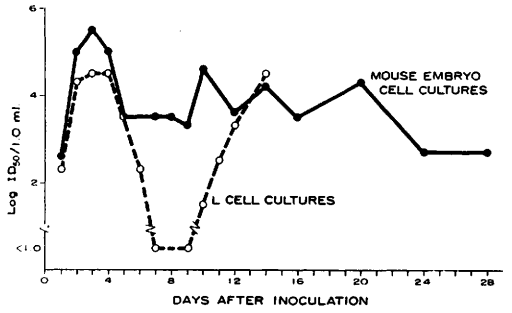

FIG. 4. Growth Curve of Mouse Hepatitis Virus in Mouse Embryo and L Cell Cultures as Determined by Assay in L Cell Cultures

detectable level only to reappear after the cell sheets had again grown out.

Discussion. Interest in mouse hepatitis viruses has derived largely from the activation of such latent agents in the attempt to transmit human hepatitis and leukemia. The discovery of these agents has also afforded a convenient model for laboratory study of problems relating to a viral hepatitis such as the effect of diet(16) and of the mechanism of transaminase elevation(17). The availability of a convenient *in vitro* method for detection of at least one member of the group should facilitate such studies.

It is of interest that a single passage of the virus under study in a susceptible cell system rendered it capable of multiplication in another system consisting of cells in which initially replication did not occur. Further investigation of this phenomenon seems desirable as the results may suggest procedures whereby adaptation of virus to an initially resistant cell can be accomplished.

Summary. The data presented indicate that MHV (Balb C) multiplies in mouse embryo cell cultures and, after passage in that system, in mouse kidney epithelium and L cells. In all these systems a cytopathic effect was observed after serial passage. These observations provide convenient techniques for studying *in vitro* this and perhaps other strains of mouse hepatitis virus.

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Urinary Mucopolysaccharide Excretion in the Sex-Linked Form of the Hurler Syndrome.* (26987)

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Hurler's syndrome (lipochondrodystrophy, gargoylism) is an hereditary aberration of the metabolism of mucopolysaccharides in human

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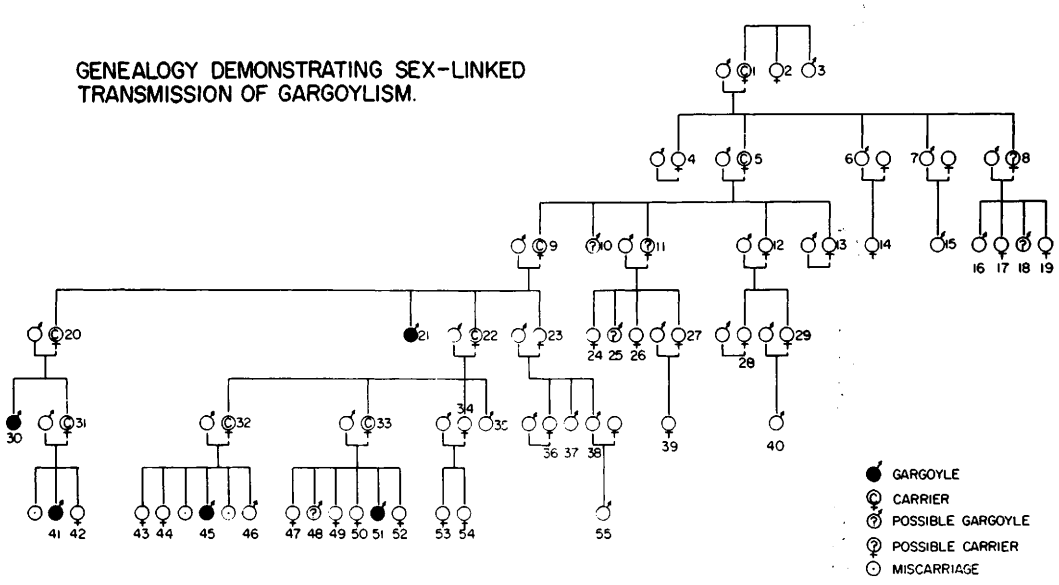

FIG. 1

beings, characterized by grossly defective mental and physical development and early, usually prepubertal, death(1). In the majority of the cases described in the literature, the disease is transmitted as an autosomal recessive trait although partial expression may occur in heterozygotes. There is also a somewhat rarer sex-linked mode of transmission in which the disease is usually manifested later in life, is restricted to males, and would appear to be clinically less severe.

