action of this hormone on ovarian weight. It shows, furthermore, that the unusually marked responsiveness of the ovary to A.P.L. which we observed during the course of pregnancy is not lost at parturition, and is still demonstrable during the first days post-partum if nursing is not allowed. A study of the uteri of these animals confirmed the findings reported above.

Summary. 1. Both the gonadotropic hormone of pregnancy urine and that prepared from pituitary tissue lead to a more marked ovarian response in the pregnant than in the non-pregnant rat. This increased responsiveness to gonadotropic hormones continues for some time post-partum if nursing is not allowed. 2. In hypophysectomized rats urinary preparations lead only to thecal luteinization, even when given during gestation. 3. Similarly in lactating and pregnant rats, the urinary preparation leads to thecal luteinization, but granulosa luteinization also occurs at the same time. Pituitary preparations do not lead to thecal luteinization during lactation. 4. Neither the urinary nor the pituitary preparation is able to produce the usual uterine reaction when given during lactation.

7752 P

A Crystalline Iron Chloride Molecular Compound of Urobilin and Stercobilin.*

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Evidence was presented previously[1] for the identity of crystalline urobilin and stercobilin as isolated from human urine[2] and feces,[3] respectively. These crystalline substances unfortunately do not have sharp melting points, nor is the "urobilin" absorption spectrum specific,[4] hence it was desirable to provide further means of positive identification, particularly for the study of substances which may be

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1 Watson, C. J., *Z. Physiol. Chem.*, 1933, **221**, 145.

2 Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1207.

3 Watson, C. J., *Z. Physiol. Chem.*, 1932, **208**, 101.

4 Fischer, H., *Ueber Blut, Blatt, u. Gallenfarbstoff. Oppenheimers Handb. d. Biochem. des Menschen u. d. Tiere. II Auflage. Ergänzungsband.* G. Fisher, Jena, 1930.

isolated subsequently. In this regard, Heilmeyer and Krebs[5] have recently mentioned certain differences in a stercobilin which they isolated by means of the procedure described by the writer. This question will be discussed in a later publication.

It has long been known that urobilin is capable of forming a number of metallic compounds. Heretofore, none of these have been isolated. A crystalline iron chloride molecular compound of mesobilirubin was described by H. Fischer, Baumgartner and Hess.[6] The corresponding compound of mesobilirubinogen is amorphous.[7]

In the present investigation it has been found that both urobilin and stercobilin yield a beautifully crystalline molecular compound with iron chloride. This crystallizes out of hot 25% hydrochloric acid in a yield of approximately 75%. Crystallization is best brought about by adding a hot, concentrated solution of $FeCl_3$ in 25% HCl, drop by drop to a tube containing the crystalline urobilin or stercobilin dissolved in hot 25% HCl, the reaction being carried out in a hot water bath with constant stirring. Immediately upon the addition of the $FeCl_3$ solution, the molecular compound comes out in an amorphous condition, but dissolves on stirring and further heating. Crystallization often commences while the solution is still hot, the crystals becoming more plentiful on cooling. They possess a glittering red-brown color. The individual crystals are tabular in habit, having a characteristic parallelopiped shape and an orange red color. The crystals obtained from the hydrochloride of urine urobilin are identical with those of stercobilin hydrochloride from the feces.

The substance is readily soluble in water, undergoing dissociation. After extraction of this water solution with chloroform, crystalline stercobilin hydrochloride may be isolated readily, indicating that the iron chloride has caused no change in the stercobilin molecule. Crystals which are allowed to stand in the air in a drop of the mother liquor eventually deliquesce. This is undoubtedly due to dissociation in the water remaining after the hydrochloric acid has in part volatilized. The molecular compound in alcoholic zinc acetate solution exhibits an intense green fluorescence. It is possible, however, that dissociation occurs in alcohol as well as water.

The preparation of this new iron chloride compound provides an added method of identification of crystalline urobilin or stercobilin. Since it is a method which is at once simple, and adaptable to as little as 2 mg. of material, it should be carried out wherever the nature of the substance isolated is at all doubtful.

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[5] Heilmeyer, L., and Krebs, W., *Z. Physiol. Chem.*, 1934, **228**, 33.

[6] Fischer, H., Baumgartner, H., and Hess, R., *Z. Physiol. Chem.*, 1932, **206**, 201.

[7] Fischer, H., and Niemann, G., *Z. Physiol. Chem.*, 1924, **138**, 293.

Elementary analyses as well as crystallographic measurements will be reported in detail in a later publication.

7753 C

Acute Agranulocytosis of Kala-Azar: Negative Effect of Urea Stibamine and Neostibosan on Blood of Normal Rabbits.

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Acute agranulocytosis, an important complication of kala-azar, has been observed in 8 of 71 patients suffering from visceral leishmaniasis.[1, 2] Six of these 8 patients exhibited a sudden and marked drop in the granulocytes at some time during treatment for kala-azar with either urea stibamine or neostibosan. Both drugs contain the benzene ring in their complex molecule. Acute agranulocytosis following the administration of certain compounds containing the benzene ring has been reported.[3] The following experiment was made in order to observe the effect of intravenous injection of urea stibamine and neostibosan on the blood of normal rabbits.

Eight normal rabbits were taken from the stock without selection. After a control period during which regular blood counts were made, 3 of the rabbits were given intravenous injections of urea stibamine, 3 others were given neostibosan and the remaining 2 were held as controls. An appropriate amount of either drug was dissolved in sufficient volume of double distilled water to make a 5% solution. The injection was given daily, except Sundays, through the marginal vein of the ear. Since urea stibamine is believed to be more toxic than neostibosan, the former was given in smaller individual doses. The total amounts were the same in all except in one (rabbit 1) in which it was double that of the others. Nothing was given to the control animals.

Counts were made on the blood obtained from the tributaries of the marginal vein of the opposite ear. These counts were done twice weekly and they included hemoglobin estimation, enumeration of total red and white blood cells and differential count of the leucocytes by the supravital method.

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[1] Zia, L. S., and Forkner, C. E., *Am. J. Med. Sci.*, in press.

[2] Zia, L. S., and Forkner, C. E., Trans. F. E. A. T. M. Ninth Congress, Nanking, China, 1934, in press.

[3] Madison, F. W., and Squier, T. L., *J. Am. Med. Assn.*, 1934, **102**, 1213.