the 2 layers support the previously advanced concept that a disulfide containing keratin precursor is present in the lower layers of the epidermis. Oxidation of –SH groups in the upper Malpighian layer accounts only for a fraction of the –S–S– bonds present in the horny layer. In other words, epidermal keratinization starts in the depth of the Malpighian layer. Preliminary experiments suggest that deviations from this normal pattern occur in pathologic specimens.

Summary. A method is described for the separation of human epidermis into a nucleated cellular and into a keratinous component. Estimations of –SH groups and –S–S– bonds in these 2 components support a previous theory that epidermal keratinization starts in the depth of the Malpighian layer.

1. Van Scott, E. J., *J. Invest. Dermatol.*, 1952, v18, 377.

2. Barrnett, R. J., and Seligman, A. M., *Science*, 1952, v116, 323.

3. Barrnett, R. J., *J. Nat. Cancer Inst.*, 1953, v13, 905.

4. Flesch, P., and Kun, E., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 249.

5. Van Scott, E. J., and Flesch, P., *Science*, 1954, v119, 70.

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Partial Protection Against Odoratism (Sweet Pea Lathyrism) by Diets High in Gelatin or Casein. (20927)

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Odoratism, the experimental lathyrism resulting from the feeding of the seed of the flowering sweet pea (*lathyrus odoratus*),* was first produced in the rat over 20 years ago(1), yet it has not been possible to decrease the toxic effect of sweet pea diets by the administration of a protective agent. Lewis and his coworkers(2,3) were unable to prevent or delay the onset of odoratism by incorporating casein into the experimental diet in levels up to 25%. For this reason it has been felt that a deficiency in amino acids or in protein is not involved in odoratism(2-5). However, it appears to this author that the tissues which are primarily affected in odoratism in the rat are those which are high in collagen: *viz.*, bone(1,3,4,6), blood vessels(4), and skin.† It seems likely, therefore, that a disturbance in collagen metabolism (or connective tissue metabolism, is involved.

Because collagen differs so markedly from other natural proteins in its hydroxyproline content, it was thought to be of interest to feed hydroxyproline to rats receiving sweet pea diets. Gelatin was used as a source of hydroxyproline. Control groups were given sweet pea diets in which the gelatin was either omitted or replaced by casein.

TABLE I. Composition of Diets.

	Diet						
	I	II	III	IV	V	VI	VII
Ground sweet peas	50	50	40	40	30	30	30
Casein*	10	10	10	10	10	10	40
Gelatin†	—	30	—	30	—	30	—
Starch	30	—	40	10	50	20	20
Brewer's yeast	4	4	4	4	4	4	4
Salts‡	4	4	4	4	4	4	4
Cod liver oil	2	2	2	2	2	2	2

* G B I casein, plain, untreated (not vitamin-free).

† Bacteriological grade.

‡ Hubbel, Mendel and Wakeman salt mixture(7).

Methods. Weanling male rats 22 days of age and 43-53 g in weight were used. The diets were made up as shown in Table I. Each diet was fed to 5 rats, individually caged. All diets were supplied *ad libitum*. *Criterion of toxicity.* Gross manifestations of skeletal change were used as the criterion of toxicity.

* The author is indebted to Mr. Raymond H. Coulter of the Ferry-Morse Seed Co., Detroit, who generously supplied the sweet peas used in this and in related studies.

† Observations to be reported elsewhere.

