which has been added i-inositol at 0.0010 g per liter, the glutamine increased to 0.200 g per liter, and the phenol red concentration reduced by a factor of 10. NCI medium is available from Grand Island Biological Co., Grand Island, N.Y.
4. Rubella virus as strain F-8 (supplied by Dr. Balsamo of the New York University Medical School) was used to develop stocks of virus. The original F-8 strain produced small HAD- plaques in 4 days, whereas passage of this strain on green monkey kidney cells in our laboratory seems to have selected for a large-plaque varient which we have designated F-8L and used as rubella stock throughout this study. We have also obtained small HAD- plaques upon direct isolation from the blood of a patient with an active case of rubella.
5. P. I. Marcus, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 351 (1962).
6. Experience with the single-cell hemadsorption technique (*5*) has demonstrated that infection with high multiplicities of myxovirus sometimes results in a small amount of red-blood-cell adsorption due to residual surface-bound virus. This "background" can be eliminated completely by exposing the NDV-infected cells to specific viral antiserum, thus coating the viral hemagglutinin. This step (addition of NDV-antiserum) is included routinely in the HAD- plaque test since it is not possible to predict a priori whether the background level of hemadsorption will be high or low for a given lot of cells.
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9. The 37-percent survival dose, D^0, for rubella virus, defined as the dose of ultraviolet light required to reduce the number of HAD- plaque-forming particles to e^{-1} (0.37 survivors = 1 lethal hit) is 110 ergs mm^{-2}. Ultraviolet-light dosimetry was based on the D^0 values obtained from the exponential inactivation curves of T2 phage and Newcastle disease virus irradiated in the same medium. T2 phage qualifies as a biological actinometer of known uniformity (*13*). For comparison, D^0 values for T2 phage and NDV are 25 and 42.5 ergs mm^{-2}, respectively.
10. P. D. Parkman, E. L. Buescher, M. S. Artenstein, J. M. McCown, F. K. Mundon, A. D. Druzd, *J. Immunol.* **93**, 595 (1964).
11. Because of the preliminary nature of this aspect of our investigation, we have deferred consideration of the possible relationship of these two types of interference to classical interferons.
12. Antiserum to rubella virus was obtained as serum from convalescent individuals who had been diagnosed as having German measles. A 1 : 50 dilution of serum neutralized more than 90 percent of the HAD- plaque-forming particle activity within 15 minutes at 37°C.
13. S. E. Luria, *General Virology* (Wiley, New York, 1953), p. 153; P. I. Marcus and T. T. Puck, *Virology* **6**, 405 (1958); H. H. Lee and T. T. Puck, *Radiation Res.* **12**, 340 (1960).
14. Part of this work was presented at the 65th annual meeting of the Amer. Soc. for Microbiol., April 1965, *Bacteriol. Proc.*, abstr. V8 (1965), p. 98. Aided by grants AI-03619-05VR and GM-12646-01A1 from NIH. One of us (P.I.M.) is a research career development awardee of the National Institute of Allergy and Infectious Diseases (2-K3-GM-15, 461-05).

18 May 1965

Serum Prealbumin: Polymorphism in Man

Abstract. *Serums from 390 Norwegian blood donors and 31 members of two families were studied by starch gel electrophoresis. Five different prealbumin phenotypes were found, indicating a genetic theory of three codominant alleles for which the terms* Pr^F, Pr^M, *and* Pr^S *are proposed.*

When human serum is subjected to zone electrophoresis in starch gel, two (*1*) or three (*2*) protein bands, the prealbumins, migrate in front of the albumins. We have examined prealbumins in serums from 390 Norwegian blood donors and from 31 members of two families, using a method based upon Poulik's (*3*) horizontal discontinuous system (gel buffer *p*H 4.95). (Details of our technical procedures are in preparation.)