Brante(2) reported that the Schiff-periodate staining material in the tissues of gargoyles was a complex acid mucopolysaccharide containing hexosamine, sulfate, and uronic acid moieties. Excretion of mucopolysaccharides in the urine of gargoyles in quantities much greater than normal has been described (3,4). The excreted material is largely composed of chondroitin sulfate B and heparin monosulfate. Although at least 2 mucopolysaccharides of differing structure and composition are affected by the disease these 2 classes of compounds do have some structures and precursors in common(5), and it is possible to postulate that a single enzymatic defect underlies the complex symptomatology.

The present study was designed to develop methods for the determination of mucopolysaccharide levels in urine and to apply them to members of a family in which the sex-linked form of gargoylism was encountered. The family is similar to that described by Beebe and Formel(6) although none of the affected individuals have lived to maturity. The possibility of using chemical methods for detecting carriers of the trait was also investigated. A preliminary report of this work has appeared(7).

The genealogy of the family studied is shown in Fig. 1. Individuals from 4 generations have been available for testing. Information about others was derived from photographs, family Bibles, and cross-checked interviews with surviving members of the family. It is a genealogy typical of the transmission of a sex-linked trait. Almost exactly 50% of the females have been carriers and nearly 50% of the males have been clinically affected. There have been no clinically affected females. There are 3 gargoyles in the most recent generation and 2 well authenticated cases in the preceding 2 generations. The genealogy indicates that 7 females, not including the first generation, have been carriers. It is not known whether the trait arose as a mutation in #1 or #5 or was present in the family at an earlier time.

Experimental. Twenty-four hour urine samples were obtained from all of the available members of the family in the direct hereditary line. Aliquots of the urine were

dialyzed exhaustively against running distilled water in the cold and diluted 1:1 with distilled water. This dialyzed and diluted urine was then analyzed for total mucopolysaccharide by the following methods.

1. *Precipitation test:* This is a quantitative modification of Dorfman's qualitative test for mucopolysaccharides(8) and is based on the fact that these highly charged macromolecules combine, at pH 3.7, with bovine serum albumin to form an insoluble complex which exists as a turbid solution for about 20-30 minutes before precipitation. A standard curve is prepared using chondroitin sulfate-A as the known mucopolysaccharide, and turbidity is measured at 600 mμ. Linear relationships were obtained at values ranging from 0-60 mg of chondroitin sulfate-A/liter. The turbidities observed with other acid mucopolysaccharides, while different, were of the same order of magnitude.

2. *Reducing test:* Mucopolysaccharides, like most other polysaccharides, have little reducing power. Upon acid hydrolysis the glycosidic bonds break, freeing most of the reducing anomeric carbons. When dialyzed urine is initially analyzed for reducing power and then acid hydrolyzed and tested again, the increase in reducing groups is proportional to the amount of mucopolysaccharide present. Large quantities of glycoproteins and mucoproteins would be the only interfering substances to be found in dialyzed urine. Since it is the change in reducing power which is being measured, large initial values such as would be obtained with the urine of diabetics will not yield false positives.

The optimum time and HCl concentration for polysaccharide hyrdolysis were determined to be 0.75 *N* HCl and 60 minutes. The Folin-Wu method(9) was used to measure reducing power.

3. *Periodate oxidation test:* Adjacent hydroxyl groups or adjacent hydroxyl and amino groups in organic compounds are quantitatively oxidized by periodate at pH 4.0. Mucopolysaccharides contain many such groups and are readily oxidized by periodate. Once again, only glycoproteins and mucoproteins, in great quantity, could interfere. The assay method used is a potentiometric titration of the iodine liberated from a KI solution by the excess periodic acid left in a mucopolysaccharide-periodic acid reaction mixture(10).

The sensitivity of this test may be approximately doubled by preliminary hydrolysis of the urine as described for the reducing test.

