Distinction Among Four Forms of Hurler's Syndrome.*† (28923)

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The evidence in this study extends the hypothesis that Hurler's syndrome is not a single entity but actually several, heritable disorders of connective tissue that are associated with abnormalities of acid mucopolysaccharide (AMPS) metabolism. Four forms were distinguished on the basis of their urinary AMPS excretion pattern.

Two of the forms studied were classical autosomal recessive Hurler's syndrome (described by Hurler(1)) and classical sex-linked recessive Hurler's syndrome (described by Hunter(2,3)). In general, these two entities are very similar clinically. Patients with either syndrome may show dwarfism, grotesque skeletal deformity, restriction of joint movement, deafness, hepatosplenomegaly, cardiac abnormality, and mental retardation. The evidence indicates that the sex-linked cases are in general less severely affected, live longer, show no cloudy cornea, and little or no gibbus(3). Both forms store an excess of chondroitin sulfate B (CSB) and heparitin sulfate (HS) in the tissues and excrete an excess of these AMPS in the urine, but no differences have been noted in their excretion pattern(15). However, because of the consistent clinical differences, the question arose whether the 2 forms could be distinguished by their AMPS excretion pattern, and if so, whether the differences could be correlated with their clinical features.

The 2 atypical forms of Hurler's syndrome studied show only a few of the features of gargoylism. This will be discussed later. In any case such variation has long been noted for other diseases. The question always arises whether the variants are the result of the same gene with variable expressivity, or the result of different genes that produce similar phenotypes. On a clinical basis, only the expression of Hurler's syndrome varied in the same pedigree could establish which of the alternatives is correct; however, some measurement closer to the metabolic defect, such as their AMPS abnormality, might show a primary genetic difference if one exists.

Methods. The urinary AMPS were isolated and purified essentially by the methods of Di Ferrante and Rich(4). The polysaccharides were then dissolved at 10 mg/ml in a solution of 2.5% calcium acetate and 0.5 N acetic acid and fractionated with ethyl alcohol. Fractionation was accomplished by adding absolute ethanol to the desired concentration (as given in the text) and storing at 3°C overnight. Uronic acid was measured by the carbazole method of Dische(5), the orcinol method of Brown(6) and a modification of the naphthoresorcinol method of Pelzer and Staib(7). The naphthoresorcinol reagent was dissolved at 0.25% rather than 0.5% and incubation time was increased to one hour. This resulted in a greater color yield (40%) for CSB, while HS and chondroitin sulfate A (CSA) still yielded about 3% (expressed per unit weight of AMPS in terms of a standard glucuronic acid solution). The ratio of glucosamine to galactosamine was determined by the ninhydrin paper method of Stoffyn and Jeanloz(8). Sulfate was determined by barium chloride titration in the presence of rhodizonate(9). Paper chromatography was accomplished with slight modification of the method of Spolter and Marx(10). The AMPS excretion pattern was studied in detail for 3 typical autosomal cases and 3 sex-linked cases. There were 2 different atypical forms of Hurler's syndrome available for study. These will be discussed more fully together with the AMPS analyses.

Results. Classical autosomal recessive cases. About 400 mg of mucopolysaccharide were

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TABLE I. AMPS Fractions from Patients with Autosomal Recessive and Sex-Linked Recessive Hurler's Syndrome.

Patient	Sex	Age (yr)	Liters urine	Alcohol fraction (%)	mg	% carbazole	% naphtho-resorcinol	% orcinol
1	♂ †	7½	1.56	20	326.7	13.1		44.0
				40	205.1	28.5		33.1
				65	93.1	21.0		26.0
2	♀ †	6	.45	25	12.8	11.7	34.2	36.0
				50	17.8	19.6	23.4	26.8
				65	5.3	17.3	13.7	19.9
3	♀ †	17	1.08	30*	25.1	14.8	43.0	55.4
				60*	26.0	22.2	22.0	44.5
4	♂ ‡	11	.500	20	38.4	13.9	32.5	42.5
				40	10.8	32.7	18.2	29.3
				65	9.7	28.5	16.4	26.0
5	♂ ‡	12	.500	20	34.0	13.5	35.3	40.5
				40	35.6	34.0	17.0	28.2
				65	18.5	29.5	17.6	24.0
6	♂ ‡	19	.500	20	40.0	17.0	38.2	44.5
				40	41.5	32.0	15.8	26.0
				65	36.5	32.8	15.2	32.2

