ing, humidity and temperature. It melts at 180°C with decomposition, but below that temperature it appears not to decompose spontaneously. It is soluble in cold water to about 1 or 2% and in hot water to about 10%. It is also soluble in alcohol.§

Summary. Thiosemicarbazide, a derivative of both thiourea and hydrazine, has proved to differ from other toxic thioureas previously studied. Instead of producing fatal pulmonary edema in a limited number of animal species and not harming others at equivalent dose levels, thiosemicarbazide caused convulsions and death within 1 to 3 hours in the 6 species tested, when given in amounts ranging from 10 to 30 mg/kg. In some individual laboratory rats, however, including those in which the convulsions had been suppressed by administration of a barbiturate, death was delayed and accompanied by the development of pulmonary edema. Thiosemicarbazide may have promise as a practical rodenticide, because of its general toxicity to rats of several species and because it appears to be readily accepted in lethal amounts when offered to rats in either water solution or bait.

It is a pleasure to acknowledge the kindness of Dr. Wayland G. Hayes of the Communicable Disease Center, U. S. Public Health Service, Savannah, Georgia, who provided the nucleus of our Alexandrine rat colony, and of Dr. R. O. Roblin, American Cyanamid Company, who has helped in many ways throughout the course of this study.

§ The analytical data on thiosemicarbazide given in this paragraph were very kindly provided by Dr. J. R. Vaughan of the American Cyanamid Company.

17036

Estimation of Dicumarol, 3, 3¹-Methylenebis (4-Hydroxycoumarin) in Biological Fluids.*

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Although the physiological activity of dicumarol has been assayed by its effect on the prothrombin time, no chemical method for its estimation has been available. A rapid chemical method would make possible a study of certain problems concerning dicumarol about which little is known. These are its physiological disposition in man, its relationship to vitamin K in the clotting mechanism, and its formation in spoiled sweet clover.

A chemical method is described below which involves isolation of the drug from the body by extraction into heptane. The drug is returned to alkali and measured spectrophotometrically at 315 mμ where the drug exhibits a pronounced peak (Fig. 1).

Reagents. 1. Standard solution of dicumarol 100 mg per liter. 100 mg of dicumarol are dissolved in 1 liter of 0.1 N NaOH. This solution is stable for at least one month when stored in the refrigerator.

2. 3 N HCl.

3. Heptane. (Paragon Testing Company). A technical grade of heptane is purified by successive washings with 1 N NaOH and 1 N HCl followed by 2 washings with water.

4. 2.5 N NaOH.

Procedure. Add 1 to 3 ml of plasma or urine (sample containing up to 50 γ of dicumarol) and 0.5 ml of 3 N HCl to 20 ml of heptane in a 60 ml glass-stoppered bottle. Adjust the aqueous volume to about 3.5 ml if necessary by the addition of water. Shake for 30 minutes on a shaking apparatus and

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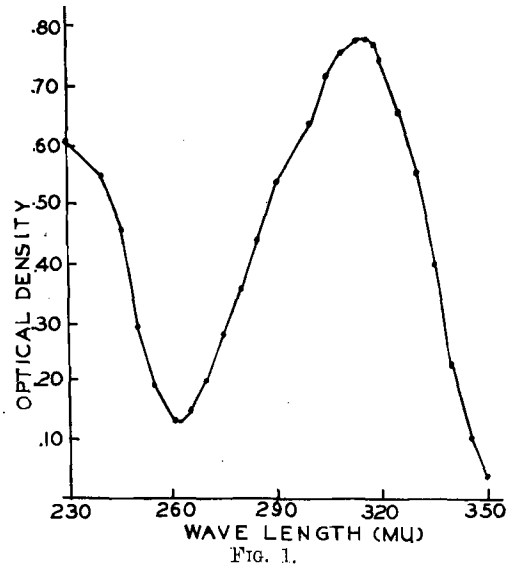

FIG. 1.

The absorption spectrum of dicumarol in 2.5 N NaOH. The concentration of the compound was 10 γ per ml. Cell thickness = 1 cm.

then centrifuge the bottle. Transfer 15 ml of the heptane phase to a 60 ml glass-stoppered bottle containing 4 ml of 2.5 N NaOH. Shake for 5 minutes. Transfer the contents to a test tube and centrifuge for 5 minutes. Remove the organic phase by aspiration with a fine-tipped pipette. Transfer about 3 ml of the aqueous phase to a quartz cuvette and determine the optical density in a spectrophotometer (Beckman) with the instrument set at the wave-length 315 mμ. A reagent blank with water substituted for plasma is run through the same procedure. This blank is used for the zero setting and should have an optical density not greater than 0.005 when 2.5 N NaOH is used for the zero setting.

Standards. The distribution of dicumarol in a heptane-acidified water system is such that at room temperature with volumes of 20 and 3.5 ml respectively, about 95% of dicumarol is in the organic phase. Standards are prepared by handling known amounts of dicumarol as in the procedure described above. The optical densities were found to be proportional to concentration. An optical density of about 0.130 is obtained in a Beckman spectrophotometer when 10 γ of dicumarol are run through the above procedure.

