endometrium. There were 22 cycles of doubtful interpretation, by which we mean that 10 or more days elapsed between the date of the biopsy and the onset of the next period of bleeding. Ovulation could, therefore, potentially occur between the date of biopsy and the onset of the flow. Forty-two to 63% of the lactating women who bleed at fairly regular intervals have anovulatory or sterile cycles.

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Crystallization of Liver Fraction Protecting Against Necrosis from Carbon Tetrachloride or Chloroform Administration.

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We have reported[1, 2] on the use of a liver preparation in the prevention of liver necrosis from carbon tetrachloride or chloroform administration. At this time we wish to report a method for the preparation of this active principle in a crystalline form.

A concentrated aqueous extract of hog liver representing approximately 10 gm. per cc., from which the heat coagulable materials have been removed, is warmed in a water bath to a temperature of approximately 60°C. To each 1000 cc. of this solution 2400 cc. of ethyl alcohol of about the same temperature is added with stirring. The precipitate which forms is filtered off after cooling and 2000 cc. of a saturated aqueous solution of ammonium sulfate added to the filtrate. The solution is then thoroughly shaken. On standing it separates into 2 layers: above, an alcoholic layer and below, a watery layer containing a great deal of precipitated ammonium sulfate. The upper layer is syphoned off and 1500 cc. of alcohol added to it to precipitate excess ammonium sulfate. The solution is filtered after being cooled in a refrigerator for several hours. The filtrate is then evaporated under reduced pressure to approximately 170 cc. It is then placed in a refrigerator and cooled over night. The precipitate is separated by centrifuging and washed in the centrifuge tubes, first with about 60 cc. of ice cold water and then

[1] Forbes, J. C., and Neale, R. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 319.

[2] Forbes, J. C., Neale, R. C., and Scherer, J. H., *J. Pharm. and Exp. Therap.*, 1936, **58**, 402.

with approximately 40 cc. of cold alcohol. The residue is suspended in 40 to 60 cc. water, depending upon the amount present, and concentrated sodium hydroxide added drop by drop with thorough mixing until needle-like crystals separate out or form when a drop of the solution is placed on a microscope slide and allowed to evaporate slightly. As a rule a satisfactory pH is approximately 9.3. After the correct amount of alkali is added the solution is set aside in a refrigerator for a number of hours and then filtered with suction. The crystalline material may be recrystallized from warm water in which it is soluble to the extent of about 30 mg. per cc. Hot water converts it slowly into an insoluble form.

The pure crystalline material is almost snow white. It gives a strong murexide test. It is precipitated by silver nitrate, picric acid, metaphosphoric acid, phenol and tricresol. Its chromogenic activity with Folin's uric acid reagent is approximately 1/350 that of uric acid. The nitrogen content of a sample recrystallized from water several times and dried at 125°C. for 24 hours was found to be 27.42%. These properties indicate that the substance is a purine derivative but apparently different from any previously reported.

The crystalline material when injected subcutaneously in doses of 100 mg. per 100 gm. of rat weight affords excellent protection against carbon tetrachloride or chloroform poisoning. Owing to its relatively low solubility in water we usually administer it, partly in solution and partly in suspension in concentrations of 50 mg. per cc. Also, in order to afford ample time for absorption, the animals are protected as a rule 18 to 24 hours prior to the time of acute poisoning. This crystalline material has also been used quite successfully in the prevention of cirrhosis from chronic carbon tetrachloride poisoning.

The nitrogen value was kindly supplied by Dr. R. C. Neale, Biochemical Research Foundation, Philadelphia, who is studying the chemical structure of the material. Frozen livers were used altogether in this investigation.