suckling rats. In previous work(1), 20% sucrose solution given under similar circumstances caused over a 100% increase in caries as compared with unsupplemented litter mates. A 20% glucose and fructose mixture given in a dosage of 1 to 1000 of body weight also increased the subsequent caries of white rats(4). If this increase in caries was due alone to dilution of the diets of suckling rats, the caries scores would be similar.

The results of the previous work are in agreement with findings of Sognnaes(5) in which rats fed high sucrose diet during calcification of teeth had higher incidence of caries over animals not so treated. Work with monkeys also showed an adverse effect of high sucrose diet during calcification on later caries experience of teeth. Reduced caries experience of children during and after World War II also correlated with reduction in sugar imports(6). These results are in contrast with those reported in this paper in which no significant change in caries resulted from addition of lactose to diet of suckling rats during calcification of their teeth.

Lactose is hydrolyzed more slowly than sucrose in the gastrointestinal tract. Since hydrolyzed sucrose is absorbed very rapidly, it might possibly produce a temporary mineral imbalance during the suckling period. This imbalance may be reflected in a significant increase in the caries developed later.

Summary. A 20% solution of lactose given 3 times a day for 18 days during calcification period of molars, resulted in no significant increase in caries of teeth of white rats over control litter mates.

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Excretion of Sulfated Mucopolysaccharides in Gargoylism. (Hurler's Syndrome)* (23714)

KARL MEYER, MELVIN M. GRUMBACH, ALFRED LINKER, AND PHILIP HOFFMAN
Departments of Medicine and Pediatrics, Columbia University College of Physicians and Surgeons, Edward Daniels Faulkner Arthritis Clinic of Presbyterian Hospital, and Babies Hospital, New York

The presence of sulfated polysaccharides has been demonstrated histologically in a number of cases of gargoylism(1) and, from their various organs, an unidentified sulfated mucopolysaccharide has been isolated(1,2). It has been established recently that the urine of 2 cases of gargoylism contained chondroitin sulfate B (ChS-B)(3,4) as well as a minor component with a positive rotation(4) designated either as heparitin sulfate(5) or heparin monosulfate(6). This paper is concerned with studies on specimens of urine from 5 patients with gargoylism and a sample of liver obtained at autopsy and preserved in a frozen state, from another patient.

Methods. The method used for isolation of sulfated mucopolysaccharides was a modification of that of Di Ferrante and Rich(7). An attempt was made to collect total urine for periods of 4 to 6 days from Cases 1-4, however, accurate collections during this entire period were not possible and the pools do not represent complete samples. The urine samples were acidified to pH 6.0 and a warm aqueous solution of 5% cetyl trimethylammonium bromide was added (1.7 ml/100 ml of urine). After standing overnight at 4° and centrifuging, the precipitate was repeatedly

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TABLE I. Mucopolysaccharide Fractions of Urine and Liver in Gargoylism.

Source of material	EtOH fractions, yield (mg)	Analysis in % — Uronic acid — Carbazole	Uronic acid — Orcinol	Ratio	Hexosamine	SO_4	$[\alpha]_D$	Aminosugar* Gal. NH_2	Gluc. NH_2
Urine of R. Do. (vol = 2.66 l)	175	14.4	37.4	.38		15.4	−55	4+	trace
	88	21.6	26.5	.81				2+	2+
	75	27.9	24.4	1.14		11.8	− 1	2+	2+
Urine of R. Br. (vol = 2.83 l)	246†	16.3	29.6	.55	21.5	13.0	−38	3+	2+
	71	31.9	24.5	1.30	22.8	15.1	+27		
Urine of P. Gr. (vol = 1.77 l)	243	12.5	40.6	.38		13.7	−60	4+	trace
	81	11.2	21.5	.52				3+	1+
Urine of J. Gr. (vol = 1.10 l)	49	12.9	36.6	.35				4+	trace
	51	15.7	21.5	.73				3+	1+
Urine of A. (vol = .97 l)	77‡	38.8	19.4	2.0	24.0	6	+60	trace	4+
Liver, 73.8 g dry wt	243	7.6	19.2						
	442‡	33.7	17.2	1.96	24.8	17.4	+61	0	4+
	974‡	46.6	17.4	2.68	28.1	11.0	+69	0	4+
	335	38.2	16.3	2.35	27.1	13.8	+60	0	4+
ChS-B pigskin		16.0	39.1	.41	24.7	13.4	−63	4+	0
heparitin sulfate (lung)		36.7	19.0	1.93	27.7	15.5	+54	0	4+

* Relative amounts of glucosamine and galactosamine were determined qualitatively by paper chromatography following ninhydrin oxidation(13).