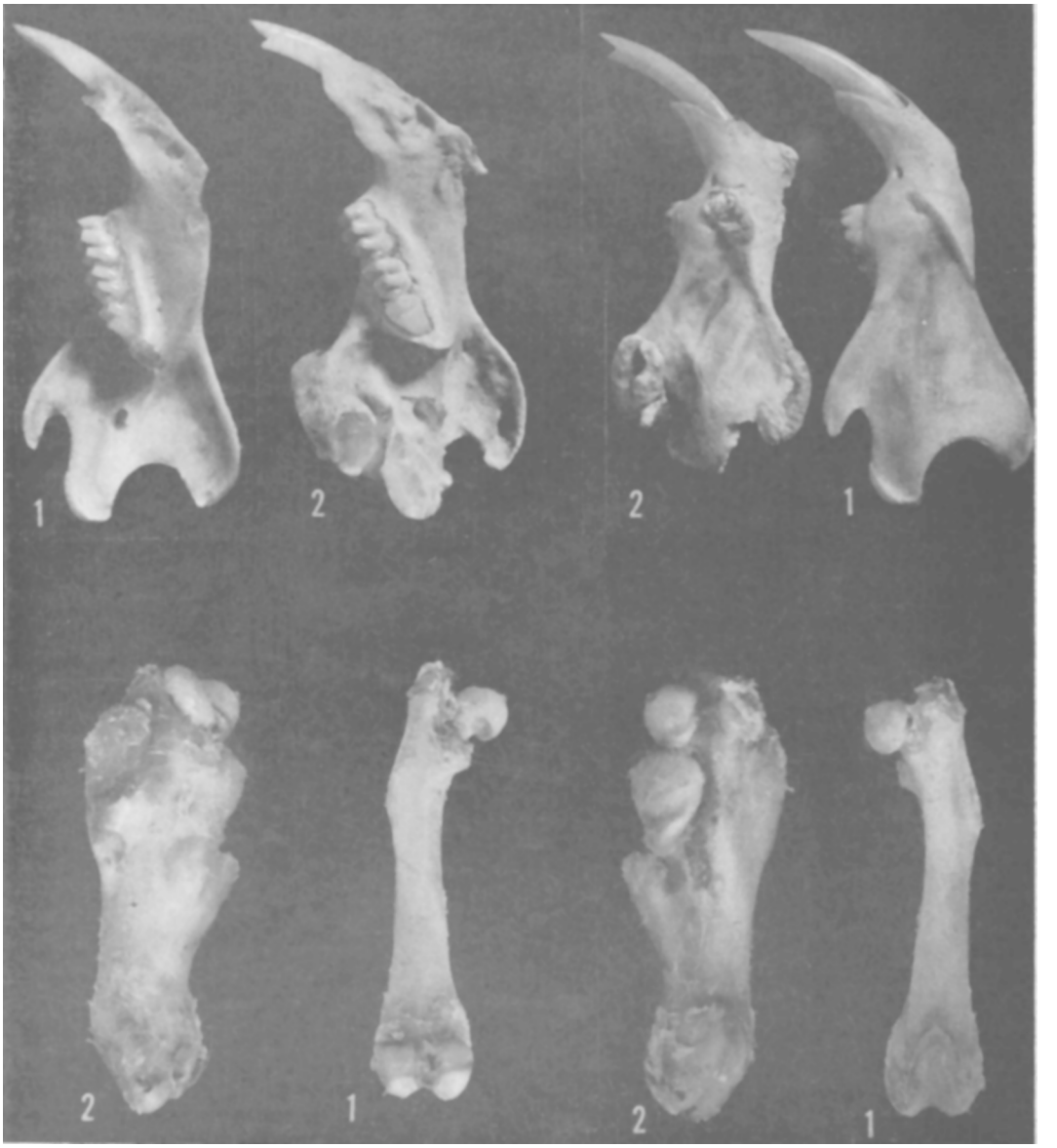

FIG. 1. Top row: Inner and outer aspects of mandibles from rats with advanced odoratism (2) and from control rats (1). Note also notching of incisors, probably mechanical in origin and induced by limited range of movement of jaw due to striking overgrowth of bone in proximal portions of mandibles. Bottom row: Opposite aspects of femur from a rat with advanced odoratism (2), and of a femur from its pair-fed control rat (1).

