Laboratory of this university. The powder method was used, employing copper Kα radiation with an exposure time of 18 hours and a plate distance of 6 cm. The diffraction pattern obtained is similar to those described in studies of the x-ray diffraction patterns of crystalline proteins. At least 4 sharp diffraction lines are visible: two of strong intensity at 88.5 A° and 43 A°, and two of weaker intensity at 39.3 A° and 29.8 A°. Although the x-ray diffraction pattern is typically that of a crystalline protein, it is recognized that the possibility of the presence of some amorphous material is not excluded on the basis of the x-ray study. Furthermore, the recent observations of Bernal and Fankuchen[5] may contribute some doubt to conclusions regarding the crystalline nature of a protein, based entirely on x-ray diffraction pattern studies.

The crystalline preparations behave like a protein in their color and precipitation reactions. Positive reactions are obtained with the biuret and xanthoproteic tests. The Millon's and the Hopkins-Cole reactions are also positive, as is the labile sulfur test. It has been reported[6] that purified prolactin preparations do not give the Millon's reaction, the xanthoproteic test or the labile sulfur test. This negative labile sulfur finding is particularly difficult to interpret in view of the fact that the same investigator reported cystine to be present in rather large amount (3.5 per cent.). The crystalline preparations are quite hygroscopic, and for micro-analysis were dried in the Pregl micro desiccator in partial vacuum in a slow stream of dry air. The following elemental composition was obtained:[7] *Carbon,* 51.11 per cent.; *hydrogen,* 6.76 per cent.; *nitrogen,* 14.38 per cent.; *sulfur,* 1.77 per cent.

The material gave no appreciable ash on ignition. The qualitative test for phosphorus was negative. A recent publication has reported[8] the presence of the latter element in purified prolactin preparations.

It is not possible at the present time to state definitely whether the crystalline protein which has been obtained is identical with the lactogenic hormone of the anterior pituitary gland, even though the evidence at hand at present would seem to indicate this conclusion. In view of the highly active, non-crystalline preparations of other investigators,[1] the discrepancies existing with respect to certain of the qualitative tests[6, 8] and the interpretation placed by Bernal and Fankuchen[5] on x-ray diffraction pattern studies, it seems best to report the present findings for purposes of record rather than of deduction. Investigations are being continued to determine whether the crystalline protein exhibits any other type of physiological activity which has been attributed to anterior pituitary extracts. Preliminary studies of the purified, non-crystalline fractions demonstrate that this material, injected at a 4 mg level daily into hypophysectomized rats, does not stimulate growth.

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THE PROTECTIVE ACTION OF CERTAIN PURINES AGAINST LIVER NECROSIS PRODUCED BY CARBON TETRACHLORIDE AND CHLOROFORM

NUMEROUS investigators have reported the occurrence of liver necrosis following the administration of carbon tetrachloride or chloroform. Many experiments have been performed in an attempt to determine the effects of changes in dietary constituents upon the histopathologic liver changes resulting from the administration of these liver poisons. Recently, Forbes and Neale[1] and Forbes, Neale and Scherer[2] reported the preparation of a liquid fractional extract of hog livers which, when administered to albino rats prior to acute poisoning with chloroform or carbon tetrachloride, exerted a protective action against these drugs. This extract contained an unknown number of substances. The detoxicating activity of the solution was found not to be due to choline or glucose content. From their solution, Forbes and McConnell[3] succeeded in preparing a crystalline substance which, in 50 to 100 mg doses given subcutaneously to rats prior to carbon tetrachloride poisoning, protected the animals from liver necrosis.

In this laboratory qualitative analysis of the crystalline substance which protected the animals showed the major portion of the product to be of a purine nature. The purine substance was separated from the other substances present and purified. This procedure did not alter the protective activity. The results of quantitative analyses for carbon, hydrogen, nitrogen and sodium indicated the empirical formula, $C_5N_4H_3O_2Na$. Further analyses and qualitative tests indicated that this substance is mono-sodium-2,6-dioxy-purine, sodium xanthine.

Hence, sodium xanthine was next prepared from

5 J. D. Bernal and I. Fankuchen, *Nature,* 139: 923, 1937.

6 E. I. Evans, *Am. Jour. Physiol.,* 119: 303, 1937.

7 The micro-analyses were conducted by Mr. W. Saschek, of Columbia University, using the micro-Pregl procedures.

8 W. H. McShan and H. E. French, *Jour. Biol. Chem.,* 117: 111, 1937.

1 J. C. Forbes and R. C. Neale, *Proc. Soc. Exper. Biol. and Med.,* 34: 319, 1936.

2 J. C. Forbes, R. C. Neale and J. H. Scherer, *Jour. Phar. and Exper. Therap.,* 58: 402, 1936.

3 J. C. Forbes and Jeannette McConnell, *Proc. Soc. Exper. Biol. and Med.* In press.

commercial xanthine, purified in this laboratory and injected subcutaneously into rats in the same quantities as the crystalline preparation from liver. Rats receiving 100 mg of sodium xanthine 20 hours before acute carbon tetrachloride poisoning by deep anesthesia for one hour and forty minutes, suffered only a slight fatty infiltration of their livers with a very limited round cell infiltration around the central veins. Ninety per cent. of the control rats, subjected to the same poisoning, died within 48 hours following the anesthesia, and after the expiration of that time the surviving rats were killed. Microscopic examinations showed very nearly complete destruction of the liver in practically all the control animals. The protection with mono-sodium-2,6-dioxy-purine was the same as that found with the crystalline preparation from liver.