† Resistant to testicular hyaluronidase.

‡ Hydrolyzed by heparin adapted enzyme(9).

triturated with a large amount of ethanol.† Completeness of the precipitation was confirmed in some instances by dialysis of the supernatant, followed by further addition of the reagent. No further fraction was obtained. The ethanol-insoluble fraction was dissolved in 50 ml of 10% aqueous Na acetate, the pH adjusted to 9.0 with a few drops of 5 N NaOH, and any insoluble material removed by centrifugation. The residue was extracted once with 50 ml of water and the combined supernatant solutions acidified to pH 5.0. The crude polysaccharide fraction was precipitated with 1.5 volumes of ethanol. The precipitated polysaccharide was left at 4° overnight, centrifuged and washed with 80% ethanol. The fraction was extracted with a small volume of water, the extract was cleared by high speed centrifugation, Ca acetate and acetic acid were added to final concentrations of 5% and 0.5 N, respectively, and then the solution was fractionally precipitated with ethanol(8). Some of the samples were further purified by treatment with Lloyd's Reagent and Kaolin and further fractionation(8). A slice of liver‡ was obtained frozen in dry ice. The liver was ground in a meat grinder, dehydrated and defatted by standing for several days first under acetone and then under ether. The air-dried material (73.8 g) was digested with pepsin and trypsin and fractionated as described previously (8). The methods of analysis used were previously described(8). In Table I, the data obtained from the different samples are recorded. For comparison, a typical analysis of a ChS-B fraction of pigskin(8) and one of heparitin sulfate from bovine lung§ is included.

Results. In Table I, the ChS-B fractions can be recognized by the following properties: a strongly negative rotation, a low carbazole/orcinol ratio, and resistance to testicular hyaluronidase, while the heparitin fractions show a positive rotation, contain glucosamine

† A large volume of ethanol, approximately 200 ml, is important since the complex is soluble in low alcohol concentration.

‡ We are greatly indebted to Dr. G. A. Jervis for this autopsy specimen and for urine from Case No. 5.

§ The heparitin sulfate(5) was a side product in production of heparin. It will be described later. We thank the Upjohn Co. for kindly supplying a sample.

instead of galactosamine, have a high carbazole/orcinol ratio, and are resistant to testicular hyaluronidase. In addition, they were digested by an enzyme obtained from a microorganism adapted to heparin(9). The urine of Case #1 yielded 175 mg or 66 mg per 1000 ml of a fraction with the typical solubility and properties of ChS-B. On increasing the alcohol concentration, 2 additional fractions were obtained representing a polysaccharide mixture of approximately the same quantity, half of which was probably heparitin sulfate. The remainder has not been identified. From the urine in Case #2, 246 mg or 87 mg per 1000 ml of an impure fraction were obtained which, on the basis of analysis and resistance to testicular hyaluronidase, consisted mainly of ChS-B. The second fraction, obtained by increasing the ethanol concentration (71 mg or 25 mg per 1000 ml), was mainly heparitin sulfate. Cases #3 and #4 were siblings. The girl (Case #4), who had as yet only minimal symptoms of the disease, excreted 49 mg or 45 mg per 1000 ml of ChS-B and 51 mg of a mixture containing apparently only a small quantity of heparitin sulfate, while the remainder was a chondroitin sulfate of undetermined type. The older brother, (Case #3) who had a more advanced form of the disease, excreted 243 mg or 137 mg per 1000 ml of ChS-B and, in addition, 81 mg of a fraction similar in composition to that of his sister.

From the urine of Case #5 no ChS-B was obtained, and only 77 mg, or 79 mg per 1000 ml of a heparitin sulfate fraction. This fraction, however, showed an unexplained low sulfate value. From the liver of Case #6, a total of 1.75 g or 2.37% of the dry weight of the defatted tissue was isolated and identified as heparitin sulfate. In addition, 243 mg of a crude polysaccharide was obtained which, on refractionation, did not contain a significant amount of ChS-B.