Our studies revealed a rather high number of prealbumin bands. However, the number of bands depends upon the technique, and only the prealbumins that were clearly recognized by our routinely used method are considered here. These prealbumins (Pr) appeared as three to four bands of varying strength and they were found in five different patterns (Fig. 1). In our hands the prealbumins in serums from most individuals appeared as a three-band pattern: one weak band in front of two relatively heavy bands. This pattern is designated MM (M = medium). Another three-band pattern, which is slower, is called SS. A phenotype which seems to be a combination of the medium three-band pattern and a hypothetical fast-moving phenotype is called FM. The last two phenotypes are called FS and MS. In combination phenotypes, the bands which do not overlap show about half the strength and staining intensity of the corresponding bands in the three-band patterns. The distance between the two main bands of each three-band pattern is largest for SS, intermediate for MM, and smallest for "FF" (as judged from the F bands in the FS phenotype).

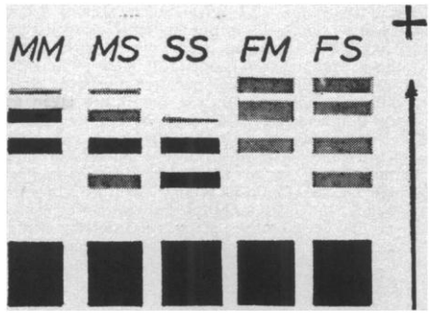

Fig. 1. Drawing of the five prealbumin phenotypes in front of the albumins.

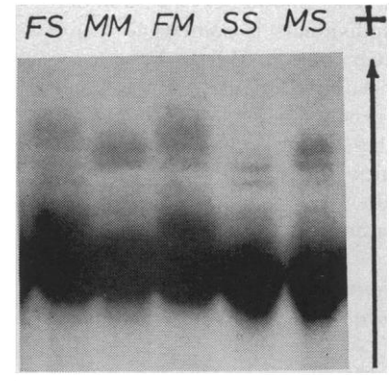

Fig. 2. Photograph of part of a stained gel where the five prealbumin phenotypes are present in front of the albumins.

Figure 2 is a photograph of part of an amido black–stained gel where samples representing the five phenotypes have been examined. Results from investigation of the families of two blood donors are shown in Fig. 3.

These findings indicate a genetic theory of three codominant alleles for which we propose the terms Pr^F, Pr^M, and Pr^S, corresponding to the fast, medium, and slow migration rates of the respective allele products. In 390 randomly selected blood donors, the phenotype MM was found in 374 individuals, while MS was found in 9, FM in 4, SS in 2, and FS in 1. These figures and the family studies fit the genetic theory advanced, the Pr^M being the most common allele and Pr^F and Pr^S rare. The FF phenotype was not found, which is according to expecta-

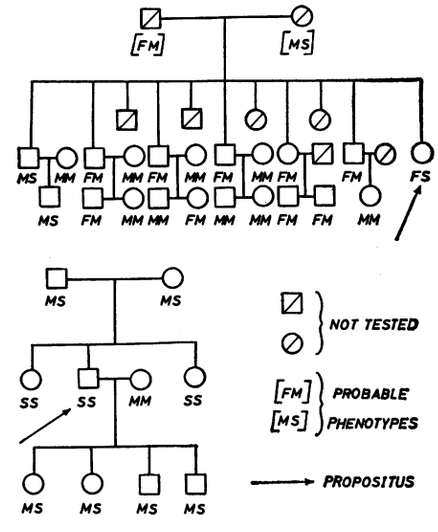

Fig. 3. Prealbumin phenotypes in members of two families.

tion. The finding of two homozygous SS individuals is somewhat surprising, but we consider this to be a matter of chance. In no case were exceptions to the genetic theory observed.

These results might open new opportunities for the study and utilization of prealbumins—for instance, in genetic research and disputed paternity cases.

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28 June 1965

Hurler's Syndrome: Demonstration of an Inherited Disorder of Connective Tissue in Cell Culture

Abstract. *Skin fibroblasts from three patients with Hurler's syndrome were grown in tissue culture and shown to contain metachromatic granules when stained for mucopolysaccharides with toluidine blue O. Similar inclusions were observed in cultures of fibroblasts from other members of the families, who appeared to be clinically normal but who were, judged from studies of pedigree, heterozygous or hemizygous for the abnormal gene.*

Tissue culture appears to provide an opportunity to study in vitro inborn errors of metabolism (*1*). If the expression of abnormal genes present in vivo can be detected in vitro at the cellular level, then such markers may be used to study both the genetic mode of inheritance of the trait and the nature of the metabolic defect. With present techniques for tissue culture, fibroblasts derived from biopsy of skin are the most consistent source of cells for studies in vitro. Inborn errors of metabolism involving connective tissue cells should be particularly amenable for study in cell culture.