Results. The results of the application of these 3 tests to the urines of members of the affected family are shown in Table I.

TABLE I. Quantitative Estimation of Urinary Mucopolysaccharides.

	Genealogy No.	Precipitation[1] Test	Reducing[2] Power	Periodate[3] Uptake	Periodate[3,4] Uptake
Gargoyles	41	122	81	7.0	9.0
	45	96	130	9.4	15.6
	51	33.5	28.2	3.4	3.6
Known Carriers	9	6.8	17.2	3.6	5.4
	20	1.8	2.5	2.6	
	31	4.8	3.8	3.7	
	32	6.2	7.5	2.6	
	33	3.7	.9	2.5	
Sib of Gargoyles					
Male	46	9.7	34.7	3.3	6.7
Female	42	8.7	1.8		
	43	13.0	13.5	3.6	
	44	9.8	17.2	3.7	
	47	10.8	15.9	3.5	
	49	6.2	1.2	3.2	
	50	6.5	6.2		
	52	10.8	7.7	3.1	

[1] Expressed as mg/L chondroitin sulfate-A.
[2] " " mg/L glucose.
[3] Milliequivalents periodate/L.
[4] Following acid hydrolysis.

TABLE II. Relationship of Urinary Mucopolysaccharides to Creatinine Excretion

Genealogy no.	Specific gravity	Creatinine mg/ml	Precipitation test mg/L	Uronic A. mg/L	Precipitation test: creatinine ratio	Uronic A.:creatinine ratio
20 (C)	1.012	.60	3	10	5	17
31 (C)	1.013	.80	8	15	10	19
	1.027	.82	10	—	12	—
32 (C)	1.013	.73	8	22	11	30
	1.012	.30	7	8	23	27
33 (C)	1.018	1.77	22	24	12	14
	1.013	.52	4	12	8	23
41 (G)	1.013	.40	166	75	415	188
	1.021	.76	112	86	147	133
42	1.022	.67	18	20	27	30
43	1.026	1.20	19	30	16	25
44	1.021	1.27	28	31	22	24
45 (G)	1.023	.66	—	69	—	105
	1.013	.33	84	92	255	280
46	1.024	.90	17	40	19	44
	1.023	.77	7	19	10	25
47	1.008	.47	4	13	8	28
49	1.021	.97	12	22	12	23
50	1.024	1.17	10	27	9	23
51 (G)	1.007	.17	62	49	365	288
	1.014	.32	60	40	188	125
52	1.008	.27	5	11	18	41

The precipitation test proved to be the most rapid method. It is clear from the table that clinically proven gargoyles excrete at least 3 times as much mucopolysaccharide as do other individuals. From a survey of a large number of normal individuals, it was ascertained that an arbitrary value of 7-9 mg chondroitin sulfate-A per liter could be set at the upper limit of normal. Usually, normal values ranged from 1-4 mg per liter.

The change in reducing power was perhaps the most sensitive of the tests with an arbitrary value equivalent to 20 mg per liter of glucose established as the upper limit of normal in a survey of a large number of non-gargoyle urines.

The periodate test is of considerably less value. Even after hydrolysis which doubled the periodate uptake, the differences between normal individuals and gargoyles were at most 1½-to 2-fold. Due to the high oxidation potential of the periodate, other substances in the urine may also be oxidized, although dialysis should have decreased such interference.

The data given represent only single determinations. Parallel determinations on the same urine samples yielded the same figures. When more than one urine sample from any one individual was tested the values, while they showed considerable variation, still fell within the characteristic range for affected or normal individuals.

A further series of studies was carried out using large pooled samples of urine from some of the individuals in the genealogy. As a means of correlating the mucopolysaccharide excretion with an independent measure of metabolic activity, the relationship of urinary mucopolysaccharide excretion to creatinine excretion was measured using the precipitation test and the uronic acid assay of Dische(11) and the creatine analysis of Bonsnes and Taussky(12). The results are shown in Table II. In the cases of known gargoyles, results of analyses of different samples are shown as examples of the variations found.

In general, the various methods for determining urinary mucopolysaccharide levels yielded parallel data which indicate that clini-

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)

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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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