In addition, 24 hour specimens of urine from the father (1790 ml) and mother (1260 ml) of Cases #3 and #4 were examined. These urines yielded 25 mg and 40 mg, respectively, of very impure fractions containing, by analysis, only 6 to 10 mg of unidentified sulfated polysaccharides. As additional controls, 3- and 5-day collections of children of 10 and 1½ years of age, admitted to the hospital for minor surgical procedures, were collected. In identical procedures of precipitation and fractionation, the urines (1.8 l and 3.9 l, respectively) yielded 20 and 30 mg, respectively, of sulfated polysaccharide with a normal ratio of carbazole to orcinol. No precipitate was obtained at 20% EtOH. We conclude from these data that these urines contained no demonstrable quantities of ChS-B. From normal urine, chondroitin sulfate fractions (7-8 mg/day) hydrolysable by testicular hyaluronidase were isolated(7). ChS-B or heparitin sulfate has not been found in normal urines. Four of the 5 patients with gargoylism excreted large amounts of sulfated mucopolysaccharides which consisted mainly of ChS-B. In addition, 4 cases excreted at least one other mucopolysaccharide fraction, apparently identical with heparitin sulfate. The latter varied in concentration from approximately 10% to 70% of the major fraction. One patient excreted only heparitin sulfate. From the liver of one autopsied case, large quantities of heparitin sulfate were isolated. A polysaccharide of apparently similar nature was isolated from the liver of a case of gargoylism by Stacey and Baker(10). In none of the patients' urines were significant quantities of ChS-A or C detected, which, at least in young individuals, are the main sulfated mucopolysaccharides of connective tissue. Table II contains the significant clinical data for each of the patients and the type of mucopolysaccharide isolated.

Discussion. ChS-B occurs normally in all connective tissue with the exception of cartilage, bone and cornea, while heparitin sulfate has been isolated only from lung, aorta, and in large quantities from amyloid liver. Dorfman and Lorincz also isolated ChS-B and a heparitin-like fraction from the urine of a female patient with gargoylism. Hence, the excretion and storage of these mucopolysaccharides appear to be a distinctive feature of this disorder.

The evidence for a genetic origin of gargoylism has been comprehensively reviewed by McKusick(11) who suggests that there are at least 2 distinct genotypes—a sex-linked recessive and an autosomal recessive form.

TABLE II. Clinical Findings of the Cases of Gargoylism Studied.

Patient	Age (yr)	Sex	Race	"Gargoyle habitus"	Skeletal lesions	Mental retardation	Corneal opacities	Hepatosplenomegaly	Cardiac abnormalities	Gibbus	Mucopolysaccharides
1.†	6	♂	N	+++ no dwarfism	++	++	0	+++	++	0	Urine, Ch-SO_4B, Hep-SO_4
2.†	12	♂	N	+++	++	+	±	+++	+++	0	*Idem*
3.*	8	♂	W	++++	+++	++	+++	+++	±	+	"
4.*	$1^{11}/_{12}$	♀	W	+	+	+	++	+	0	0	"
5.	7	♂	W	++	++	++	0	yes	?	?	Urine, Hep-SO_4 only
6.‡	10	♂	W	++++	+++	+++	0	"	"	"	Liver, Hep-SO_4 only

Severity of lesions: 0 - ++++. ±, cardiac abnormalities: organic murmur and cardiac enlargement.

* Sibling pair; 2 normal male siblings. † History of a similarly affected maternal uncle and normal female sibling(s). ‡ Parents' first cousins; affected male siblings.

The pedigrees of Cases #1 and #2, and of Cases #3 and #4, respectively (Table II), are compatible with these two different modes of inheritance. An analysis of the chemical findings in these 4 patients, according to the postulated genotype, did not show an obvious difference in the pattern of urinary mucopolysaccharides.

It is difficult to explain gargoylism in terms of a single metabolic defect since two mucopolysaccharides of different composition and structure appear to be involved. Further, the absence, in these cases, of significant quantities of other mucopolysaccharides excreted in the urine of normal individuals, such as ChS-A and C, and hyaluronic acid, appears to exclude a general aberration in the metabolism of mucopolysaccharides, hexuronic acids, hexosamines or sulfate.

