HURLER'S SYNDROME

A Genetic Study in Cell Culture*

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Plates 1 to 3

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Hurler's syndrome (gargoylism) is a rare inborn error of mucopolysaccharide metabolism which results in an accumulation of mucopolysaccharides in various tissues of the body (1–3). The main clinical features of this syndrome are dwarfism, grotesque skeletal deformity, restriction of joint movements, deafness, hepatosplenomegaly, cardiac abnormalities, and mental retardation (4). Family studies suggest that the condition may be inherited in an autosomal recessive fashion, the classical Hurler's syndrome (5), or in an X-linked recessive form that is sometimes called Hunter's syndrome (6). Clinical, genetic, and biochemical considerations indicate that the autosomal recessive form of the disease may be further subdivided into at least four subtypes (7).

Development of the methods of tissue culture has provided an opportunity to study this group of inherited diseases of metabolism at the cellular level. This paper extends and amplifies a preliminary report in which the cells of normal subjects, affected individuals, and clinically normal carriers of the abnormal gene were cultured *in vitro* (8).

Materials and Methods

Seven families with members affected with various forms of Hurler's syndrome have been studied. All but one (L. R.) had been investigated previously in this laboratory as part of a clinical and biochemical study of Hurler's syndrome (8a). Two families (P. H., M. H., and L. R.) with the autosomal recessive form of Hurler's syndrome were studied (5). P. H. and M. H. excreted increased amounts of chondroitin sulfate B and heparitin sulfate in the urine (8a) whereas L. R. excreted an increased amount of chondroitin sulfate B while the excretion of heparitin sulfate was normal. In two families (R. B. and J. M.) the affected individuals were physically only mildly affected although they showed severe mental retardation. They excreted increased quantities of heparitin sulfate in the urine, a characteristic finding in the clinical variant of the disease called Sanfilippo's syndrome (9). In three families (M. P., F. S.,

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and F. F.), children with clinical and biochemical features compatible with the X-linked recessive form of the disease were studied. M. P. had increased amounts of chondroitin sulfate B and heparitin sulfate in the urine. Information on the urinary excretion of mucopolysaccharides of F. S. and F. F. is presently unavailable.

Skin biopsies were obtained from normal subjects, patients with the various forms of Hurler's syndrome and certain of their relatives. Split thickness biopsies were taken without anesthesia from the extensor surface of the upper arm. When duplicate biopsies were obtained they were always taken from the contralateral arm. Each biopsy (approximately 1 x 3 mm) was then cut into several pieces (10–20) and cultured in a plasma clot in a Carrel flask (10). After several weeks the explants were surrounded by a dense halo of fibroblastic growth. When this became evident the cultures were treated with trypsin to disperse the cells into a uniform suspension and transferred to flasks for subsequent propagation as monolayer cultures on glass. The cultures were grown in reinforced Eagle's medium (10) with 10 per cent newborn calf serum. The initial inoculum per flask was approximately 0.5×10^4 cells per ml.

Coverslips were introduced into the flasks at the time of subculturing for cytological studies. One to 7 days later the coverslips were removed and the cells stained. Each coverslip was washed twice in warm balanced salt solution, fixed in methanol for 5 minutes, and air dried. Two dyes were used to stain the intracellular mucopolysaccharides: the metachromatic dye, toluidine blue 0 (11) and the phthallocyanine dye, alcian blue (12).

Toluidine blue 0 used was obtained from Matheson Coleman and Bell, East Rutherford, New Jersey (color index, No. 52040, total dye content 89 per cent). Each preparation was stained for 5 minutes (0.1 per cent toluidine blue in 30 per cent methanol), cleared with acetone, acetone: xylene, and xylene reagent grade (Merck) and mounted in permount (11).

When alcian blue was employed, the preparation was transferred to distilled water and then stained (1 per cent aqueous solution alcian blue saturated with thymol) for 1 minute. The slide was washed in distilled water and immersed for 24 hours in 0.5 per cent borax in 80 per cent ethanol, dehydrated, and mounted in permount (12).

Cytological evaluation of all preparations was based on the examination of 100 fields each containing approximately 100 cells. The intracellular metachromatic granules were graded in a semiquantitative fashion: (*a*) absent granules; (*b*) granules present but scanty; (*c*) many granules; and (*d*) "gargoyle" cells (13).

Chromosomal analyses were performed (14) on cell lines at the same time as the cytochemical studies.

Examination of the lymphocytes was performed on all individuals who had skin biopsies. Blood smears were prepared directly from peripheral blood obtained by pricking an index finger. The blood smears were air dried, fixed in methanol, and stained by the methods used for the cell preparations (11, 12).

RESULTS

Living Cultures.—The time required to establish a cell line from cultured skin varied from 50 to 90 days and appeared to be dependent on the age of the individual from whom the skin was obtained and the size of the original explants. No significant differences in cellular activity between the cultures from affected and normal individuals were observed. The cells from affected individuals were usually larger than those from control subjects; this difference was frequently sufficient to identify a culture as having been derived from a patient with Hurler's syndrome. During the first 7 days in culture, sheets of epithelial tissue migrated from the explant. In the 2nd week these epithelial sheets

retracted to the explant and thereafter showed no further evidence of cellular activity. After a variable latent phase, fibroblasts in large numbers migrated from the explant.

Two different morphological types of fibroblasts, spindle-shaped and epithelioid, were seen in the living cultures (Fig. 1). The origin of these fibroblasts is not known; they are assumed to have arisen from the dermis, although some of the fibroblasts may have migrated from blood vessels.

