

flavin and the "filtrate" or "rat growth" factor fed to rats. The symptoms described are similar to those reported here, but differ in certain essentials.

The skin lesions observed in the mouse differ in several respects from conditions of dermatitis described in the literature.⁸ The paws, ears, nose and tail, and in most cases the eyes appear normal. In a few cases a sticky exudate is observed about the eyes. The lesions are preceded by loss of hair on the abdomen, closely followed by the appearance of shiny dry skin in the inguinal region. This in turn is soon followed by a scaly dandruff-like appearance beginning almost simultaneously on the back of the neck and in the inguinal region. If the animal survives this stage of the deficiency, the pelt frequently begins to come off in large plaques, particularly on the back, leaving a dry but otherwise normal-appearing hairless skin. In some cases it has been possible to keep the animals alive until they became completely denuded except for a

slight amount of fuzzy hair around the head. In some cases, the peeling off of the pelt does not occur, but instead the dandruff-like appearance spreads over the body and the hair gradually falls out, leaving the animals covered with dry white scales. Early in the course of the deficiency the animals usually assume a hunched position with the hind feet drawn far up underneath the body. Histopathological examination of all organs and sections of the skin is now being made. At autopsy, the animals are found to be emaciated, but otherwise normal in gross appearance.

Mice require a water-soluble factor other than thiamin, nicotinic acid, pyridoxin, riboflavin and the "filtrate factor."⁹ The required factor is present in yeast and liver. A study of the properties of this substance is now in progress.

EARL R. NORRIS

JAMES HAUSCHILDT

UNIVERSITY OF WASHINGTON

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SUBSTITUTE FOR EDESTIN

EDESTIN, the globulin of hemp-seed, has been regarded as a standard example of the seed globulins for many years. Osborne¹ subjected this protein to more detailed and extensive study than any other globulin, and his demonstration with Mendel² of its adequacy as the sole source of protein in the diet of animals has led to extensive use of edestin in certain types of animal experimentation. It serves, for example, as a convenient protein relatively free from phosphorus.

In recent years, many amino-acid determinations³ have been made upon edestin, with the result that the composition of this protein is better known than is that of any globulin in its molecular weight class; in the cases of only a few other proteins are higher summations of amino-acids available. Furthermore, much attention has been given to its physical properties,⁴

and the preparation of a sample of this protein has long been a standard exercise in laboratory courses in biochemistry.

The passage of the Marihuana Law of 1937 has placed restrictions upon trade in hemp-seed that, in effect, amount to prohibition. The seed may be purchased only under license, and transfer is subject to a tax of one dollar an ounce (if illegal, one hundred dollars an ounce). On application to the Collector of Internal Revenue, properly accredited persons may obtain exemption from this tax, and provisions are made in the law so that scientific research on marihuana (the legal definition of which includes all parts of the plant, and all products, save fiber, oil and seed cake prepared from it and from its seed) shall not be impeded.

Hemp-seed is produced only in small quantities in the United States, chiefly in Kentucky, and the plant may be grown only under license. Most of the hemp-seed is imported from the Orient, and there are only a few importers in the country who are licensed by the Federal Narcotics Division to engage in this trade. The seed is devitalized by heat treatment on receipt by the importers and may then be sold without restriction. It is used chiefly in various special feeds such as bird-seed.

The general effect of these restrictions is to render the purchase of hemp-seed for the preparation of edestin a time-consuming and troublesome process. The devitalized seed is useless, since the yield of protein is reduced to less than one twentieth of that from untreated seed. Although some laboratories may be willing to face the difficulties involved, the licensed

⁹ S. Lepkovsky, T. H. Jukes and M. E. Krause, *Jour. Biol. Chem.*, 115: 557, 1936.

⁸ N. Halliday and H. M. Evans, *Jour. Nutrition*, 13: 657, 1937.