* Fractionated as sodium salt. † Autosomal recessive. ‡ Sex-linked recessive.

isolated per liter of urine from the male patient and about 80 mg and 50 mg were isolated from the 2 female siblings, respectively (Table I). The excretion rate for the male was the highest encountered in this study, and is also higher than any reported in the literature.

a. *Acid mucopolysaccharides of the lower alcohol fractions.* The high naphthoresorcinol and orcinol values in relation to the low carbazole values indicate that very little, if any, of the material in the low alcohol fractions is heparitin sulfate, and that it is composed almost exclusively of chondroitin sulfate B (Table I). A typical ammonium formate paper chromatogram(10) as shown in Fig. 1, indicates that the 20% alcohol fraction from the male patient is composed of 2 components of chondroitin sulfate B. To determine the nature of these two components, this fraction containing 326 mg of mucopolysaccharide was refractionated as the calcium salt with ethyl alcohol. It was demonstrated that about one-half of the material precipitated with 10% alcohol and that a large share of the remainder precipitated with 25%. It is interesting that the 25% fraction was not precipitable thereafter with 20% alcohol and that some material did not precipitate until alcohol concentrations in excess

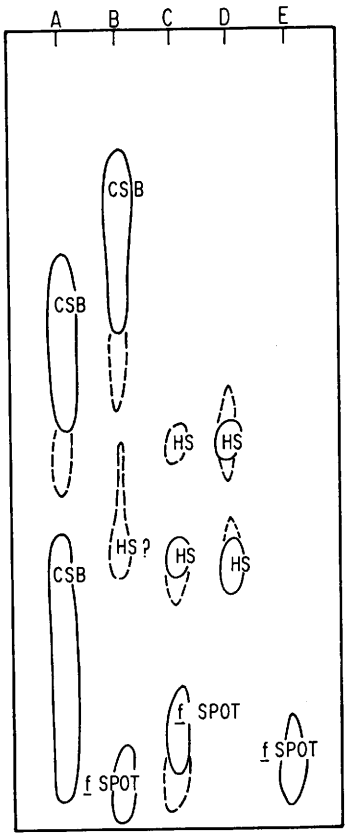

FIG. 1. Typical ammonium formate chromatogram of AMPS from autosomal cases. (A) 20% fraction, (B) chondroitin sulfate B, (C) 40% fraction, (D) heparitin sulfate, (E) 65% fraction.

TABLE II. Urinary Acid Mucopolysaccharide Analyses Reported for Autosomal Recessive Cases.

Source	Patient	Sex	mg/liter	mg/fraction	% CSB*	% HS*	% carbazole	% orcinol	$GalNH_2$	$GluNH_2$	Optical rotation
Meyer(15)	3	♂	182	243	95	5†	12.5	40.6	4+	Trace	−60
				81			11.2	21.5	3+	1+	
	4	♀	91	49	88	12†	12.9	36.6	4+	Trace	
				51			15.7	21.5	3+	1+	
Meyer(16)	2	♂	58		74	26	Estimates obtained mainly from carbazole to orcinol ratios and $GalNH_2$ to $GluNH_2$ ratios, but values were not published.				
	3	♀	78		78	22					
	7	♀	74		90	10					
	9	♀	132		72	28					
Meyer(14)		♀	81		90	10	Estimates made on same basis as Meyer(16).				
Dorfman(17)		♀			Major	Minor	Estimates obtained by slab electrophoresis.				
Sanfilipo(11)		♀			"	"	Estimates obtained by paper chromatography.				

* Relative amounts of chondroitin sulfate B and heparitin sulfate.
† Values estimated by the present authors.

of 25% were reached. As expected it was shown that the 25% fraction exhibited a faster mobility on the ammonium formate paper chromatograms than the 10% fraction; however, they both show almost identical chemical analyses which indicate they are chondroitin sulfate B (in each case the 10% fraction is given first): Carbazole 12%, 15.9%; orcinol 42%, 46%; sulfate 13.8%, 13.5%; and optical rotation $[\alpha]_D^{28} = -61$ (Cl, H_2O) and $[\alpha]_D^{28} = -57$ (Cl, H_2O). This would indicate that the difference might be one of molecular weight. A preliminary analysis indicates a slightly lower molecular weight for the material which precipitated at higher alcohol concentration (7,000-11,000 as opposed to 11,000) as determined by the ultracentrifuge.