Stained Cultures.—

Normal subjects: Cultures of fibroblasts derived from 19 normal individuals contained cells predominantly showing no cytoplasmic metachromasia (Figs. 1 and 2, Table I). However, cultures contained an occasional cell showing metachromatic granules (Table I). Skin cultures from 13 of the normal individuals showed that approximately 0.1 per cent of the fibroblasts contained metachromatic granules. These cells were usually large, occasionally multinucleated, and had the appearance of wandering cells rather than classical fibroblasts. Cultures derived from the remaining 6 normal individuals showed an inconstant number of metachromatic cells varying from 1 to 6 per cent. These cells could not be distinguished from those containing metachromatic granules in cultures derived from affected individuals. Skin fibroblasts from normal subjects occasionally contained vacuoles whose contents stained pink with toluidine blue O and could be mistaken for metachromatic cytoplasmic material under low magnification. When examined under higher magnification however, it could be seen that the pink material was intravacuolar and distinct from any metachromatic granules present. Occasionally the cultures contained fibroblasts with irregular cell membranes which stained deep purple.

TABLE I

Metachromatic Granules in Skin Fibroblasts from Normal Individuals (24 Hours after Subculturing)

Normal individuals	Sex	No.	Age	Metachromatic cells (1000 cells counted)	
				Range	Average
			yrs.	*per cent*	*per cent*
Adults	Male	8	23–45	0–6	1.3
	Female	5	28–40	0–5	1.8
Children	Male	2	0.2–12	0–1	0.5
	Female	4	1.3–11	0–3	1.7
Total.....		19	0.2–45	0–6	1.5

Fibroblasts from normal individuals stained with alcian blue showed little cytoplasmic staining except for an occasional greenish blue granule.

"Gargoyle" cells were never found in cultures from normal individuals.

Hurler's syndrome: Cultures derived from the skin of patients with Hurler's syndrome contained large numbers of fibroblasts with metachromatic granules in the

cytoplasm (Figs. 1 to 3, Tables II to VI). Cell lines from the same biopsy showed a remarkable constancy in the proportion of cells containing metachromatic granules. Different biopsies from the same area from a single individual showed slight variations in the type of fibroblasts produced in culture. Two biopsies were taken on different occasions from 1 patient with the X-linked type (M. P.). The first biopsy produced a line having predominantly spindle-shaped fibroblasts whereas the second produced a line containing both spindle-shaped and epitheloid fibroblasts (Fig. 1). Although the degree of cellular metachromasia varied in the three types of Hurler's syndrome (Tables II to VI), the size and distribution of the metachromatic granules were similar

TABLE II

Metachromatic Granules in Skin Fibroblasts from the P. H. Family (Autosomal Recessive Form of Hurler's Syndrome)
(24 Hours after Subculturing)

Generation*	No.	Metachromatic cells (1000 cells counted)		Degree of metachromasia of positive cells		
		Negative	Positive	Very few granules	Many granules	"Gargoyle" cells
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
I	1	78	22	80	19	1
	2	78	22	60	38	2
	3	94	6	100	0	0
II	1	99	1	100	0	0
	2	75	25	80	18	2
	4	66	34	90	8	2
	5	71	29	59	39	2
	6	74	26	70	25	5
III	1	99	1	100	0	0
	2	92	8	100	0	0
	3	95	5	100	0	0
	4	80	20	80	18	2
	5	0	100	50	45	5
	6	0	100	46	50	4

* See Text-fig. 1.

(Figs. 1 to 3). In skin biopsies derived from patients with the X-linked syndrome, 60 to 100 per cent of the fibroblasts contained metachromatic granules whereas the other two types, presumed to be inherited in an autosomal recessive fashion, only 60 to 70 per cent of the fibroblasts contained demonstrable metachromatic granules.

The metachromatic granules appeared of uniform size and, with the exception of the juxtanuclear area (Figs. 2 and 3), were usually evenly distributed throughout the cytoplasm. Occasionally the granules appeared in vacuoles in stellate clusters.

"Gargoyle" cells (2 to 5 per cent) were found in all cultures from affected individuals (Tables II to VI, Fig. 3). These cells had the same morphological appearance as "gargoyle" cells described in histological sections of skin from affected individuals (13).

Such cells had ruffled cell membranes, poorly visualized cytoplasmic structure, and, as the cytoplasm was loaded with metachromatic granules, they were readily seen under low power microscopy.

Throughout 8 months of continuous growth, cultures derived from patients with Hurler's syndrome showed a slight decrease in the percentage of fibroblasts containing metachromatic granules (Table VII) although there was no detectable decrease in metachromatic granules in each positive cell. The varieties of fibroblasts and the presence of "gargoyle" cells remained constant.

TABLE III

Metachromatic Granules in Skin Fibroblasts from the L. R. Family (Autosomal Recessive Form of Hurler's Syndrome) (24 Hours after Subculturing)

Generation*	No.	Metachromatic cells (1000 cells counted)		Degree of metachromasia of positive cells		
		Negative	Positive	Very few granules	Many granules	"Gargoyle" cells
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
I	1	97	3	100	0	0
	2	68	32	50	47	3
II	1	99	1	100	0	0
	2	99	1	100	0	0
	3 Biopsy 1	40	60	67	29	4
	Biopsy 2	30	70	60	33	7
	4 Biopsy 1	70	30	90	5	5
	Biopsy 2	79	21	88	10	2
III	1	96	4	100	0	0
	2	76	24	60	37	3
	4	27	73	90	8	2
	5	97	3	100	0	0

* See Text-fig. 2.

The cytological findings obtained when alcian blue was used were similar to those with toluidine blue O.