Hurler's syndrome (gargoylism) is a rare inborn error of metabolism (*2*) that results in an accumulation of mucopolysaccharides in various tissues of the body; its clinical features are dwarfism, grotesque skeletal deformity, restriction of joint movements, deafness, hepatosplenomegaly, cardiac abnormalities, and mental retardation (*3*). Studies of families indicate that the condition may be inherited in an autosomal recessive fashion—classical Hurler's syndrome (*4*); or in an X-linked recessive form—Hunter's syndrome (*5*). Despite some clinical overlap (*6*), the X-linked type is milder and, characteristically, is not associated with corneal clouding. The autosomal recessive form of the disease has been further subdivided into three separable clinical entities (*7*).

Skin was obtained by biopsy from three patients with various forms of Hurler's syndrome and from certain of their relatives. The first, a 4-year-old girl, had the clinical stigmata, including the presence of cloudy corneas, of the classical, autosomal recessively inherited type of Hurler's syndrome. The other two patients had already been investigated in this laboratory during a clinical and biochemical study of Hurler's syndrome (*8*). One was a 6-year-old boy with the clinical and biochemical signs of the X-linked, recessive form of the disease, Hunter's syndrome; he was only mildly affected, with no corneal clouding, and excreted increased amounts of chondroitin sulfate B and heparitin sulfate in the urine. The third patient, a 13-year-old boy, rather mildly affected physically but severely retarded mentally, excreted an increased quantity of heparitin sulfate in the urine. These symptoms and biochemical findings characteristically are associated with one of the autosomal-recessive forms of the disease sometimes known as the Sanfilippo syndrome (*9*). Skin was obtained by biopsy from the parents of the first two patients and, where possible, from other normal members of the families. Thirteen normal individuals (seven males and six females) served as controls. Each sample of skin (1 by 3 mm) was taken without anesthesia from the extensor surface of the upper arm. The skin was cut into 10 to 20 tiny pieces and cultured in a plasma clot in a Carrel flask by standard techniques (*10*).

After several weeks the explants were surrounded by dense halos of fibroblastic growth; the cultures were then treated with trypsin and transferred to flasks where they became established as monolayer strains. These cultures were grown in reinforced Eagle's medium (*10*) with 10-percent newborn calf serum. No significant differences in cellular activity between the cultures from normal and affected individuals were observed. Karyotypic analysis revealed a normal chromosomal constitution.

At the time of subculturing, coverslips were introduced into the flasks; from 1 to 7 days later the coverslips were removed and the cells were stained. Each coverslip was washed twice in warm balanced-salt solution, fixed in methanol for 5 minutes, and air dried. The preparation was then stained with the metachromatic dye, toluidine blue O (*11*) (0.1 percent toluidine blue O in 30-percent methanol), for 5 minutes; cleared with acetone,

Table 1. Metachromasia in skin fibroblasts 24 hours after subculturing.

Source of fibroblasts	Incidence in 1000 cells (%)	Extent (%)		
		Granules		Gargoyle cells
		Few (<50 per cell)	Many	
	Hurler, autosomal recessive			
Proband	73	90	8	2
Father	60	67	29	4
Mother	30	90	5	5
	Hurler (Sanfilippo), autosomal recessive			
Proband, biopsy 1	60	65	30	5
biopsy 2	69	87	11	2
biopsy 3	76	70	24	6
	Hurler (Hunter), X-linked			
Proband, biopsy 1	99	42	50	8
biopsy 2	93	68	24	8
Father	0	0	0	0
Mother	49	74	22	4
Paternal grandmother	0	0	0	0
Maternal grandmother	22	80	14	6
Paternal aunt	0	0	0	0
Maternal uncle	3	100	0	0
	Normal			
Controls, averages of 13	0.7	100	0	0