¹ T. B. Osborne, *Am. Chem. Jour.*, 14: 662, 1892; *Jour. Am. Chem. Soc.*, 21: 486, 1899; 24: 28, 39, 1902; T. B. Osborne and I. F. Harris, *Jour. Am. Chem. Soc.*, 25: 837, 1903; *Am. Jour. Physiol.*, 14: 151, 1905; T. B. Osborne and L. M. Liddle, *Am. Jour. Physiol.*, 26: 295, 1910.

² T. B. Osborne and L. B. Mendel, *Jour. Biol. Chem.*, 13: 233, 1912.

³ O. Folin and V. Ciocalteu, *Jour. Biol. Chem.*, 73: 627, 1927; O. Folin and A. D. Marenzi, *Jour. Biol. Chem.*, 83: 89, 1929; H. B. Vickery and C. S. Leavenworth, *Jour. Biol. Chem.*, 76: 707, 1928; H. B. Vickery and A. White, *Jour. Biol. Chem.*, 99: 701, 1933; D. B. Jones and O. Moeller, *Jour. Biol. Chem.*, 79: 429, 1928; H. D. Baernstein, *Jour. Biol. Chem.*, 106: 451, 1934; K. Bailey, *Biochem. Jour.*, 31: 1396, 1937; H. B. Vickery, *Jour. Biol. Chem.*, 132: 325, 1940.

⁴ E. J. Cohn, *Physiol. Rev.*, 5: 349, 1925; N. F. Burk and D. M. Greenberg, *Jour. Biol. Chem.*, 87: 197, 1930; C. F. Failey, *Jour. Am. Chem. Soc.*, 54: 2367, 1932; R. W. G. Wyckoff and R. B. Corey, *SCIENCE*, 81: 365, 1935; E. J. Cohn, *Chem. Rev.*, 24: 203, 1939.

importers are not enthusiastic about cooperation, since they think in terms of tons and they are also fully alive to the penalties that are risked if unauthorized persons secure some of the seed.

It seems clear that the long and important career of the protein edestin is coming to a close in the United States. A substitute must be found, and it is the purpose of this communication to draw attention to the problem this presents. The substitute for edestin must be a seed globulin of approximately the same solubility; it should be a protein that is easily prepared in crystalline and therefore presumably pure form; it should be derived from a seed that is an important crop not only in America but elsewhere, and it is desirable that it should be relatively cheap. A survey of possibilities in this field has led, largely by elimination on one ground or another, to the plants of the family Cucurbitaceae. Many species are commonly grown, and considerable study has been given to the proteins. Squash-seed globulin was prepared by Osborne⁵ in 1892 and has been occasionally investigated since. Cantaloupe seed has been studied by Jones and Gersdorff.⁶ Hirohata⁷ has examined the globulins of some thirty-eight varieties and species of eight genera of this family and has drawn attention to the close similarity, if not identity, of the globulins from closely allied species. Kiesel and his collaborators⁸ and also Krishnan and Krishnaswamy⁹ have studied the globulin of watermelon seed.

Considerations of availability have led us to the tentative suggestion that the globulin of the pumpkin seed (*Cucurbita pepo*)¹⁰ may be found to fulfil most of the requirements of a substitute for hemp-seed edestin. Numerous varieties are grown, and the seed is a waste product of the canning industry in some states. It is always available on the market at a price that is usually a small fraction of that commanded by most cucurbit seeds.

⁵ T. B. Osborne, *Am. Chem. Jour.*, 14: 662, 1892.

⁶ D. B. Jones and C. E. F. Gersdorff, *Jour. Biol. Chem.*, 56: 79, 1923.

⁷ R. Hirohata, *Ztschr. f. physiol. Chem.*, 212: 1, 1932.

⁸ A. Kiesel, A. Belozersky, P. Agatow, N. Biwschich and M. Pawlowa, *Ztschr. f. physiol. Chem.*, 226: 73, 1934.

⁹ P. S. Krishnan and T. K. Krishnaswamy, *Biochem. Jour.*, 33: 1284, 1939.