Results. Recoveries of dicumarol added to plasma in amounts of 5 to 50 γ were quite satisfactory (100 $\pm$ 3%). The sensitivity is more than adequate for the plasma levels which are obtained after therapeutic doses of the drug.

Assay of Specificity. There is a negligible amount of material in normal plasma and urine which assays as dicumarol in the analytical procedure described above. The possible interference by metabolic products of the drug was examined by a distribution technic previously described by one of us.[1,2] It involves a comparison of the distribution of the substance extracted from plasma with that of the authentic substance in a two-phase system consisting of an organic solvent and water at various pH values. Dissimilar distributions indicate the presence of a substance different from the authentic compound. To escape detection a transformation product would have to have not only a similar dissociation constant but identical solubility characteristics in two solvents.

The examination in the case of dicumarol was made with heptane extracts of the pooled plasma of 2 subjects who had received the drug. The plasma was obtained 23 hours after the oral administration of a 500 mg

TABLE I.

Distribution of Dicumarol and Apparent Dicumarol Between Heptane and Water at Various pH Values.

The apparent dicumarol was obtained by extraction with heptane of the acidified plasma of 2 subjects who had received dicumarol. The compound was returned to dilute alkali. Aliquots of this solution and of an authentic dicumarol solution were adjusted to various pH values and shaken with 2 volumes of heptane. The fraction of the compound extracted at various pH values is expressed as the ratio of the amount of compound in the organic phase to total compound.

pH	Authentic dicumarol	Apparent dicumarol from plasma
1	.95	.94
6	.93	.91
6.5	.90	.87
7.0	.81	.80
7.5	.53	.52
8.0	.36	.35

1 Brodie, B. B., and Udenfriend, S., *J. Biol. Chem.*, 1945, **158**, 705.

2 Brodie, B. B., Udenfriend, S., and Baer, J. E., *J. Biol. Chem.*, 1947, **168**, 299.

dose. The distributions of dicumarol between heptane and water at various pH values were compared with those of the apparent compound extracted from plasma. The results showed that within experimental error the apparent and authentic compound had the same solubility characteristics and were, therefore, presumably the same compound (Table I).

Basic organic drugs do not interfere in the procedure for dicumarol since they are not extracted at an acid pH. The following acidic or neutral drugs were tested for their interference in the procedure for dicumarol: acetanilide, phenacetin, antipyrine, phenobarbital, sulfanilamide, sulfadiazine, sulfathiazole, penicillin, vitamin K, nembutal, pentothal, and salicylic acid. Only pentothal and salicylates interfered in the procedure and consequently these substances should not be present when analyses for dicumarol are being made.

Summary. A simple and sensitive spectrophotometric method for the estimation of dicumarol in plasma and urine is described. Dicumarol is isolated from the biological material by extraction into heptane. The drug is returned to alkali and measured spectrophotometrically at 315 mμ. The method is specific in that it does not include metabolic products of the drug.

17037 P

Seasonal Variations in the Choline Content of Human Serum.

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In the literature there are but few reports of studies on serum choline content (Guggenheim and Loffler,[1] Sieburg and Patzschke,[2] Luecke and Pearson,[3] and Schlegel[4]).

It appears from these studies that the concentration of choline in human serum ranges from about 0.2 to 2 mg %. As far as has been ascertained, no records are available of studies presenting evidence of seasonal variations in serum choline content.

The present study comprises 142 duplicate determinations of serums obtained from both men and women in the period from May 1, 1947 to May 1, 1948. Determinations of choline were made according to the method of Abdon and Ljungdahl-Ostberg[5] by converting choline into acetylcholine, which physiologically is about 100,000 times as active as choline. Its action was determined

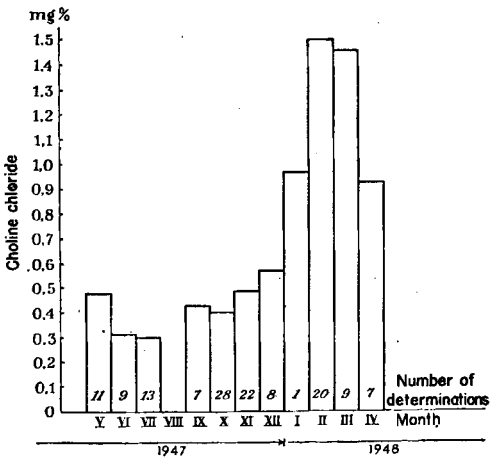

FIG. 1.

The average serum choline content during the different months of the year.

[1] Guggenheim, M., and Loffler, W., *Biochem. Z.*, 1916, **74**, 303.

[2] Sieburg and Patzschke, *Z. d. ges. exp. Med.*, 1923, **36**, 324.

[3] Luecke, R. W., and Pearson, P. B., *J. Biol. Chem.*, 1944, **153**, 259.

[4] Schlegel, J. U., Variationer i Serumcholinindholdet hos Mennesker, Copenhagen 1948 (Thesis).

[5] Abdon, N. O., and Ljungdahl-Ostberg, K., *Acta Physiol. Scandinav.*, 1944, **8**, 103.