The other 2 autosomal cases possessed a fraction of chondroitin sulfate B precipitable with 10% alcohol, and fractions of chondroitin sulfate B precipitable with higher alcohol concentrations were obtained. Also, because of these different fractions of chondroitin sulfate B, the acid mucopolysaccharides that precipitate at less than 25% alcohol show a good deal of streaking on the ammonium formate paper chromatograms. This probably accounts for the long complex obtained with the ammonium formate paper chromatograms by Sanfilippo and Good(11) when they chromatographed the mucopolysaccharides isolated from the urine of gargoyles.

b. *Acid mucopolysaccharides that precipitate at higher alcohol concentrations.* The material that precipitates at higher than 20% alcohol accounts for approximately 50% of the total AMPS isolated from the urine samples. The uronic acid values for these fractions suggest the presence of about equal amounts of choidroitin sulfate B and heparitin sulfate (Table I). For this reason, it was surprising that with ammonium formate paper chromatography most of the material migrated like the fastest moving spot (*f* spot) of the chondroitin sulfate B lung preparation (to be discussed), although some corresponded to heparitin sulfate (Fig. 1). It was established by this method that not more than 10% of the total material excreted corresponded to heparitin sulfate for the male and even less for the 2 females. However, the proportion of glucosamine to galactosamine, estimated for the male patient, indicates that roughly 20% of the total AMPS excreted is heparitin sulfate (Fig. 1). (This corresponds closely to the results published by Meyer *et al,* for 7 autosomal recessive cases, Table II.) This suggests that some of the fastest moving component, the *f* spot as shown by the paper chromatograms, is heparitin sulfate.

A preliminary analysis of the *f* spot material also indicates that it may be similar to the CSB-like material isolated by Hoffman, Linker and Meyer from pigskin(12). Furthermore, the analogous component of the

TABLE III. Urinary Acid Mucopolysaccharide Analyses Reported for Sex-Linked Recessive Cases.

Source	Patient	Sex	mg/ liter	mg/ fraction	% CSB*	% HS*	% carbazole	% orcinol	$GalNH_2$	$GluNH_2$	Optical rotation
Meyer(15)	1	♂	150	175			14.4	37.4	4+	Trace	−15
				88	60	40‡	21.6	36.5	2+	2+	
				75			27.9	24.4	2+	2+	− 1
Meyer(15)	2	♂	112	246	53	47‡	16.3	29.6	3+	2+	−38
				71			31.9	24.5			+27
Meyer(16)	5	♂	154		61	39	Estimates obtained mainly from carbazole to orcinol ratios and $GalNH_2$ to $GluNH_2$ ratios.				
	6†	♂	33		50	50					

* Relative amounts of chondroitin sulfate B and heparitin sulfate.
† Patient that McKusick(3) has shown to be a sex-linked recessive case.
‡ Values estimated by present authors.

CSB standard presents a similar chemical analysis. It would seem quite likely that a third AMPS is being excreted in excess by these individuals. This requires further investigation.

Sex-linked recessive Hurler's syndrome. The acid mucopolysaccharide fractions obtained from these children are shown in Table I. The youngest child (age 11) excreted about 98 mg of acid mucopolysaccharide per liter of urine; his full brother (age 12) excreted approximately 176 mg per liter; and their half brother (age 19) excreted 236 mg per liter. The carbazole values (approximately 13%) and naphthoresorcinol values (about 35%) for the 20% alcohol fraction indicate mostly chondroitin sulfate B. A typical ammonium formate paper chromatogram for these individuals demonstrates that the 20% fraction is composed of 2 components of chondroitin sulfate B. It was found by refractionation of the 20% fraction that, like the autosomal cases, it was possible to obtain a 10% fraction and a 25% fraction. These probably correspond to the original 2 spots distinguished by the ammonium formate paper chromatograms. These data indicate no obvious difference between the chondroitin sulfate B excreted by the sex-linked and autosomal cases. However, in contrast to the AMPS of the autosomal cases, only 30 to 35% of the total material precipitated at 20% alcohol.