Relatives of patients with Hurler's syndrome: Metachromatic inclusions were seen in cultures of fibroblasts derived from members of the families who appeared clinically normal but who were considered by pedigree studies to be heterozygous or hemizygous for the abnormal gene (Fig. 2, Text-figs. 1 to 3, and Tables II to VI). No difference between the metachromatic granules in the affected individuals and the unaffected carriers could be detected. "Gargoyle" cells were consistently seen in the cultures of carriers of the abnormal gene and represented from 1 to 7 per cent of the total cell population (Tables II to VI).

Chromosomal analysis revealed a normal chromosomal constitution in all affected individuals as well as in the healthy members of the families.

Lymphocytes: The lymphocytes obtained from the peripheral blood of normal, affected individuals, and their relatives were studied for the presence of metachromatic cytoplasmic inclusions (Table VIII). In the 19 normal individuals studied no abnormal cytoplasmic inclusions were seen. In all 7 patients studied some of the lymphocytes contained metachromatic inclusions. The proportion of cells containing inclusions was similar in both small and large lymphocytes.

The lymphocytes of relatives, who were considered by pedigree studies to be heterozygous or hemizygous for the abnormal gene, contained no detectable metachromatic inclusions or any other morphological abnormality (Table VIII).

TABLE IV

Metachromatic Granules in Skin Fibroblasts from the R. B. and J. M. Families (Autosomal Recessive Form of Hurler's Syndrome, Sanfilippo's Syndrome) (24 Hours after Subculturing)

Subjects	Metachromatic cells (1000 cells counted)			Degree of metachromasia of positive cells		
		Negative	Positive	Very few granules	Many granules	"Gargoyle" cells
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
R. B. family						
Propositus.....	Biopsy 1	40	60	65	30	5
	Biopsy 2	24	76	70	24	6
	Biopsy 3	31	69	87	11	2
Father.....		64	36	75	20	5
Mother.....		84	16	80	18	2
J. M. family						
Propositus.....		70	30	70	25	5
Father.....		68	32	68	31	1
Mother.....		78	22	70	28	2

DISCUSSION

Since Hurler's syndrome is an inherited disease which affects connective tissue, it was hoped that studies of human fibroblasts would provide an opportunity to investigate the mode of inheritance of the abnormal trait as well as the primary biochemical error. In earlier studies, Hambrick and Scheie (15) had shown that the skin from affected individuals contains metachromatic material and striking vacuolization of cells in the basospinous layers of the epidermis. They also demonstrated the presence of metachromatic material in fibroblasts, "gargoyle" cells and the intercellular ground substance of the dermis.

As a necessary prerequisite for studying the genetic and biochemical aspects of Hurler's syndrome in tissue culture the morphology and activity of the cells

of the dermis from the skin biopsies of normal and affected individuals were studied. In cell cultures derived from skin biopsies from both normal and affected individuals, the three cell types described in tissue sections by Hambrick and Scheie (15) were seen. The spindle-shaped fibroblasts were predomi-

TABLE V

Metachromatic Granules in Skin Fibroblasts from the M.P. Family (X-linked Recessive Form of Hurler's Syndrome)
(24 Hours after Subculturing)

Generation*	No.	Metachromatic cells (1000 cells counted)		Degree of metachromasia of positive cells		
		Negative	Positive	Very few granules	Many granules	"Gargoyle" cells
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
I	1	99	1	100	0	0
	2	74	26	80	16	4
II	1	99	1	100	0	0
	2	100	0	0	0	0
	4	78	22	80	14	6
	6	64	36	78	20	2
III	1	98	2	100	0	0
	2 Biopsy 1	100	0	0	0	0
	Biopsy 2	100	0	0	0	0
	3 Biopsy 1	49	51	74	22	4
	Biopsy 2	55	45	70	25	5
	4	97	3	100	0	0
	5	95	5	100	0	0
	7	99	1	100	0	0
	8	54	46	75	20	5
IV	1 Biopsy 1	7	93	68	24	8
	Biopsy 2	1	99	42	50	8

* See Text-fig. 3.

nant but epithelioid fibroblasts were also observed (Fig. 1). These two morphologically distinct fibroblasts have previously been noted in tissue culture of human diploid cells and have been considered to be genetically distinct (16).

To determine if the increased intracellular metachromasia seen *in vivo* persisted *in vitro*, cell preparations were stained with toluidine blue O and alcian blue. Toluidine blue O is a cationic dye which stains mucopolysaccharides metachromatically due to the availability of consecutive regularly spaced

TABLE VI

Metachromatic Granules in Skin Fibroblasts from Two Families with the X-linked Recessive Form of Hurler's Syndrome (24 Hours after Subculturing)

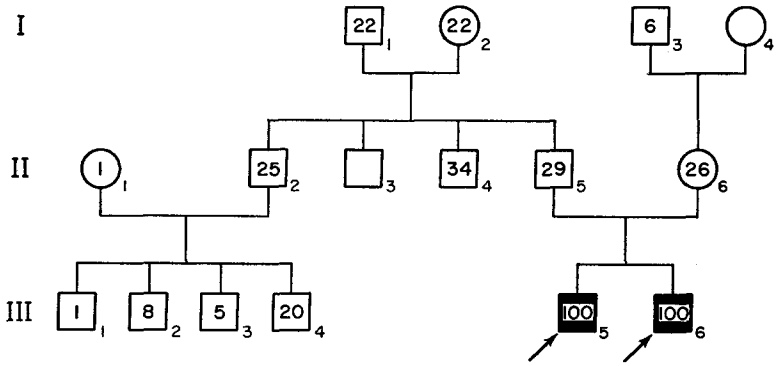
Subjects	Metachromatic cells (1000 cells counted)		Degree of metachromasia of positive cells		
	Negative	Positive	Very few granules	Many granules	"Gargoyle" cells
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Family F. S.					
Propositus	2	98	60	36	4
Father	99	1	100	0	0
Mother	55	45	55	42	3
Family F. F.					
Propositus	40	60	60	35	5
Father	95	5	100	0	0
Mother	34	66	70	27	3
Sister	52	48	60	36	4

