cally affected gargoyles excrete significantly increased quantities of mucopolysaccharide. The known carriers and most of the siblings are in or near the normal range. Based on these quantitative data it is not yet possible to identify carriers by these procedures.

Since the analytical methods employed yield values for total mucopolysaccharide without distinguishing among the various types which may be present, the present results do not preclude the existence of qualitative differences between carriers and normal individuals which would not be quantitatively evidenced. Such qualitative differences are currently being investigated.

Summary. The urinary mucopolysaccharide excretion of a family with the sex-linked form of gargoylism has been quantitatively studied, using several methods. The results indicate that neither carriers nor unaffected siblings excrete significantly higher than normal quantities of total acid mucopolysaccharides.

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Hexosamine and Hydroxyproline Alterations in Chronically Sun-Damaged Skin.* (26988)

J. GRAHAM SMITH, JR., EUGENE A. DAVIDSON, JOHN P. TINDALL, AND W. MITCHELL SAMS, JR. (Introduced by E. A. Stead, Jr.)

Division of Dermatology, Department of Medicine, Department of Biochemistry, and the Center for the Study of Aging, Duke University Medical Center, Durham, N. C.

The wrinkling, loss of elasticity, and other changes in human skin associated with aging are markedly accentuated by chronic sun damage. Unexposed skin of elderly persons does not show these alterations to the same degree as exposed areas and may look decades younger than uncovered skin(10). Histochemically, the dermis of sun-damaged skin (actinic elastosis) displays increased staining for acid mucopolysaccharides (*e.g.* Alcian blue, Mowry colloidal iron) and elastin-like material (*e.g.* orcein, Verhoeff-VanGieson) (6,9). The relationship to true elastin and collagen of the latter fraction is still uncertain(7). This study was undertaken to quantitate hexosamine and hydroxyproline in sun-damaged skin as compared to unexposed skin, and to localize the abnormality.

Materials and methods. Chronically sun-damaged skin of the forearms of 3 volunteers was removed with a Brown dermatome under local anesthesia; elliptical areas of similarly damaged skin were excised from the forearms or neck of 6 other long-time residents of central Florida.† Control skin was removed at autopsy from the Y incision of the abdomen of 12 adults whose deaths resulted from a variety of causes. All skin was frozen until

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† Skin from human volunteers was obtained through the cooperation of Dr. Charles C. Tindall, Kissimmee, Fla.

TABLE I. Hexosamine and Hydroxyproline of Chronically Severely Sun-Damaged and Control Skin

	Chronic sun-damaged dermis	Control dermis
No.	9	12
Age	67.8 ± 9.3*	53.5 ± 12.5
Hexosamine (μm/100 mg dry wt)	3.4 ± .9	2.0 ± .5
Hydroxyproline (μm/100 mg dry wt)	55.0 ± 11.1	66.0 ± 3.6
Hexosamine/hydroxyproline ×'s 10	10.7 ± 4.3	3.0 ± .9

* Mean and standard deviation.

ready for use. Small pieces of the skin from all groups were fixed for 24-48 hours in 10% formalin for histological and histochemical study. Skin removed with the Brown dermatome was between 0.3 to 0.38 mm in thickness. The upper 0.3 mm of the other skin specimens was removed using the Castroveijo keratotome(2).

A similar 0.3 mm section of skin from the lower-most dermis of 5 specimens of excised sun-damaged defatted skin was also removed with the keratotome to compare with the upper section of dermis from the same individual, since histologic changes are demonstrable only in the upper ⅓ to ½ of sun-damaged dermis(9). The epidermis was heat separated from the dermis using a modification of the Baumberger method(1). The dry weight of dermis was obtained by desiccation in a vacuum oven and the dermis hydrolysed with 4 N hydrochloric acid for 15 hours at 100°C in sealed tubes. Neutralized aliquots of the hydrolysate were taken for hydroxyproline(8) and hexosamine(4) determinations. Paper chromatography was used to confirm the identity of the amino sugars.

