worthy that this effect was prominent after sublethal doses of X-ray indicating that some metabolic function of the liver is markedly altered by X-irradiation. The present biochemical studies have thus revealed metabolic changes in the livers of irradiated animals which are apparently not accompanied by detectable pathological changes(15,16). The observation that normal female rats can accumulate citrate in the liver after fluoroacetate treatment in contrast to normal male rats suggests that this effect is regulated by the sex hormones. Citrate accumulation in the livers of X-irradiated male rats thus suggests that some of the acute effects as well as delayed effects may result from alteration of steroid hormones by X-ray.

Summary. The technic of Potter(5) for measuring the effects of toxic agents on citrate accumulation *in vivo* was applied to studies on X-irradiation. Lethal doses of X-ray (800 r) markedly inhibited citrate accumulation in spleen, thymus, ileum, pancreas and testes of fluoroacetate-treated rats but exerted no significant effect on brain and heart. Sublethal doses of X-ray markedly inhibited citrate accumulation in spleen and thymus. The extent of inhibition was dependent upon the dose of X-ray and was reversible after sublethal doses and irreversible when lethal doses were given. Accumulation of citrate occurred in the livers of irradiated rats following fluoroacetate treatment in contrast to the inability of livers of normal male rats to accumulate citrate following fluoroacetate treatment.

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Colorimetric Measurement of Serum Cholinesterase. (18512)

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Immense quantities of phosphorus-containing anticholinesterase agents are currently in use in this country as agricultural insecticides. The high toxicity of these materials to man has resulted in a number of cases of poisoning (1) including some fatalities among people inadvertently exposed during the manufacture and use of these compounds. Since some of these agents, especially parathion and octamethyl pyrophosphoramide (OMPA, Pestox III), may exert a cumulative effect(2-4), serious poisoning can occur in individuals who have been exposed to relatively small amounts of these materials repeatedly. Periodic measurements of the cholinesterase levels of the blood of workers handling the anticholinesterase agents provide the only known means of detecting exposure before the advent of symptoms. The Warburg manometric method is entirely suitable for these measurements, but the necessary special equipment and personnel trained in its use are often not available under the conditions of practical usage of these agents. A need has, therefore, arisen for a simple, accurate, and economical method for measuring the cholinesterase activity of the blood of persons exposed to anticholinesterase agents. In efforts to devise a suitable procedure we have found that the colorimetric esterase method of Gomori(5) can be adapted as an assay procedure for the pseudocholinesterase of serum. This procedure is not applicable to measurements of the true

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cholinesterase of red cells, but since inhibition of serum cholinesterase generally occurs sooner than inhibition of the enzyme activity of other tissues after exposure to anticholinesterase agents, values for serum may be used as an indication of exposure.

The present communication describes the colorimetric measurement of serum pseudocholinesterase and to illustrate the reliability of this procedure a comparison of values obtained by this method and the manometric technic is presented. The applicability of the assay is shown by data obtained on serum of patients under treatment for myasthenia gravis with octamethyl pyrophosphoramide.

Methods. Manometric assays were made according to the method of DuBois and Mangun(6). The colorimetric determinations of cholinesterase activity were made using the esterase method of Gomori(5) with minor modifications. The procedure as employed in our studies was carried out as follows:

Preparation of a standard curve. For this cholinesterase method phenyl benzoate is employed as the substrate and the amount of phenol liberated through hydrolysis by pseudocholinesterase is measured. A stock aqueous solution of phenol (reagent grade) containing 100 μg/ml was prepared. For the standard curve amounts from 2.5 to 25 μg of phenol were diluted to 6 ml with water in colorimeter test tubes. To each of the tubes 4 ml of a saturated borax solution was then added. The borax solution was prepared by adding 35 g of powdered $Na_2B_4O_7$ $10H_2O$ to 1 liter of 15% ethanol and allowing the mixture to dissolve over a 24-hour period. To each tube 0.5 ml of diazo reagent (prepared by dissolving 0.25 g of DuPont Naphthanil Diazo Red B salt in 100 ml of ice-cold water and filtering) was added. The samples were mixed and allowed to stand 10 minutes and were then read against a reagent blank in a Klett-Summerson photoelectric colorimeter with a No. 50 blue-green filter. A straight line relationship was obtained by plotting phenol concentrations against Klett readings.

Serum cholinesterase measurements. For colorimetric measurements of cholinesterase activity, 1 ml of serum was diluted to 100 ml with water and 1 ml of diluted serum (0.01 ml of serum) was placed in a colorimeter tube. Five ml of a buffered phenyl benzoate solution was then added. The buffer was prepared by dissolving 5.35 g of $Na_2HPO_4 \cdot 12H_2O$ and 7 g of $NaH_2PO_4 \cdot H_2O$ in 1 liter of water. Into 500 ml of this buffer 1 ml of stock substrate solution (2 g of phenyl benzoate in 100 ml of methanol) was added. After addition of the substrate to the serum samples they were incubated at 37°C for 1 hour. The samples were then placed in ice-cold water and the borax and dye reagent was added as described above. Blank samples were included in which the serum was added after the incubation at 37°C. The micrograms of phenol liberated per 0.01 ml of serum represented the serum cholinesterase activity after deducting the small blank values. The borax solution and the phosphate buffer will keep indefinitely. However, the buffered substrate and the dye reagent must be kept in a refrigerator and will then only keep about 2 weeks.