TABLE VII

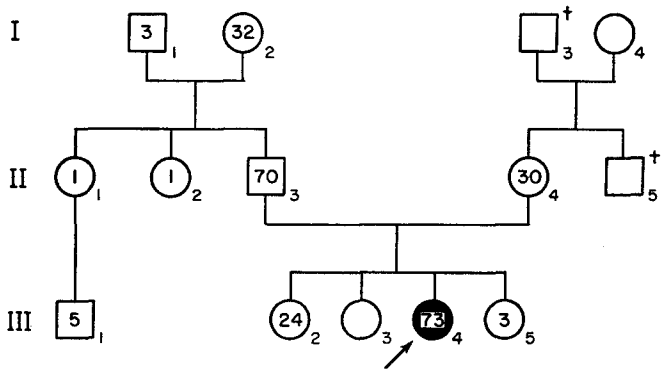
Metachromasia of Skin Fibroblasts during Continuous Culture

Subjects	Time in culture	Metachromatic cells (1000 cells counted)		Degree of metachromasia of positive cells		
		Negative	Positive	Very few granules	Many granules	"Gargoyle" cells
	months	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
M. P.	2	1	99	42	50	8
	5	36	64			
	8	28	72	38	56	6
L. R.	2	27	73	90	8	2
	5	28	72			
	8	31	69	92	7	1
R. B.	2	40	60	65	30	5
	5	47	53			
	8	40	60	60	38	2
Father of M. P.	2	100	0	0	0	0
	5	100	0			
	8	100	0	0	0	0
Mother of M. P.	2	49	51	74	22	4
	5	55	45			
	8	67	33	68	27	5

anionic groups along the carbohydrate chain (17). Nucleic acids and acidic lipids of high molecular weight stain purple or violet with toluidine blue O whereas acid mucopolysaccharides stain a strong pink (18). Alcian blue is a phthallocyanine dye with a tetravalent cation that directly stains areas con-



TEXT-FIG. 1. Pedigree of patients P. H., No. 20,417, and M. H., No. 20,418 (autosomal recessive form of Hurler's syndrome). Numbers inside the male and female symbols indicate the per cent of fibroblasts in a cell culture that contained increased intracellular mucopolysaccharides.

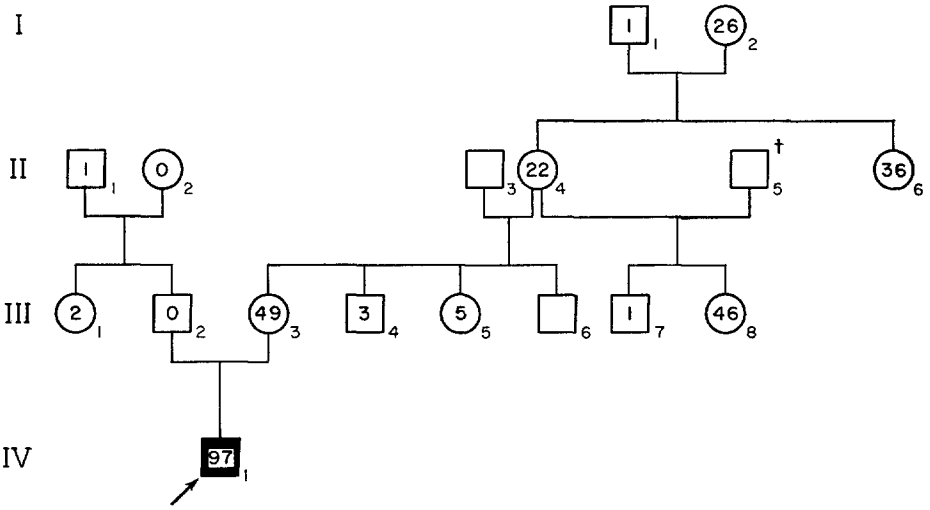


TEXT-FIG. 2. Pedigree of patient L. R., No. 20,227 (autosomal recessive form of Hurler's syndrome). Numbers inside the male and female symbols indicate the per cent of fibroblasts in a cell culture that contained increased intracellular mucopolysaccharides.

taining polysaccharides (12). A close correlation has been found between alcian blue, toluidine blue O and S^{35} autoradiographs of tissue containing sulphated mucopolysaccharides (19).

Cells from various tissues obtained from affected individuals have been shown to contain increased amount of mucopolysaccharides *in vitro*. The lymphocytes of the peripheral blood (20–23) and certain cells in the bone marrow (24, 25)

have been found to contain metachromatic material in cytoplasmic vacuoles. Cellular exudates induced by abrading the epidermis of affected individuals have been reported to contain mononuclear cells with metachromatic material (26). None of these cellular sources show cytological abnormalities in members of the families considered to be carriers for the abnormal gene (20–26). In agreement with other studies (20–23), lymphocytes from all patients with Hurler's syndrome showed metachromatic granules (Table VIII). Healthy members of the affected families however showed no abnormality in their white blood cells.



TEXT-FIG. 3. Pedigree of patient M. P., No. 20,440 (X-linked recessive form of Hurler's syndrome). Numbers inside the male and female symbols indicate the per cent of fibroblasst in a cell culture that contained increased intracellular mucopolysaccharides.

Skin fibroblasts from normal subjects rarely disclosed metachromatic granules in culture whereas fibroblasts from patients with Hurler's syndrome were consistently packed with metachromatic granules (Fig. 2, Tables I to VI). The age of the patient and the cultural conditions employed probably modify, to some extent, the selection of cells which migrate from the biopsy explant and constitute the cell population studied. The absence of detectable metachromasia in some fibroblasts probably reflects a variation in intracellular mucopolysaccharides during the cell cycle. These results support the suggestion of Meyer and coworkers who suggest that the fibrocyte is the cell responsible for the synthesis of mucopolysaccharides (3).