¹⁰ *Cucurbita pepo* includes the common field, pie and cattle pumpkin of North America and also the vegetable marrow. There are several varieties recognized in systematic works, and many varieties are commonly grown. *C. moschata* is a closely allied species that includes the cushaw and crookneck squashes and other varieties known both as pumpkins and as squashes. *C. maxima*, also closely allied, includes such well-known squash varieties as Hubbard and Boston Marrow as well as certain very large varieties commonly called pumpkins. Whether varietal or even specific differences in these plants are reflected in the main storage globulins of the seeds is unknown. Uncertainty on this point presents no graver problem, however, in this case than is presented by any other protein of vegetable or for that matter of animal origin.

A series of proteins from commonly available cucurbit seeds is under investigation in this laboratory. Although there are minor differences in the behavior of the different seeds, all yield well-crystallized globulins when the ground, whole seed is extracted by means of a hydraulic press with warm 10 per cent. sodium chloride solution. After removal of the emulsion of fat which readily rises from the warm solution, the filtered aqueous phase is diluted to about 2 per cent. sodium chloride concentration at 60° C. This clear solution is slowly cooled to about 5° C., when the protein deposits almost entirely in the form of octahedral crystals. Reprecipitation under similar circumstances gives, as a rule, a very fine product. The yield is of the order of 10 per cent., somewhat higher than that usually secured from hemp-seed.

A report on the preparation and nutritive properties of one or more of these globulins and on certain of their chemical properties will form the subject of a later communication.

HUBERT BRADFORD VICKERY

EMIL L. SMITH¹¹

LAURENCE S. NOLAN

CONNECTICUT AGRICULTURAL

EXPERIMENT STATION

¹¹ John Simon Guggenheim Memorial Fellow.

BOOKS RECEIVED

- ALLEN, GLOVER M. *The Mammals of China and Mongolia. Part 2 of Vol. XI of Natural History of Central Asia.* Pp. xxvi + 621-1350. Illustrated. American Museum of Natural History. \$10.00.
- BELL, E. T. *The Development of Mathematics.* Pp. xiii + 583. McGraw-Hill. \$4.50.
- BOGERT, L. JEAN and MAME T. PORTER. *Dietetics Simplified; The Use of Foods in Health and Disease.* Second edition. Pp. xi + 742. 76 figures. Macmillan. \$3.00.
- CLARK, C. C. and R. H. HALL. *This Living World.* Pp. x + 519. Illustrated. McGraw-Hill. \$3.25.
- HAPP, STAFFORD C., GORDON RITTENHOUSE and G. C. DOBSON. *Some Principles of Accelerated Stream and Valley Sedimentation.* Pp. 134. Illustrated. U. S. Department of Agriculture. Superintendent of Documents, Washington. \$0.75.
- LANDIS, CARNEY and others. *Sex in Development.* Pp. xx + 329. Hoeber. \$3.75.
- KENDALL, JAMES I. *The Microscopic Anatomy of Vertebrates.* Second edition. Pp. 342. 197 figures. Lea and Febiger. \$3.75.
- KLEINER, ISRAEL S. and LOUIS B. DOTY. *Laboratory Instructions in Biochemistry.* Looseleaf. Mosby. \$1.50.
- PETERS, CHARLES C. and WALTER R. VAN VOORHIS. *Statistical Procedures and Their Mathematical Bases.* Pp. xiii + 516. Illustrated. McGraw-Hill. \$4.50.
- Report on the Agricultural Experiment Stations, 1939.* Prepared by the Office of Experiment Stations. Pp. 265. U. S. Department of Agriculture. Superintendent of Documents, Washington. \$0.25.
- SHUSTER, CARL N. *A Study of the Problems in Teaching the Slide Rule.* Pp. v + 103. Illustrated. Teachers College, Columbia University. \$1.85.
- YOUNG, KIMBALL. *Personality and Problems of Adjustment.* Pp. x + 868. Illustrated. Crofts. \$4.25.