Spectacular skeletal changes take place in rats receiving sweet pea diets(1,3,4,6). Fig. 1 illustrates extreme examples of malformations which occur in femurs and in mandibles of growing rats receiving a diet containing 50% sweet peas and 10% casein for 21 days. When this diet is fed to weanling rats, exostoses on the ventral and lateral aspects of the mandibles can be palpated in all rats at the end of one week. These become progressively worse. Spinal curvatures and sternal curvatures which may assume fantastic proportions occur in 2 to 3 weeks. Paralysis of the rear legs and urinary distention of the bladder (incontinence) frequently occur after about 3 weeks on the diet.

TABLE II. Average Weekly Weights of Rats Receiving 30% Sweet Peas.

No. of days	Diet V, g	Diet VI (high gelatin), g	Diet VII (high casein), g
0	45.7	48.0	46.1
7	71.5	60.6	68.4
14	96.4	81.9	97.9
21	120.2	110.3	140.0
28	122.5	127.8	161.2

Results. High levels both of gelatin and of casein were found to be partially protective against the toxicity of sweet peas. Casein gave better protection than gelatin. Growth records of the rats receiving 30% sweet peas are given in Table II. The following is a summary of the observations.

Diet V (30% sweet peas, 10% casein; 5 rats). *Two weeks.* Palpable exostoses on mandibles of all rats. Sternal curvature or groove in thoracic cage in 4 rats; in one of these, the sternal curvature is severe. *Three weeks.* Moderate to severe spinal and sternal curvatures in all rats. Decreased muscle tone. One rat paralyzed. Prominent mandibular exostoses in all rats. *Four weeks.* One rat dead; 2 others paralyzed, one of the latter is incontinent. Living rats have all classical symptoms of advanced odoratism including very prominent palpable mandibular exostoses and spinal and sternal curvatures.

Diet VI (30% sweet peas, 30% gelatin, 10% casein; 5 rats). *Two weeks.* Palpable thoracic groove or sternal curvature in 3 rats. Slight or questionable palpable mandibular exostoses in same 3 rats. *Three weeks.* Rats much more active than preceding group (Diet V). Good muscle tone. Mandibular exostoses absent or questionable in all rats. Severe sternal curvature in one rat, slight to moderate in 3 others. *Four weeks.* All rats living and active. Mandibular exostoses absent, slight, or questionable. No paralysis. All have more or less spinal and sternal curvatures, but much less severe than preceding group (Diet V). Better muscle tone than preceding group.

Diet VII (30% sweet peas, 40% casein; 5 rats). *Two weeks.* No mandibular exostoses. Four rats entirely normal. One rat has palpable sternal curvature. *Three weeks.* No mandibular exostoses. Three rats entirely normal. Two rats have moderate to severe sternal curvatures; one of these has a spinal curvature. *Four weeks.* No mandibular exostoses. Good muscle tone. Three rats have slight spinal or sternal curvature (one of these has both). Two rats have moderate to severe spinal and sternal curvatures.

Diets III and IV (40% sweet peas). These groups confirmed the protective action of gelatin. In fact, throughout the 4-week period, rats receiving 40% sweet peas plus 30% gelatin were in much better condition than the rats receiving 30% sweet peas without the added gelatin (Diet V).

Diets I and II (50% sweet peas). The protective action of gelatin at this level of sweet peas was questionable. Both groups exhibited advanced odoratism before the end of 3 weeks.

Discussion. In these experiments, both casein and gelatin delayed the onset of odoratism, but did not prevent it. Rats receiving the high casein diet or the high gelatin diet (30% sweet peas), when continued beyond the 4-week period, deteriorated further and developed advanced symptoms of the disease including severe spinal and sternal curvatures and, in some cases, eventual paralysis. Casein did, however, entirely prevent the formation of palpable exostoses on the mandibles, even in the most advanced cases, the jaws remaining indistinguishable by palpation from those of normal rats. Gelatin also prevented the formation of the usual prominent mandibular exostoses, although in the case of gelatin some roughness of the jaw could be detected.