Fibroblasts from affected individuals and clinically normal carriers of the abnormal gene, contained metachromatic granules suggesting that mucopolysaccharide synthesis persisted *in vitro* over 8 months of culture (Table VII). During this period the fibroblasts remained diploid and did not undergo any

tissue culture transformation such as an alteration in morphology or an increase in growth rate. It is well known that fibroblasts from various tissues, including the skin, produce mucopolysaccharides *in vitro* (27, 28). The persistence of mucopolysaccharide synthesis by fibroblasts in culture has been shown by Davidson (29) for a heteroploid line of rat eye connective tissue cells in continuous culture for over 3 years. As synthesis and secretion of mucopolysac-

TABLE VIII

Metachromatic Granules in Blood Lymphocytes

Subjects	Metachromatic Lymphocytes (100 cells counted)				Total	
	Small		Large			
	Positive	Negative	Positive	Negative	Positive	Negative
	per cent	*per cent*	*per cent*	*per cent*		
Hurler: autosomal recessive						
P. H.	19	47	15	19	34	66
M. H.	13	32	15	40	28	72
L. R.	26	26	22	26	48	52
Hurler: (Sanfilippo) autosomal recessive						
R. B.	29	16	28	27	57	43
J. M.	32	25	26	17	58	42
Hurler: X-linked recessive						
M. P.	20	14	31	35	51	49
F. S.	44	41	5	10	49	51
F. F.	17	47	18	18	35	65
Normal Individuals (average of 19)	0	42	0	58	0	100
Clinically normal relatives of patients with Hurler's Syndrome (average of 41)	0	46	0	54	0	100

charides are considered characteristic of connective tissue cell types *in vivo* (30, 31), it will be of interest to determine if the fibroblasts from affected individuals and carriers produce the same mucopolysaccharides in increased amounts *in vitro*.

Previous studies (8 *a*, 32–34) suggest that there is considerable overlap in the clinical and biochemical findings between the autosomal and X-linked recessive types of Hurler's syndrome. Moreover, examination of an individual pedigree frequently does not permit critical discrimination between the two genetic modes of inheritance. If an affected male is born to unrelated parents, it is frequently impossible to determine the mode of inheritance unless the affected

individual reproduces. Analysis of the urinary mucopolysaccharides of presumed carriers has failed to reveal any conclusive evidence that biochemical abnormalities can be detected in the carriers of the gene for Hurler's syndrome (8 *a*, 35) although Teller *et al.* (36) using rather imprecise methods have claimed that one or both parents and certain other relatives of patients with Hurler's syndrome have an abnormal excretion of chondroitin sulfate B. Three of the families (P. H., J. M., and R. B.) studied previously in this laboratory were thought to represent the autosomal recessive form of Hurler's syndrome on genetic, clinical, and biochemical evidence (8 *a*). The parents who were assumed to be heterozygous carriers appeared clinically normal and no increased urinary excretion of mucopolysaccharides could be detected. The skin fibroblasts grown in culture from all 6 parents contained demonstrable metachromatic granules as well as "gargoyle" cells (Tables II and IV). Thus it appears that in these three families the heterozygous state, which could not be recognized from clinical examination, from cytochemical staining of lymphocytes from peripheral blood (Table VIII), or from urinary excretion of mucopolysaccharides (8*a*), could be detected in tissue culture. However, the degree of cellular metachromasia could not be used to distinguish affected individuals from heterozygous carriers of the abnormal gene (Text-figs. 1 and 2, Tables II to IV).

In two of the families with the autosomal recessive form of Hurler's syndrome (P. H. and L. R.) it was possible to trace the abnormal gene through unaffected generations (Figs. 1 and 2). In the P. H. family, both paternal grandparents had fibroblasts containing metachromatic granules and were thus presumed to be heterozygous for the abnormal gene. The probability that a rare gene is present in both marriage partners is low, but is made more likely when consanguinity is present. Remote consanguinity could be reasonably suspected as both paternal grandparents came from the same farming community in Ireland. On the maternal side of the family the mode of inheritance strongly suggested the autosomal recessive form. The maternal grandmother had five normal brothers. The probability of five normal brothers is approximately six times more likely if the gene is on an autosome than on an X chromosome.

Reports in the literature suggest that the subtype of Hurler's syndrome in which heparitin sulfate is the only mucopolysaccharide excreted in excess in the urine (9) can be inherited in an autosomal (32) or X-linked form (33). In the present study the parents of the two affected individuals with this form of the disease (J. M. and R. B.) showed metachromasia in the cultures of fibroblasts and were therefore considered to be heterozygous for the abnormal gene (Table IV).

All three families of the presumed X-linked syndrome showed that fibroblasts cultured from the skin of the fathers contained no metachromatic granules whereas those of the hemizygous mother contained both metachromatic granules and "gargoyle" cells (Tables V and VI). In one family, M. P., the skin fibroblasts from the maternal great-grandmother, maternal grandmother,

maternal great-aunt, and maternal half-aunt all contained positive cells (Text-fig. 3, Table V). Thus in this family the abnormal gene could be faithfully traced for three generations. The clinically normal sister of F. F. had metachromatic fibroblasts and was presumed to be a hemizygous carrier (Table VI); in the F. S. family there were no living maternal relatives or sibs. Thus in these three families the hemizygous state, although not demonstrable *in vivo*, could be clearly detected *in vitro*.