Results. To test the applicability of the colorimetric method for serum cholinesterase, determinations were made on the serum of 40 normal persons. Simultaneous manometric measurements were made on the same samples. By the colorimetric method the average cholinesterase activity expressed in μg of phenol liberated by 0.01 ml of serum/hour was 23 with individual values varying from 16 to 30. The average value by the manometric method was 36 expressed as μl of CO_2 liberated/5 min/0.1 ml of serum and individual values ranged from 27 to 46. The variation of in-

TABLE I. Comparison of Colorimetric and Manometric Methods for Measuring Inhibition of Serum Cholinesterase *in Vivo*.*

% of control activity			
Manometric method	Colorimetric method	Total mg of OMPA	Avg No. days of treatment
100	100	0	0
75	74	12.6	1.5
55	48	36.8	3.5
35	31	70.2	6.6

* Avg values for 5 patients under treatment with octamethyl pyrophosphoramide (OMPA) 8 to 12 mg/day.

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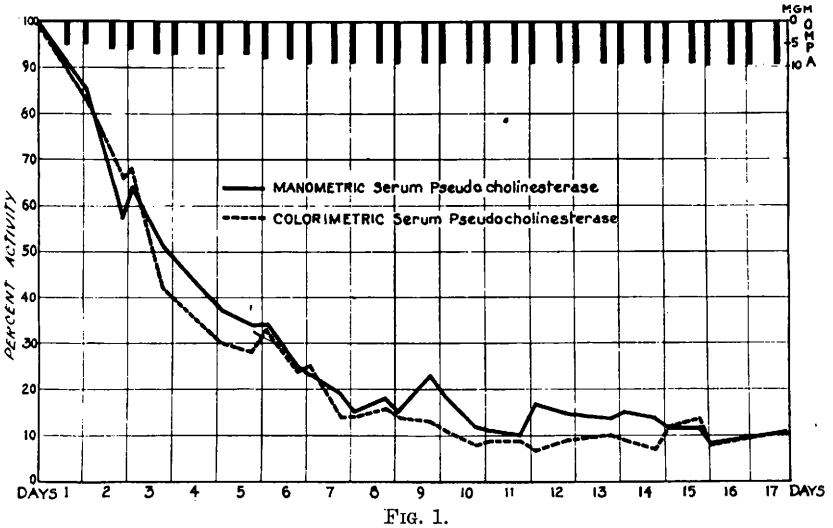

FIG. 1.
Depression of pseudocholinesterase by OMPA as determined manometrically and colorimetrically.

dividual samples from the average was similar with both methods. Thus, high or low cholinesterase activity among different individuals could be detected by either method.

In vitro experiments with neostigmine and di-isopropyl fluorophosphate (DFP) demonstrated that the inhibitory effect of the phenylcarbamic acid esters, as well as the organic phosphates on serum cholinesterase, can be measured by the colorimetric method. Thus, a final concentration of 7.5 x 10^{-7} M neostigmine produced 50% inhibition of serum cholinesterase by the colorimetric method, whereas 6 x 10^{-7} M neostigmine produced 50% inhibition using the manometric technic when the inhibitor was mixed with the serum before addition of the substrate. The final concentrations of DFP which produced 50% inhibition of serum cholinesterase were 6.3 x 10^{-8} M and 4.5 x 10^{-8} M by the manometric and colorimetric methods respectively.

The practical application of the colorimetric determination of cholinesterase activity was studied using serum from 5 patients with myasthenia gravis who were under treatment(4) with octamethyl pyrophosphoramide (OMPA). After measurements of the normal serum cholinesterase activity were made on each patient, oral administration of OMPA was begun and continued at an average dose of 8 to 12 mg/day in 2 divided doses. Serum cholinesterase was usually determined twice each day by the colorimetric and manometric methods. The data in Table I give a comparison of the average % depression of enzyme activity in the 5 patients as measured by both methods. It may be seen that there is a close correlation between the inhibition observed by the two methods.

A more detailed comparison of the daily depression of cholinesterase as measured by the colorimetric and manometric methods is shown in Fig. 1 which shows the decrease in enzyme activity following daily administration of OMPA to a patient with myasthenia gravis. Throughout the observation period there was good correlation between the results obtained by the two methods which indicates that the colorimetric procedure may be used as a reliable index of exposure to anticholinesterase agents.

Summary. The colorimetric esterase method of Gomori(5) was applied to studies on the cholinesterase activity of serum. The method was found to be applicable to studies on the action of anticholinesterase agents *in vitro*. It is also suitable for detecting exposure of humans to organic phosphorus-containing insecticides and for following serum cholinesterase levels during therapy with anticholinesterase drugs.

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