The increased rate of growth of the rats receiving the high casein diet as contrasted to those receiving the high gelatin diet is no doubt due to the amino acid deficiencies of gelatin. The apparent superiority of the protective action of casein over that of gelatin, however, is probably not a result of the better growth on the high casein diet since all past experience indicates that the more rapidly growing animal develops symptoms of odoratism at least as fast as the slower growing animal.

Any hypothesis attempting to explain the partial protective action of high levels of

casein or of gelatin in these experiments would be purely speculative at this point. It seems impossible that a deficiency of an essential amino acid could be involved in odoratism because the animals continue to grow. Further, the symptoms of odoratism are profoundly different from those produced by any known amino acid deficiency.

Summary. High levels of gelatin and of casein delayed, but did not prevent, the onset of odoratism (experimental lathyrism) in the rat. Casein exerted a greater protective action than gelatin.

The technical assistance of Miss Mildred Mosby is gratefully acknowledged.

1. Geiger, B. J., Steenbock, H., and Parsons, H. T., *J Nutrition,* 1933, v6, 427.

2. Lewis, H. B., and Esterer, M. B., PROC. SOC. EXP. BIOL. AND MED., 1943, v53, 263.

3. Lewis, H. B., Fajans, R. S., Esterer, M. B., Shen, C., and Oliphant, M., *J. Nutrition,* 1948, v36, 537.

4. Ponseti, I. V., and Baird, W. A., *Am. J. Path.,* 1952, v28, 1059.

5. Lee, J. G., *J. Nutrition,* 1950, v40, 587.

6. Vivanco, F., and Diaz, C. F., *Bull. Inst. Med. Res., Madrid,* 1951, v4, 1.

7. Hubbel, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition,* 1937, v14, 273.

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Macrocytic Anemia and Leucocytosis of Guinea Pigs with Muscular Stiffness Disease.* (20928)

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A deficiency syndrome characterized by muscular stiffness was described by Wulzen and Bahrs(1) in guinea pigs raised on skim milk diets supplemented by minerals and the known vitamins. Physiological, chemical, and pathological aspects of the disease were studied(2-6).

The present investigation was undertaken to determine whether hematological changes occurred in guinea pigs deficient in the anti-stiffness factor. Preliminary results of these studies have been published(7).

Methods. Guinea pigs used to establish the normal blood values were segregated as to sex and raised on diet of rolled barley, liberal amounts of kale or grass, iodized salt, and wheat straw *ad lib.* None showed wrist stiffness. Gravid animals were not tested. Experimental animals were fed the following diet deficient in the anti-stiffness factor(8). A.M.: skim milk powder 20.0 g, water 80.0 g, copper sulfate 0.078 mg, ferric chloride 0.048 mg, ascorbic acid 10.0 mg; P.M.: skim milk powder 20.0 g, water 80.0 g. Fat- and water-soluble vitamins were added alternately to the evening diet to make the average daily consumption per animal for each vitamin as follows: thiamin hydrochloride 0.20 mg, pyridoxine hydrochloride 0.10 mg, riboflavin 0.50 mg, nicotinic acid 1.0 mg, pantothenic acid 0.10 mg, inositol 10.0 mg, para-aminobenzoic acid 2.0 mg, choline 50.0 mg, biotin (SMA conc. S200) 0.01 mg, beta-carotene 150 I.U., viosterol 40 I.U., alpha-tocopherol 0.10 mg, 2-methyl-1,4-naphthoquinone 0.10 mg, folic acid 0.003 mg. All received straw and iodized salt *ad lib.* Blood samples were taken from the ear which was gently stroked or warmed to cause an hyperemia(9). Incisions were made with Bard-Parker "11" blades. The animals were tame from frequent handling in the laboratory and did not have to be confined in any way when samples were taken. Erythrocyte counts were made after diluting the blood with Toisson's solution. Hemoglobin estimations were made with the Sahli-Haskins technic (10). The hemoglobin coefficient, according

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