As had been found in the autosomal type of the disease, the degree of metachromasia could not be used to distinguish the affected individual from the hemizygous carriers (Tables V and VI). The cultures from two propositi (M. P. and F. S.) had almost homogeneous populations of metachromatic fibroblasts

TABLE IX

The Distribution of Affected and Non-Affected Offspring According to Mode of Inheritance and Mating Type

Mode of inheritance and mating type	No.	Children				χ^2 1 d.f.	*P*
		Positive		Negative			
		Observed	Expected	Observed	Expected		
X-Linked recessive Mother (positive) × father (negative)	4	5	3.5	2	3.5	1.2857	0.2–0.3
Autosomal recessive Positive × positive	3	7	6.75	2	2.25	0.0371	0.8–0.9
Autosomal recessive Positive × negative	2	2	3.5	5	3.5	1.2857	0.2–0.3

whereas those from the hemizygous mother consistently showed approximately half the cell population containing metachromatic granules. (Whether the locus for Hurler's syndrome participates in the "Lyon" effect (38) can be elucidated by cloning the cells of the hemizygous mother. Such studies are underway). The fibroblasts grown from the other propositus (F. F.) and his mother showed a similar degree of metachromasia.

The distribution of affected and non-affected offspring in families with Hurler's syndrome will depend on the mode of inheritance of the abnormal trait. Calculation of the expected and observed distributions in families with the autosomal and X-linked recessive form of Hurler's syndrome are illustrated in Table IX. In both forms of inheritance a good agreement between the observed and expected number of affected and unaffected offspring was found.

Although it has long been recognized that Hurler's syndrome may be inherited in both an X-linked and an autosomal recessive form, there is no reliable

information on the relative frequency of the two modes of inheritance. The results from this investigation suggest that, provided the father of an individual with Hurler's syndrome is available for clinical and cytological examination, an estimate of the relative frequency of the two modes of inheritance can be made. In addition, it seems likely that the application of the techniques of cell culture will enable genetic counselling for this disease to be placed on a more precise basis. It is evident that the preservation of the biochemical abnormality of Hurler's syndrome in affected individuals and carriers of the abnormal gene in cell culture should permit an investigation of this inherited mucopolysaccharidosis at the cellular level. The disclosure of a biochemical defect in the carriers of the abnormal gene in the X-linked variety of the disease has, in effect, converted a conventional X-linked recessive trait to an X-linked dominant trait. This "conversion" will greatly amplify the usefulness of this trait in studies of the linkage of genes on the human X chromosome.

SUMMARY

Seven families affected with Hurler's syndrome have been studied using the methods of cell culture. Skin fibroblasts obtained from the skin of 7 patients with Hurler's syndrome contained metachromatic granules when stained for mucopolysaccharides with toluidine blue O and alcian blue, whereas fibroblasts from normal subjects contained no metachromatic granules.

In four families skin cultures of the clinically normal parents showed fibroblasts which contained demonstrable metachromatic granules and "gargoyle" cells and were considered to be heterozygous for the abnormal gene. Fibroblast cultures from certain other members of these families showed metachromasia. These findings were also considered to indicate heterozygosity for the abnormal gene.

Three families of the X-linked type of the disease were studied. Fibroblasts cultured from the father contained no metachromatic granules whereas those of the hemizygous mother contained both metachromatic granules and "gargoyle" cells. In one family the abnormal gene could be traced through unaffected individuals for three generations. The prolonged preservation of the biochemical trait in tissue culture will permit studies to be performed designed to clarify the primary action of the abnormal genes which result in Hurler's syndrome, as well as to increase the usefulness of this trait in mapping the human X chromosome.

