TABLE II. Plasma Alloxan and Glucose in 6 Patients.

Initials	G.M.	S.M.	S.S.	I.G.	S.W.	E.S.
Diagnosis	Necr. of pancreas	Adrenal hypotens.	Glom. nephr.	Diab. & nephr.	Diab.	Cirrhosis & Diab.
Alloxan (hr)						
Fasting	.150	.200	.120	.235	.277	.235
½	.210	.150	.105	.265	.240	.090
1	.210	.110	.125	.330	.280	.195
2	.165	.135	.110	.285	.225	.200
3	—	.140	.130	.240	.240	.200
Glucose (Folin-Wu) (hr)						
Fasting	90	103	107	93	140	190
½	146	172	250	198	218	310
1	94	216	294	240	316	300
2	94	162	280	183	254	382
3	94	165	193	128	250	398

significant increase in plasma alloxan was provoked by a glucose meal in man and dog.

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Method for Separation of Human Epidermis into Cellular and Keratinous Components.* (20926)

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One of the major difficulties which has hampered experimental work on human epidermis is its heterogeneous composition. Experiments done on whole epidermis do not differentiate between the cellular components and the inert keratinous fibers. This paper describes a simple method for the separation of pulverized, dry human epidermis into its keratinous and cellular layers.

Methods. Human epidermis from autopsy or surgical material was separated from the corium with the stretch method(1), dried, pulverized in a mortar and passed through a 200-mesh sieve. The resulting powder was defatted by washing 3 times with ether in a centrifuge tube. Before the last washing the epidermal powder was allowed to settle and then centrifuged at moderate speed, whereupon 2 layers developed in the precipitate. After drying, the 2 layers could be separated easily. The upper layer was lightly pigmented and of chalk-like appearance, while the lower layer was darker and granular (Fig. 1). Powder from each of the 2 layers was embedded in paraffin, sectioned and stained with various histochemical reagents, such as Schiff's reagent and the Barrnett-Seligman stain for sulfhydryl groups(2,3), and with Giemsa stain. Sulfhydryl groups and disulfide bonds in each layer of the dry powder were determined with Bennett's reagent(4,5) quantitatively.

Results. Microscopic studies showed that the upper layer consisted mainly of Schiff-

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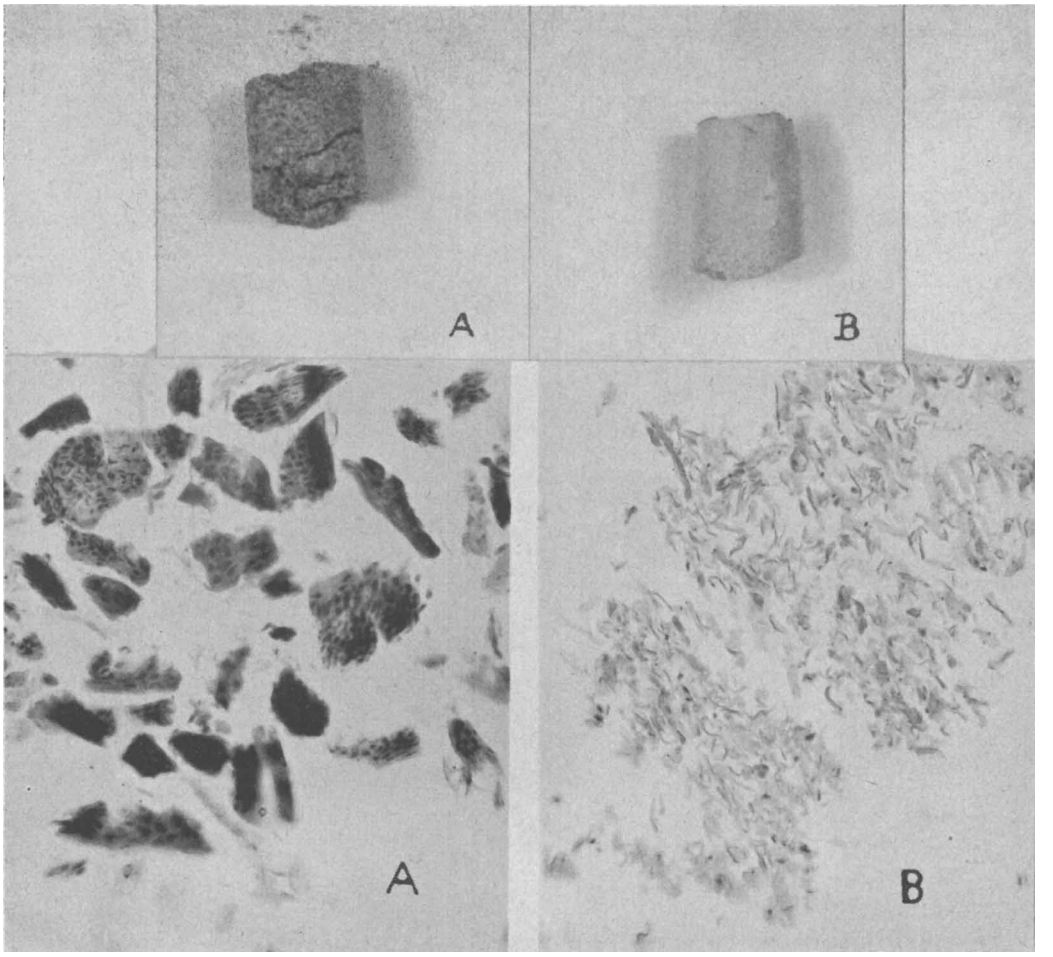

FIG. 1. Separation of pulverized human epidermis into two fractions. A. Lower dark cellular component. B. Upper light keratinous component.

FIG. 2. Microscopic appearance of components of pulverized human epidermis (Giemsa stain, 200× enlarged). A. Lower cellular fraction. B. Upper keratinous fraction.

negative particles with an occasional scattered nuclear remnant, while the lower layer contained mostly clumps of deeply staining nucleated Schiff-positive cells. Similar observations were made with the Giemsa stain (Fig. 2). The histochemical sulfhydryl reaction was positive in sections from both layers.

Preliminary estimations of sulfhydryl and disulfide in powders from both layers of 8 samples of human white abdominal epidermis extended previous data obtained on plantar epidermis(5). There was approximately a 20% lower –S–S– content in the lower layer. The values of the –SH groups of the upper layer were two-thirds that of the lower layer. These values comprised only a fraction of the "total" (–SH+–S–S–) sulfur, about 20% in the lower and 9% in the upper layer.

Discussion. The present method permits the study of epidermal components in various stages of their differentiation. It opens up new avenues for the investigation of various epidermal functions, such as pigment formation, keratinization and enzymatic activities. The latter studies are made possible by the fact that upon centrifugation, incompletely homogenized wet epidermis also separates into keratinous and cellular fractions. Since components of the same tissues are used, the same epidermis can serve as its own control.

Estimations of –SH and –S–S– groups in

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.

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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.

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† Observations to be reported elsewhere.