Results. All sections of sun-damaged skin showed increased staining in the upper dermis with the Mowry colloidal iron and the orcein stains as compared to the control material. Hexosamine in the sun-damaged skin was significantly increased over that in the control skin, whereas the hydroxyproline content was significantly lower than in the controls (Table I). The hexosamine/hydroxyproline ratio of the controls was lower than that of sun-damaged skin.

The comparison of the upper and lower-most dermis of 5 individuals is shown in Table II. Differences between hexosamine, hydroxyproline, and hexosamine/hydroxyproline ratios are statistically significant.

Paper chromatography of the amino sugars indicated that glucosamine exceeded galactosamine in both sun-damaged and control skin samples.

Discussion. The hexosamine and hydroxyproline alterations of elastotic skin represent a reversal of the pattern found in unexposed skin with age(11). The decrease in hydroxyproline accompanying epidermal carcinoma induction by topical application of the carcinogen methylcholanthrene(5) is interesting in view of the similar finding in sun-damaged skin whose susceptibility to development of malignancy is well-documented(3). The re-

TABLE II. Hexosamine and Hydroxyproline in Upper and Lower Dermis of 5 Individuals with Chronically Severely Sun-Damaged Skin

	Upper dermis	Lower dermis
Hexosamine (μm/100 mg dry wt)	3.6 ± 1.1*	1.6 ± .14
Hydroxyproline (μm/100 mg dry wt)	41.1 ± 6.8	58.1 ± 9.0
Hexosamine/hydroxyproline ×'s 10	8.9 ± 3.1	2.8 ± .5

* Mean and standard deviation.

duced level of hydroxyproline does not, however, define the identity of the elastin-staining material, since either an increase of true elastin or a degradation of collagen could result in the lower value.

The excess of glucosamine over galactosamine combined with the hyaluronidase lability of the acid mucopolysaccharide staining in sun-damaged skin(9) suggest hyaluronic acid is the major mucopolysaccharide in sun-damaged skin.

Summary. 1. The upper dermis of chronically sun-damaged human skin contains more hexosamine and less hydroxyproline than the upper dermis from unexposed areas. 2. The increased hexosamine confirms the presence of increased acid mucopolysaccharides seen histologically in sun-damaged skin, but the decreased hydroxyproline does not identify the elastin staining material as either elastin or "degraded" collagen. 3. The upper dermis of sun-damaged skin has higher hexosamine and lower hydroxyproline levels than the lower-most dermis of the same specimen, thus the localization of the biochemical alteration corresponds with the localization of the histologic abnormality.

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Is There a Heme-Stimulating Factor in Human Plasma?* (26989)

S. KRAMER,† ANN C. LOCHHEAD, AND A. GOLDBERG
(Introduced by G. E. Cartwright)
University Department of Medicine, Gardiner Institute, Western Infirmary, Glasgow, Scotland

Many recent observations have strengthened the original studies of Carnot and Deflandre(1) in experimental animals, suggesting a humoral mechanism controlling erythrocyte production. Plasmas from patients with various hematological disorders have also been found to stimulate erythropoiesis in experimental animals(2). Linman *et al*(3) have suggested that there are probably 2 factors in plasma which exert control over erythropoiesis; one, a thermostable factor which stimulates division of erythroblasts and another factor which is only relatively thermostable, which enhances hemoglobin synthesis.

Various attempts have been made to demonstrate the hemoglobin stimulating factor *in vitro*. Normal rabbit serum has been shown to have a marked stimulating effect on rate of incorporation of C^{14}-labelled glycine into heme by rabbit bone marrow(4). This heme-stimulating factor in serum was thermolabile. There are however conflicting reports regarding the effect of plasma on hemoglobin formation as measured by uptake of radioiron into heme. Using an hemolysate of chicken red cells as the enzymic source and glycine as substrate, Goldberg *et al*(5) failed to show any difference in uptake of radioiron into heme between deproteinized plasmas of normal chickens and of chickens made anemic by

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† MacKenzie McKinnon, Research Fellow of Royal Colleges of Physicians and Surgeons, England.