Examination of the uronic acid values for the 40% and 65% fractions shows, in contrast to the 1:1 ratio of carbazole to naphthoresorcinol found for the autosomal cases, a C/N ratio of 2:1. This finding, along with that of less chondroitin sulfate B in the 20% fraction, indicates that the AMPS of the sex-linked cases consists of considerably more heparitin sulfate. This conclusion is substantiated by the greater amount of glucosamine in proportion to galactosamine in the higher alcohol fractions and by the ammonium formate paper chromatograms. It was shown by the latter method that the two higher alcohol fractions contain heparitin sulfate and material that migrates like *f* spot. It was estimated by this method that approximately 30% of the total material excreted by these individuals is heparitin sulfate. However, this is probably an underestimate since total glucosamine content amounts to 45% to 50% of the hexosamine. This estimate is close to the results published for 4 sex-linked cases (Table III).

Distinction between the sex-linked and autosomal cases. The data presented indicate that the sex-linked cases may excrete significantly more heparitin sulfate in proportion to total amount of acid mucopolysaccharide excreted than do the autosomal cases. These data are fully substantiated by analyses reported in the literature for similar patients. Table II shows a summary of the results reported for females (who are presumably autosomal recessive cases) and known autosomal recessive male patients. Table III shows the analyses for the sex-linked recessive cases reported in the literature. These data serve to distinguish between the 2 hereditary forms.

Finally, the above findings indicate that it should be possible to distinguish between

TABLE IV. Comparison of C/O and C/N Ratios Obtained for Patients with Hurler's Syndrome.

	Carbazole/orcinol		Carbazole/naphthoresorcinol	
	Sex-linked	Autosomal	Sex-linked	Autosomal
	.53	.53	.72	.60
	.79	.45	1.03	.57
	.87	.51	1.17	.49
	.62*	.34*		
	.70*	.49*		
Mean	.70	.46	.97	.55

* Meyer, K. *et al*, PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 275.

these 2 disorders on the basis of the carbazole:orcinol ratios (C/O) and carbazole: naphthoresorcinol ratios (C/N) computed for the total acid mucopolysaccharide sample. This was found to be the case. In Table IV the ratios are shown for 5 sex-linked cases and 5 autosomal cases. The ratios for 4 of these patients were obtained from the data of Meyer and his associates as shown in Tables II and III. The other ratios were calculated from the values presented in Table I. The 5 sex-linked cases show a mean C/O ratio of 0.70 and a range of 0.53 to 0.87. The C/O ratios for the autosomal cases have a mean of 0.46 and a range of 0.34 to 0.53. A group comparison test indicates a highly significant difference between the means of the 2 samples ($p<0.01$). The C/N ratios seem to be a better means of distinguishing between these 2 groups; for example, the C/N ratios distinguish between the one sex-linked and the one autosomal case that had overlapping C/O ratios.

Atypical Hurler's syndrome patients clinical symptoms. Patient 1. A white female who recently died at age 17 with respiratory infection. She has one normal brother and one similarly affected brother (patient 2) who are still alive. The parents are both normal and unrelated. There are no other known cases on either side of the family.

This girl began walking and talking in a normal manner, but by 3 years of age, she became hyperkinetic and "withdrawn" from her surroundings. From this time progressive neurological deterioration was manifested by loss of memory, vocabulary, comprehension, and orientation. At 4 years of age, there was nothing suggesting a significant anomaly of bony development. When examined at 10 years of age the patient had lost the ability to walk. She also showed marked peripheral vascular instability manifested by dark cyanotic feet. Other significant clinical features presented by this patient were stiff neck, inability to swallow foods normally, hepatosplenomegaly, and profuse growth of dark hair. The patient remained bedridden until death.

Patient 2. This child, like his sister, seemed to progress well in neurological achievement during his first 2 years of life. At 2½ years of age, he became acutely ill with poliomyelitis. By 5½ years of age, the clinical symptoms were similar to those of his sister. The most significant finding was a general neurological impairment manifested by mental deficiency and irregularly distributed motor disability. The child also had hepatosplenomegaly and profuse hair growth. It is perhaps significant that at 2 years of age the skin showed a rash which was due to ruptured blood vessels.

Two other males were found with this disease but were not observed by the authors.