Sodium guanine, injected into rats in doses of from 50 to 100 mg, exerted the same protective action as did the sodium xanthine and the preparation from liver. Adenine sulfate given in equivalent doses protected the animals to a certain extent but seemed to be definitely toxic itself in the amounts injected. Further experiments using chloroform poisoning showed both sodium guanine and sodium xanthine to protect the livers of rats. Liver sections from animals protected with these substances and subjected to two hours of deep chloroform anesthesia showed no histological changes except for an infiltration of a few fibroblasts intercellularly. There was no fatty infiltration or degeneration. Sections of the livers of control animals exhibited typical central necrosis extending toward the periphery of the lobule involving 50 per cent. of the liver lobule. Many of the control animals died.

Other purine bases and derivatives, both natural and synthetic, are being tested for protective action against carbon tetrachloride, chloroform and other liver poisons. A more complete report will appear in the near future, giving complete chemical identification of the active crystalline substance from liver and showing the effects of purines on the toxicity of the more common poisons.

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CORTICO-ADRENAL AND NEURAL EFFECTS ON GONADOTROPIC ACTIVITY OF THE PITUITARY[1]

IN the rabbit and cat ovulation usually occurs only after mating and is dependent upon the secretion of the anterior hypophyseal gonadotropic hormones. The data recently published by Brooks[2] and by Haterius and Derbyshire[3] suggest that in the rabbit coitus stimulates the pituitary through nerves in the infundibular stalk. Previously, only one neural pathway to the cells of the anterior hypophysis was recognized, *viz.*, the cervical sympathetic fibers.

In 1935, Friedgood and Pincus[4] and Friedgood and Cannon[5] stimulated electrically the cervical sympathetics of normal estrous rabbits. Such stimulation resulted in ovum maturation in the majority of rabbits, but rarely did it result in ovulation, and then only of a limited number of ova. The simultaneous intravenous injection of adrenalin did not enhance the effect of these electrical stimuli. Quantitatively, therefore, the ovarian response was similar to that evoked by injections of subovulatory doses of FSH and LH. It was concluded that stimulation of the pituitary through its sympathetic innervation increased, to a limited extent only, the normal rate of secretion of its gonadotropic hormones. Collin and Hennequin[6] later reported that stimulation of these nerves resulted in marked cytological changes in the anterior pituitary.

In searching for another factor which might influence the rate of secretion of the gonadotropic pituitary hormones, the possibility of a humoral mechanism was considered. The cat was used as an experimental animal, instead of the rabbit, because its survival period after adrenalectomy is longer. Estrous cats go out of heat and their ovaries atrophy after bilateral adrenalectomy, even if the animals are maintained in apparent good health by daily administration of a potent preparation of cortin[7] containing the life-sustaining hormone. A preliminary unilateral adrenalectomy was therefore carried out on 12 anestrous cats. After objective evidence of the estrous state developed in these animals (4 to 12 weeks later), 9 of them were subjected to a second adrenalectomy from 15 to 55 minutes after mating. This procedure prevented the occurrence of ovulation in every instance. In the remaining 3 cats, adrenalectomy was delayed until 6 hours postcoitum. All 3 animals ovulated. These experiments indicate either that adrenalectomy within one hour after mating interferes with the usual response of the anterior hypophysis to the coital stimulus, or that the ovary is unable to respond normally to

[1] From the Department of Physiology in the Harvard Medical School, Boston. Aided by a grant from the National Research Council Committee for Research in Problems of Sex.

[2] C. McC. Brooks, *Proc. Am. Physiol. Soc.* In press.

[3] H. O. Haterius and A. J. Derbyshire, *ibid.* In press.

[4] H. B. Friedgood and G. Pincus, *Endocrinology*, 19: *Physiol.*, 116: 54, 1936.

[5] H. B. Friedgood and W. B. Cannon, *Am. Jour. Physiol.*, 116: 54, 1936.

[6] R. Collin and L. Hennequin, *Compt. rend. Soc. de biol.*, 121: 84, 1936.

[7] We are indebted to Dr. David Klein, of the Wilson Laboratories, who furnished the cortico-adrenal extract for these experiments.