Dr. W. H. Bradley, senior geologist, U. S. Geological Survey, likes the word "sapropol" previously suggested by H. Potomie in 1908.

Dr. Glover M. Allen, Cambridge, Mass., suggested "ilyonic food."

Dr. Denis L. Fox, Scripps Institution, La Jolla, Calif., likes "ilytrophic food"—from *ilytrophon* (mud food).

Dr. L. O. Shapolano, of Stanford University, would qualify "benthotic food" as littoral, sub-littoral, profundal and abysmal benthotic. Dr. Wm. Rienhoff, Sr., also suggested the necessity of qualifying the term benthotic according to depth.

Dr. Agnes De Sales, College of Mount St. Joseph on the Ohio, suggests "acropelotic"—*akros*, top; *pelos*, mud.

Dr. A. Willey, Mille Isles, Quebec, agrees with Dr. Glover M. Allen—"ilyonic food" to compare with planktonic food.

My own choice would lie between "ascion," suggested by Dr. Rienhoff, and "sapropol," suggested by Dr. Bradley.

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SPECIAL ARTICLES

A CRYSTALLINE PROTEIN WITH HIGH LACTOGENIC ACTIVITY*

DURING the course of chemical studies of anterior lobe fractions of the pituitary gland, it has been possible to isolate in crystalline form a protein having marked prolactin (lactogenic) activity. The method of preparation of the prolactin fraction from the gland is essentially that described by Lyons.[1] The purified prolactin preparations have been obtained in crystalline form from pyridine-acetic acid mixtures, using a procedure which is essentially the same as one employed for the crystallization of insulin.[2] One hundred milligrams of the purified fraction are dissolved in a centrifuge tube by the addition of 2 cc of 13 per cent. acetic acid. The material dissolves slowly. When solution is complete, 2 cc of 10 per cent. pyridine are added and the mixture centrifuged. The supernatant fluid, which is usually slightly turbid, is set aside and the precipitate dissolved in 2 cc of acetic acid and 2 cc of pyridine solution added as before; the mixture is then centrifuged. This procedure is repeated 10 times. The mother liquors are combined, and from this solution a crystalline precipitate can usually be obtained in either one of two ways: (1) The pyridine-acetic acid solution slowly deposits a crystalline material on standing for several days in the ice-box; (2) the pyridine-acetic acid solution is treated carefully with one per cent. ammonium hydroxide solution. The latter is added until a distinct, heavy turbidity results. Any material settling out immediately is centrifuged off and the turbid mother liquor placed in the ice-box over night. Microscopically, the crystals appear for the most part as cylindrical rods of varying length, with the rounded edges usually characteristic of protein crystals. The precipitate may be prepared in dry form by centrifuging, and washing at the centrifuge twice with 2 cc portions of ice-cold water, followed by washing once with a mixture of equal parts of absolute alcohol and dry ether, and finally washing two times with dry ether. It is dried in a vacuum desiccator over sulfuric acid.

In recrystallization the material may be treated exactly as described above by means of the pyridine-acetic acid procedure. The lactogenic activities of the various crystalline fractions and residues were determined by bioassays on one-month-old squabs, using the 2-day "local" test.[3] Some of the data obtained are shown in Table I.

TABLE I

ASSAY OF CRYSTALLINE PROLACTIN PREPARATIONS BY THE "LOCAL" OR INTRADERMAL TEST

Preparation	Crystallized	Extinction point*
A	Once	< 0.25 gamma
A	Twice	< 0.125 "
A	Thrice	0.0625 "
B	Once	< 0.25 "
B	Twice	< 0.125 "
C	Once	0.10 "
C	Twice	< 0.0625 "

* The extinction point is designated as the dosage below which a positive response to the injection can not be detected.

It will be seen from the data in Table I that after two recrystallizations, the preparations attain a fairly constant level at which a positive reaction is still obtained, *i.e.*, between one tenth and one twentieth of a gamma. When tested by the "systemic" test,[4] these preparations were found to have an average minimal effective dose of 0.1 mg. It is interesting to note how closely the results of the bioassays agree with the activities reported by Lyons recently[1] for his purified mammotropic hormone. It is evident that the latter investigator has a preparation of a high degree of purity.

A study of the x-ray diffraction pattern of the once crystallized product was kindly conducted by Professor L. W. McKeehan, director of the Sloane Physics

* This study was made possible by a grant from the Fluid Research Fund of Yale University School of Medicine.

1 W. R. Lyons, *Proc. Soc. Exp. Biol. and Med.*, 35: 654, 1936–37.

2 V. du Vigneaud, H. Jensen and O. Wintersteiner, *Jour. Pharm. Exp. Ther.*, 32: 367, 1927–28.

3 W. R. Lyons and E. Page, *Proc. Soc. Exp. Biol. and Med.*, 32: 1049, 1935.

4 O. Riddle, R. W. Bates and S. W. Dykshorn, *Am. Jour. Physiol.*, 105: 191, 1933.

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.