It was demonstrated that the 4 patients just described excrete mostly heparitin sulfate in the urine. Approximately 70 mg of mucopolysaccharide were isolated per liter from patient #1, 87 mg per liter from patient #2, 100 mg per liter from patient #3, and about 32 mg per liter from the fourth patient.

In Table V is shown the analysis for the acid mucopolysaccharide isolated from urine of the second patient after testicular hyalu-

TABLE V. Testicular Hyaluronidase Treated Fractions from Patient with "Heparitin Sulfate Disease."

Alcohol fraction (%)	mg	% carbazole	% orcinol	% sulfate	A.F.*
30	6	36.3			slow spot
40	26	45.1	12.9	19	mostly slow
50	32	42.1	12.8		both spots
65	25	28.2	10.0	16	fast spot

* Ammonium formate paper chromatograms show 2 spots for heparitin sulfate.

TABLE VI. Urinary Acid Mucopolysaccharides from 2 Atypical Hurler's Syndrome Patients.

	Liters urine	Alcohol fraction (%)	mg	% carbazole	% orcinol	Optical rotation	% sulfate
Male, age 31	1.4	25	22.5	17.0	45.5	−47°	14
		65	51.2	20.5	27.0		
Female, age 29	1.4	25	23.4	15.0	46.6	−42°	13
		65	38.3	22.2	25.5		

ronidase treatment. Previous to this treatment it was shown that the material contained a spot that corresponded to chondroitin sulfate A on the ammonium formate paper chromatograms, although most of the material migrated like heparitin sulfate. After enzyme treatment the only spots present correspond to the standard heparitin sulfate. It is interesting that even though the 30% and 40% fractions contain mainly the slower moving spot for heparitin sulfate, while the 65% fraction contains mainly the faster moving spot, there are no obvious chemical differences between these fractions. Also there are no differences between these fractions and samples of heparitin sulfate isolated from other sources. However, since the ammonium formate paper chromatograms do not distinguish between heparin and heparitin sulfate adequately, the fractions were also tested by electrophoresis(13) to determine whether any of it corresponded to the former material. No heparin could be demonstrated.

A survey of the literature shows 7 other similar patients have been reported where an AMPS analysis has been carried out, although only a short note recently by Meyer *et al*(14) indicates that they show different clinical symptoms. Sanfilippo *et al*(11) reported 2 females and one male with this disorder; Lorincz(18) reported one female and a sibling of unknown sex; and Meyer *et al* (14) reported one male and one other patient of unknown sex. If these data are included with those of this series, then there are a total of 4 females and 5 males where the sex is known. Since the parents are normal and since there are an equal number of affected males and females, there is little doubt that these individuals are the result of an autosomal recessive gene. Also there can be no doubt that it is an entirely different genetic disease than the classical autosomal recessive Hurler's syndrome, since the apparent abnormality obviously reflects a different metabolic defect.

Patients with atypical Hurler's syndrome. ("Adult form" of Hurler's syndrome.) The clinical features of the patients in this group were discussed previously by Scheie and his associates(19). The following is a preliminary chemical analysis of the acid mucopolysaccharides excreted by 2 of these patients. One of these is a 31-year-old male and the other is his 29-year-old sister. Their parents are normal and unrelated. They also have 2 normal siblings. Both are highly intelligent and show only mild features of Hurler's syndrome. They were referred to Dr. Scheie(19) for familial corneal dystrophy.

Approximately 53 mg of acid mucopolysaccharide were isolated per liter from the male patient and 44 mg per liter from the female. Since these 2 are so mildly affected, it was somewhat surprising to find that the ammonium formate paper chromatograms indicated both chondroitin sulfate B and heparitin sulfate. This finding was substantiated by the chemical analysis as shown in Table VI. However, it was demonstrated that when the material was dissolved at 10 mg per ml in the calcium acetate buffer, 2.5% calcium acetate and 0.5 N acetic acid, none of the material precipitated with either 10 or 20% alcohol when left overnight at 3°. This is in contrast to the chondroitin sulfate B isolated from the typical Hurler's syndrome patients and from pigskin.