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EXPLANATION OF PLATES

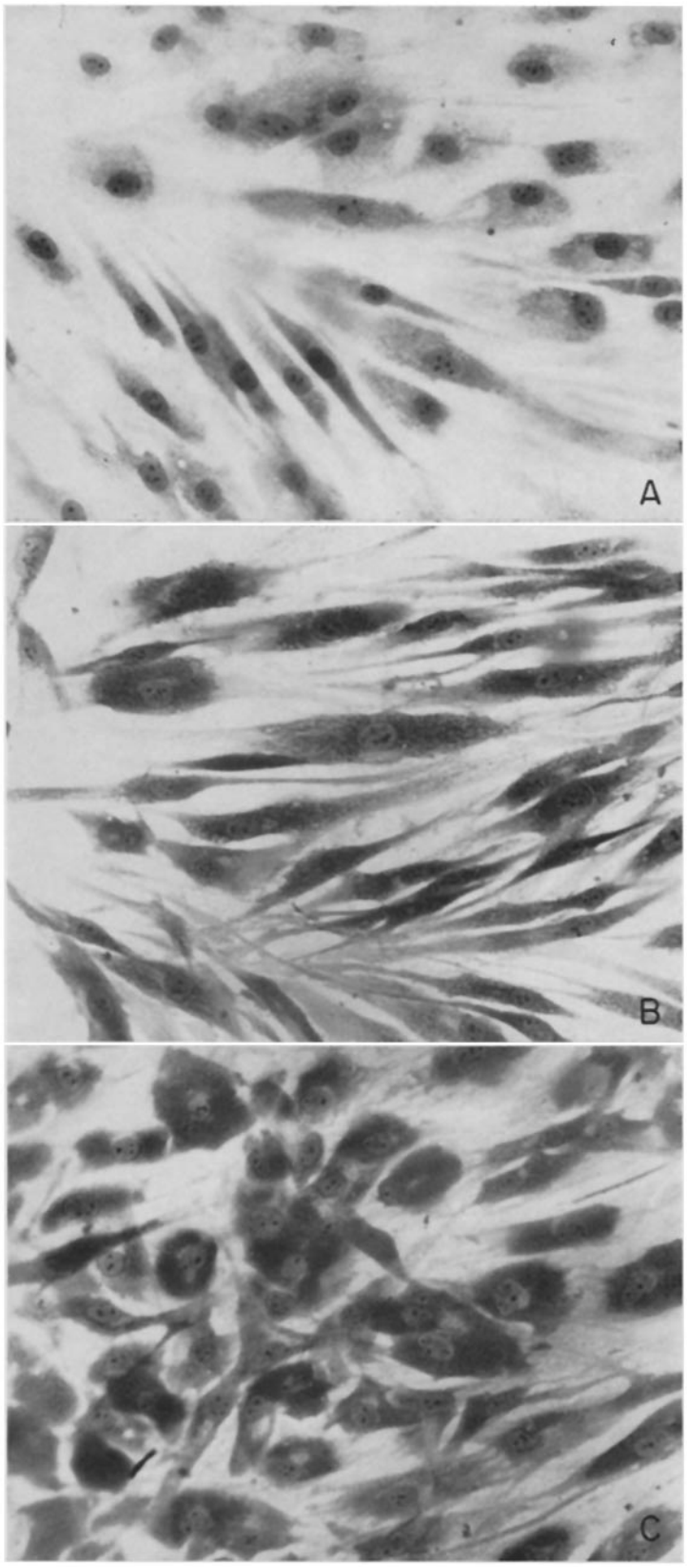
PLATE 1

FIG. 1. Monolayers of skin fibroblasts grown in tissue culture. Preparations stained with toluidine blue O to demonstrate intracellular acid mucopolysaccharides which stain metachromatically (pink).

A. Fibroblasts from a normal individual. General field, nuclei and cytoplasm stained light blue. × 1000.

B. Fibroblasts from a patient, M. P., with the X-linked type of Hurler's syndrome. General field, spindle-shaped fibroblasts. × 1000.

C. Fibroblasts from a patient, M. P., with the X-linked type of Hurler's syndrome, General field, epithelioid fibroblasts. × 1000.



(Danes and Bearn: Hurler's syndrome)

PLATE 2

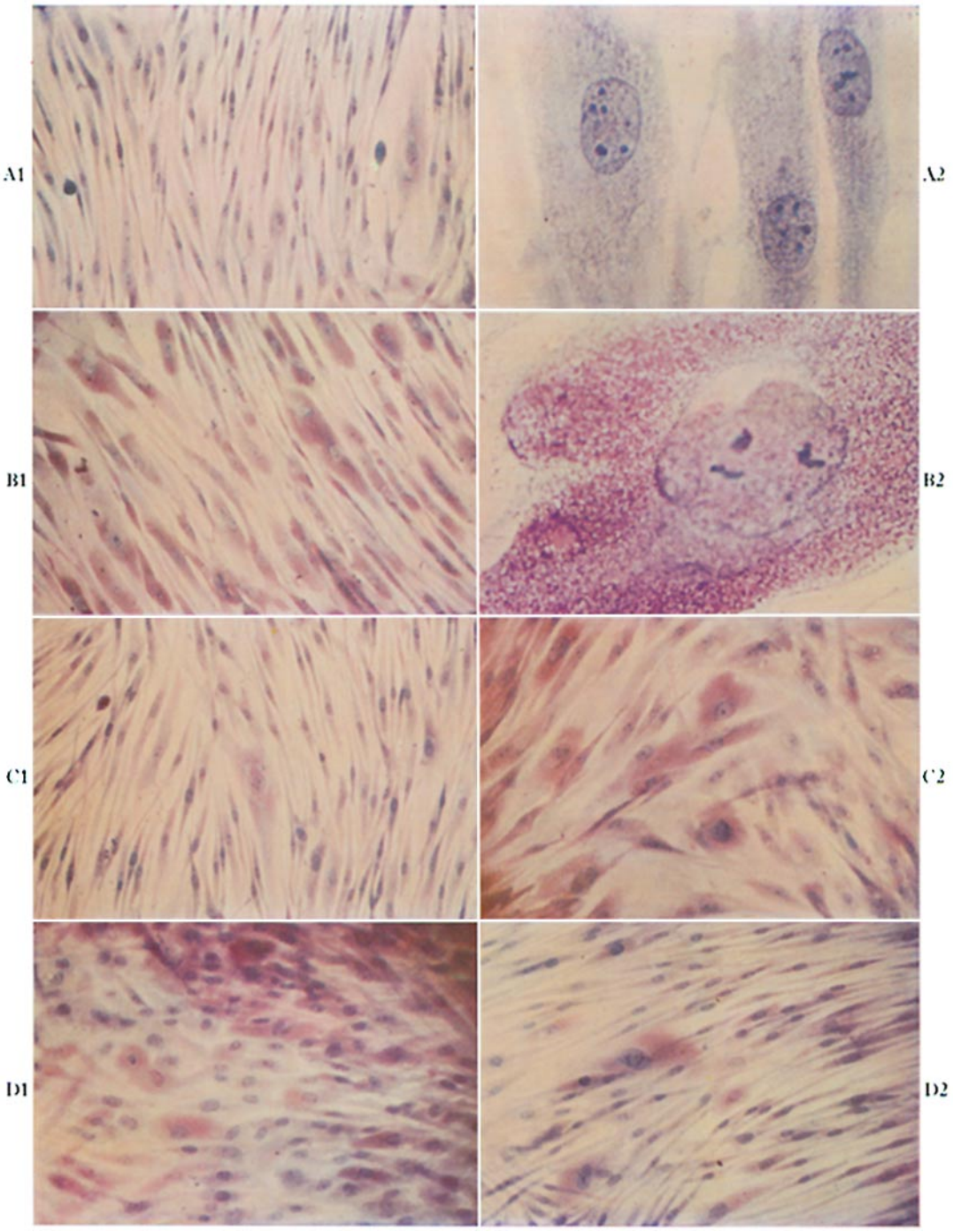
FIG. 2. Monolayers of skin fibroblasts grown in tissue culture. Preparations stained with toluidine blue O.