In view of the large quantities of mucopolysaccharides excreted in the urine, it seems probable that in gargoylism these substances are produced in excess. Other possibilities include an inability to bind the polysaccharides at their normal sites or a defect in their metabolic breakdown.

It has been pointed out previously(8) that the mucopolysaccharides produced by connective tissue in different sites fall into distinct patterns. Furthermore, tumors of mesodermal origin contain only one mucopolysaccharide and not mixtures of various polymers (8). Therefore, it seems possible that connective tissue cells each produce only one specific type of product, *e.g.*, cells producing hyaluronic acid cannot produce any of the chondroitin sulfates. A tentative explanation of what appears to be an overproduction of certain normal polysaccharides in gargoylism might be sought in a genetically determined error of differentiation of the fibroblasts. The excessive production of polysaccharides results in the accumulation of these substances in connective tissue cells as well as the cells of many organs and the excretion of large quantities in the urine.‖ This hypothesis is consonant with the pathologic observations of Lindsay(12), *et al.* and others (see 11) which suggest that gargoylism is both a generalized disorder of the connective tissue and a storage disease. The precise nature of these two aspects has not been defined.

Summary. 1. From the urine of 4 out of 5 patients with gargoylism, a mixture of mucopolysaccharides was isolated which could be identified as ChS-B and heparitin sulfate—with the former predominating. The urine of one patient, and the liver of another, yielded only heparitin sulfate. 2. The type of polysaccharide excreted by these patients had no obvious correlation with the severity of the disease or the postulated mode of inheritance suggested by the history. 3. It is suggested that gargoylism represents an overproduction of certain mucopolysaccharides due to a genetically determined error of differentiation

‖ Storage of lipid, especially in brain and, in part, in liver has been regarded by Uzman(2) and by Brante(1) as a secondary effect which results from the derangement of normal cellular function.

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Failure to Sensitize to Autologous Skin.* (23715)

STANLEY A. ROSENTHAL, RUDOLF L. BAER AND BLANKA HAGEL
(Introduced by M. B. Sulzberger) (With technical assistance of Gerald Kaplan)
Department of Dermatology and Syphilology, New York University Post-Graduate Medical School and Skin and Cancer Unit, University Hospital, N. Y. City

The possibility that human beings could develop allergic sensitivity to their own skin, either alone or in combination with foreign materials, has been considered for many years. In clinical dermatology this concept is so ingrained that the diagnoses of autosensitization dermatitis and autoeczematization are in common use in some dermatological departments. Detailed descriptions of the phenomena which denote "autosensitization" have been given (Whitfield(1), Haxthausen (2)) and studies by means of skin and serologic tests using extracts of skin have been carried out (Templeton(3), Cormia(4)). There is, however, a scarcity of basic immunologic studies in this highly important segment of dermatologic investigation. Hecht, Sulzberger and Weil(5) produced precipitating antibodies in rabbits against homologous skin by injecting them with skin adsorbed on aluminum cream, and with staphylococcus toxin. They found that rabbits with these antibodies in their blood developed skin lesions when their skin was subjected to certain non-specific traumas, while rabbits not having such circulating antibodies did not react in such a manner. Voisin and Maurer (6) injected 6 rabbits with homologous skin and adjuvants. When subsequently they grafted the injected rabbits with skin from the donor animals, they observed the development of peculiar cutaneous lesions. Such lesions did not develop in animals which had received the skin-adjuvant mixture, but no skin grafts. Furthermore, 4 of the 6 test animals subsequently rejected autografts, an event which Voisin and Maurer attributed to autosensitization to skin.

In the present studies in guinea pigs we used technics similar to those which have been reported to have been successful in engendering experimental sensitization to homologous or autologous organs in experimental animals, using a variety of tissues: kidney(7), lens(8), nervous tissue(9-12), tumor(13) and testes (14).

Method. Emulsions of autologous skin were prepared as follows: guinea pigs, after having been clipped and shaved on the flank using a germicidal soap lather, were placed under general anesthesia with sodium pentothal. After sponging the skin with 0.1%

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