Discussion. The evidence indicates there are 3 forms of Hurler's syndrome that excrete mostly a mixture of chondroitin sulfate B and heparitin sulfate, and that a fourth form excretes only heparitin sulfate in excess. Since they share a similar AMPS abnormal-

ity, it is not surprising that they all resemble the classical cases of Hurler's syndrome. However, the results of this study demonstrate that all 4 can be distinguished by their acid mucopolysaccharide excretion pattern.

It is estimated that the autosomal recessive patients excrete approximately 80% chondroitin sulfate B (and chondroitin sulfate B-like material) and about 20% heparitin sulfate. These values are the average for those observed in this study and the data presented by Meyer *et al*(Table II).

The chondroitin sulfate B excreted by these individuals shows considerable streaking on the ammonium formate paper chromatograms and precipitation over a wide range of alcohol concentration. It is interesting that a large proportion precipitates as the calcium salt with 10% alcohol. This is in contrast to the 18% reported in the literature for chondroitin sulfate B from other sources. Further studies are needed to establish the significance of this observation. The chondroitin sulfate B may also be composed of more than one closely related AMPS. It was observed that a large proportion of the material present in the higher alcohol fractions has a mobility similar to the fast moving component of β-heparin (this has been referred to as *f* component). Preliminary analysis indicates a CSB-like AMPS similar to a fraction isolated from pigskin(12).

The AMPS excreted by patients with the sex-linked form of inheritance were demonstrated to be approximately 45% heparitin sulfate and about 55% chondroitin sulfate B (about 20% of it migrated like the *f* component). Again, these values are about the average for the patients in this study and for those of Meyer *et al*(Table III). There were no apparent differences in chondroitin sulfate B or heparitin sulfate excreted by these patients and the autosomal cases. However, the proportion of heparitin sulfate is more than twice that estimated for the autosomal cases. This observation is supported by the ammonium formate paper chromatograms, hexosamine values, and by the uronic acid values. It was demonstrated by the latter that the ratio of total mg of orcinol (or naphthoresorcinol) to carbazole is significantly higher for the sex-linked cases.

If the only difference in the AMPS excreted by the classical autosomal and sex-linked forms resides in the proportion of heparitin sulfate and chondroitin sulfate B, it is difficult to understand why the former are more severely affected and why they tend to have cloudy corneas and pronounced gibbus that are not associated with the latter. It would seem likely that a more fundamental reason exists for this difference. It is possible that the chondroitin sulfate B complex excreted by the 2 groups is dissimilar; however, there are no data to support this suggestion. Another and possibly more attractive theory is that the primary defect is associated with specific tissues that tend to be different for the 2 disorders; namely, it is not the accumulation of AMPS *per se* that causes, for example, cloudy corneas and gibbus, but rather a disturbance of associated tissues—tissues not specifically involved in the sex-linked cases.

It has been suggested by Meyer and his associates(14) that since chondroitin sulfate B and heparitin sulfate are unrelated chemically the genetic defect involves only the former and the over-production of heparitin sulfate is a secondary phenomenon. This is supported by experiments which show an increase of glucosamine containing polysaccharide when chondroitin sulfate B is injected into experimental animals(14). Considering that the sex-linked cases excrete about twice the amount of heparitin sulfate in relation to chondroitin sulfate B (even though the amount of chondroitin sulfate B may be as high), it would seem that the above explanation is inadequate, or the primary defective site differs which could possibly lead to a greater or lesser stimulus of heparitin sulfate excretion.

Other explanations that have been suggested by Meyer *et al*(14) are: (1) "the failure of a degrading enzyme specific for both polysaccharides; (2) the fault in synthesis of an acceptor protein, common to both polysaccharides, which binds the polysaccharides in the tissue; (3) (previously discussed); (4) a fault in the mechanism of embryologic differentiation of mesenchymal

cell lines, leading to an abnormal number and to abnormal sites of cells specialized for the production of the two polysaccharides . . ."

The last possibility has been rejected by Hessburg(20), on the basis that the AMPS and collagen synthesizing cells are derived from the same connective tissue which suggests there should be marked collagen problems, but this is not the case.