A. Fibroblasts from a normal individual. A 1, general field, × 320; A 2, fibroblasts, × 2000.

B. Fibroblasts from a patient, M. P., with the X-linked type of Hurler's syndrome. B 1, general field, × 320; B 2, spindle-shaped fibroblasts with cytoplasm filled with metachromatic granules, × 2000.

C. Fibroblasts from parents of a patient, M. P., with the X-linked type of Hurler's syndrome. C 1, father, cytoplasm shows no metachromatic granules × 320; C 2, mother, approximately half the cell population contains metachromatic cytoplasmic granules × 320.

D. Fibroblasts from parents of a patient, L. R., with the autosomal recessive form of Hurler's syndrome. D 1, father, approximately 70 per cent of the cells contain with metachromatic granules × 320; D 2, mother, approximately 30 per cent of the cells are loaded with metachromatic granules × 320.



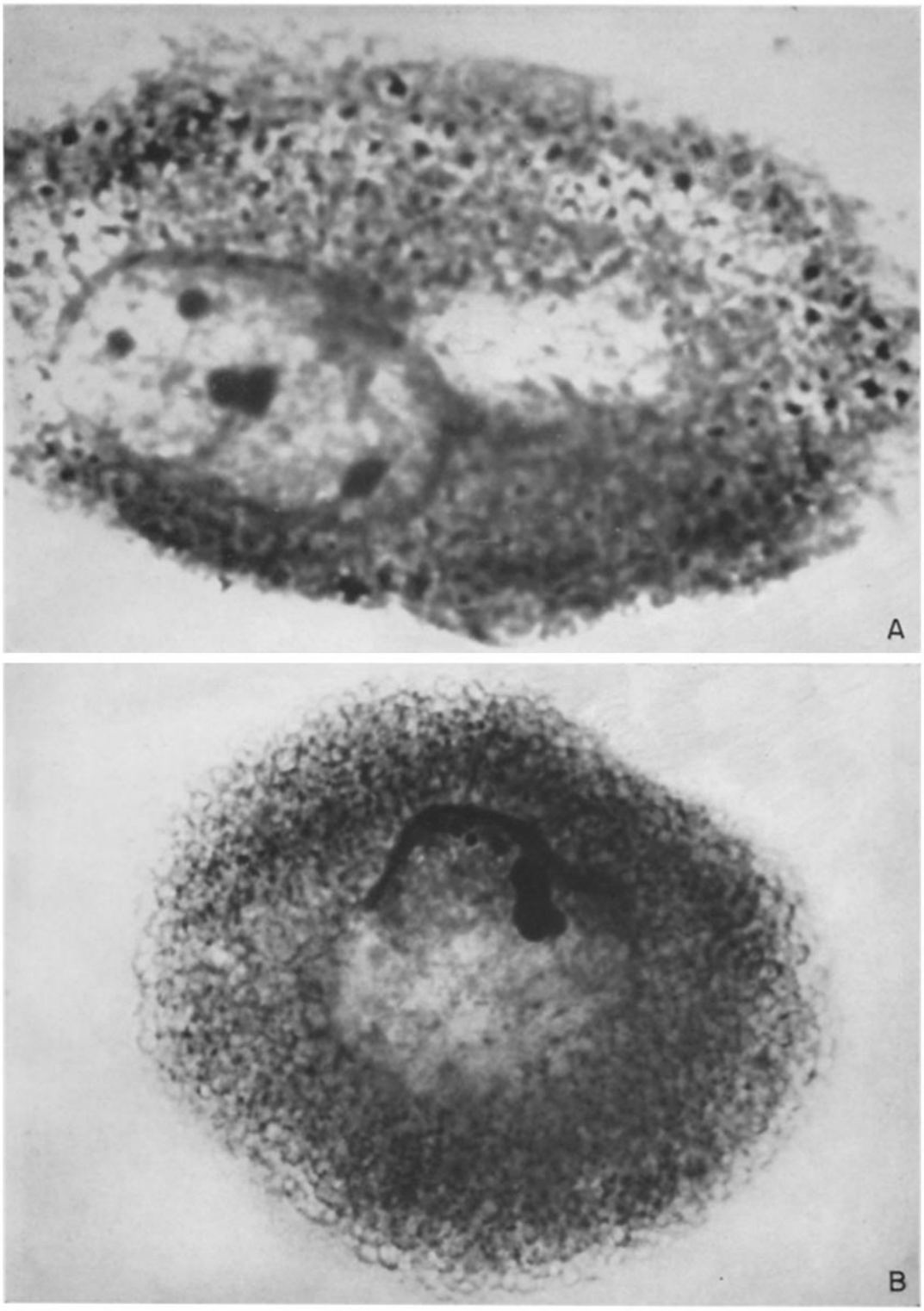
(Danes and Bearn: Hurler's syndrome)

PLATE 3

FIG. 3. Cells from the skin biopsy from a patient, L. R., with the autosomal recessive form of Hurler's syndrome. Preparation stained with toluidine blue O to demonstrate intracellular acid mucopolysaccharides which stain metachromatically (pink).

A. Spindle-shaped fibroblast. Discrete metachromatic granules in the cytoplasm Note clear juxtanuclear area. × 5000.

B. "Gargoyle" cell. Cytoplasm loaded with metachromatic granules, cell membrane ruffled and other cytoplasmic structures poorly visualized, × 3500.



(Danes and Bearn: Hurler's syndrome)