incubation for 30 minutes at 37° C the reaction was stopped, and the remaining unchanged protein precipitated, by the addition of 2 ml of Bromsulphalein solution. The remainder of the procedure was carried out as in Section 1.

Fig. 2 shows a typical curve relating optical density of a solution of remaining unchanged protein to dry weight of proteolytic preparation. The amount of Bromsulphalein precipitated by the proteolytic preparation alone was in this instance negligible.

Comment. In addition to casein, all the protein preparations tested, including human serum albumin, crystalline bovine plasma albumin (Armour), ovalbumin, hemoglobin, bovine gamma globulin (Armour), normal human serum, and lysozyme also precipitate with Bromsulphalein from acid solution. In the case of gelatin, high speed centrifugation may be used to pack a finely divided precipitate. Curves similar to Fig. 2 can be obtained with solutions of crystalline pepsin, commercial papain, and ficin preparations, and the proteolytic reaction may be carried out at the optimum pH for the enzyme tested provided the precipitation is conducted at or near pH 2.5. It is also possible to detect as little as 10 μg of protein, measuring by difference, the amount of color removed from a known quantity of Bromsulphalein. It should be noted that the concentration of Bromsulphalein must be carefully adjusted in these experiments, as large excesses of this indicator may result in incomplete precipitation of protein.

Summary. From acid solutions, Brom-

sulphalein is precipitated in amounts proportional to the protein present. This reaction has been used to measure proteolytic activity, and could be adapted to the quantitative determination of minute amounts of protein.

Received November 17, 1950. P.S.E B.M., 1950, v75.

Effect of Eserine and Atropine on ACTH Release. (18354)

FRANCO DORDONI* AND CLAUDE FORTIER.† (Introduced by Hans Selye.)
From the Institut de Médecine et de Chirurgie Expérimentales, Université de Montréal.

Recent observations suggest that hypothalamic centers might take part in the control of the corticotrophic function of the hypophysis, through the release into the pituitary portal system of an unknown stimulating agent(1,2). Adrenalin and histamine have been so far investigated in this respect with negative results; adrenolytic(2-4) as well as antihistaminic(5) agents failing to prevent a stress-induced release of ACTH. The aim of the following experiments was to study the role of acetylcholine in the regulation of ACTH release. Using Sayers' test(6) as an index of corticotrophic function, we have measured, in the rat, the adrenal ascorbic acid response to the independent and simultaneous administration of a parasympathomimetic drug (eserine) and an anticholinergic agent (atropine).

One hundred twenty male piebald rats of an average weight of 130 g were subdivided into groups of 8 animals. Eserine salicylate was subcutaneously injected at a dose level of 0.03 mg/100 g, atropine sulphate at a dose of 20 mg/100 g. The animals, previously fasted for 24 hours, were killed by bleeding one hour after the injection and the adrenal ascorbic acid determined according to Roe and Kuether's method.

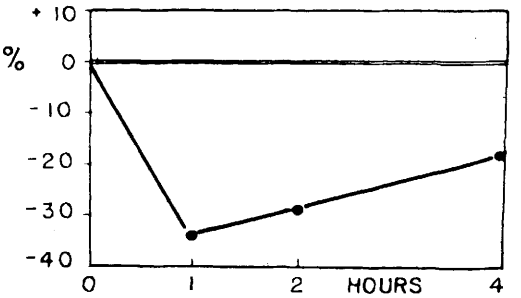

FIG. 1.
Effect of eserine salicylate (0.03 mg/100 g) on adrenal ascorbic acid concentration.

A maximal ascorbic acid depletion (Fig. 1) was observed one hour after the administration of eserine; it was followed in the next hours by a progressive return to normal level.

Atropine (Table I), far from preventing the eserine-induced depletion of adrenal ascorbic acid, increased it significantly and was by itself responsible of an intense discharge.

In order to eliminate the possibility of a direct adrenal ascorbic acid depleting effect of atropine, eventuality suggested by di Mattei's(8) and Frommel's(9) observations on the devitaminizing action of certain drugs, we studied in a final experiment the adrenal ascorbic acid response to atropine, 48 hours after hypophysectomy.

A complete inhibition (Table II) of the

* Canada-UNESCO Research Fellow.

† This work was done during the tenure of an American Heart Association Research Fellowship.

1. Harris, G. W., and De Groot, J., *Fed. Proc.*, 1950, v9, 57.

2. Fortier, C., *Proc. Canad. Physiol. Soc.*, 1950, v14, 13.

3. Seifter, J., Ehrich, W. H., Begany, A. J., and Hydyma, G. M., *Fed. Proc.*, 1949, v8, 331.

4. Ronzoni, E., and Reichlin, S., *Am. J. Physiol.*, 1950, v160, 490.

5. Fortier, C. and Guillemin, R., Unpublished data.

6. Sayers. M. A., Sayers, G., and Woodbury, L. A, *Endocrinology*, 1948, v42, 399.

7. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.

8. Di Mattei, P., *Questioni di Attualita Mediche E. chirurgiche*, 1949, v1, 1.

9. Frommel, E., Picquet, J., Quénot, C. L., et Loufti, M., *Helv. Physiol. Acta*, 1945, v3, 431.