The third type of Hurler's syndrome, which has been referred to as the "adult form" for lack of a more suitable name, is also inherited in an autosomal recessive manner. The results so far obtained indicate that these patients can be distinguished from the classical cases of Hurler's syndrome by virtue of the fact that they excrete no demonstrable chondroitin sulfate B that precipitates as the calcium salt with 10% or even 20% alcohol concentration, although the results indicate that they excrete an excess of CSB and HS. It will be of considerable interest to determine whether other similar patients show the same pattern. However, it would seem significant that these individuals are atypical Hurler's syndrome patients who also seem to show a different excretion pattern than the classical autosomal cases.

It is of interest to consider the fourth form of Hurler's syndrome, those that excrete only heparitin sulfate in excess. Since it has been shown that heparitin sulfate, like heparin, is associated with blood vessels, it is not surprising that these individuals show very little skeletal defect. There is, however, mental and motor retardation, profuse hair growth, and what seem to be abnormalities associated with the peripheral blood vessels. Nothing is known concerning the pathology of this disease. However, such knowledge would provide insight into the physiological role of heparitin sulfate in the disease state and also may provide some insight into its normal metabolism. Since the disorder is clearly different and since the excretion pattern among siblings is similar, there is little doubt that this is an entirely different genetic entity. That this disease is the result of an autosomal recessive gene is shown by the ratio of affected males to affected females (1:1) and by the association of normal parents with more than one affected sibling of different sex. In the records of the Salt Lake City hospitals only 3 patients were shown to have this disorder. These patients probably account for no more than one out of every 100,000 to 200,000 people. This would correspond to a gene frequency of about 0.001 to 0.002, indicating the rarity of this disorder.

Summary. Analysis of the urinary AMPS from individuals with Hurler's syndrome indicates there are 4 forms that can be distinguished by their excretion pattern. The classical autosomal recessive form was shown to excrete significantly more chondroitin sulfate B in proportion to heparitin sulfate than the classical sex-linked cases. Both forms were shown to excrete a fraction of chondroitin sulfate B that would precipitate as the calcium salt with 10% alcohol which is in contrast to the behavior of this substance isolated from other sources. An atypical "adult form" (autosomal recessive) was shown to excrete chondroitin sulfate B and heparitin sulfate in excess, but no fraction of condroitin sulfate B was obtained that would precipitate as the calcium salt with 10% alcohol. Another atypical form, which shows primarily degeneration associated with the nervous system, was shown to be inherited in an autosomal recessive manner and to excrete only heparitin sulfate in excess.

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Effect of Restricted Access to Feed on the Serum and Liver Lipids of Swine. (28924)

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The pattern of food intake has been reported by several investigators to exert an important influence on the manner in which it is metabolized. Cohn and co-workers(1) found that chicks permitted access to food for an hour in the morning and for another hour in the afternoon after 5 weeks had higher serum cholesterol levels than chicks allowed *ad libitum* access to food. In addition, the thoracic aortas of the chicks eating twice a day had a higher incidence of atherosclerosis, which was also more severe.

Okey, *et al*(2) and Lis and Okey(3) reported that changes in the feeding pattern affect the composition of both plasma and liver lipids of rats, with most of the alterations noted in the plasma lipids. However, the total amounts of serum and liver cholesterol, triglyceride and phospholipid were not appreciably altered by feeding method.

The alterations in lipid metabolism observed in these studies as a consequence of restricted feeding are of particular interest in human nutritional studies since the dietary habits of many humans resemble the pattern of food intake observed in the restricted-fed laboratory animal. Because of the physiological similarities between the pig and the human, the following experiment was conducted to determine what effect the pattern of food intake would have on the blood and liver lipids of swine.

Materials and methods. One hundred eight weanling pigs of mixed sex and breeding were randomly assigned to 12 concrete feeding floors each with identical feeding and watering facilities. All pigs received a balanced growing ration containing 16.4% protein and a fat content of 2.7%, of which 85% was unsaturated fat from a plant source (ground milo). Four pens of 9 animals each were assigned to each of the following feeding schedules: 1) *Ad libitum* access to feed, 2) One 2-hour feeding period (8:00 a.m.-10:00 a.m.), 3) Two one-hour feeding periods (8:00-9:00 a.m., 4:00-5:00 p.m.).

The first phase of the experiment was terminated at the end of 84 days, at which time all animals were bled *via* heart puncture and the blood analyzed for serum lipids. Six animals from each of the 3 feeding schedules were selected to continue the experiment for another 90 days, with care being taken to equalize sex and breed between treatments.

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