It is a matter of some interest that gamma globulin given intraperitoneally in mice may require as much as four days to achieve maximal protection. The explanation for the lag-period is not entirely clear. Kramer(11) found that when convalescent serum was inoculated into mice intraperitoneally, the level of poliomyelitis neutralizing antibody in the circulation was high the next day and fell sharply within the following 3 days. However, Morgan, Schlesinger, and Olitsky(14) and Kramer(11) presented evidence that the concentration of neutralizing antibody in the central nervous system—the *locus minoris resistentiae*—was a more important criterion of immunity to intracerebral inoculation of virus than was circulating antibody. Appearance of antibody in the brain and spinal fluid lagged behind antibody in the blood(14).

It is important then to consider that experiments on passive protection with gamma globulin or immune serum administered a customary 24 hours before intracerebral virus challenge, may not constitute a test under conditions of maximal prophylactic effect.

Summary. 1. The dosage of gamma globulin required to protect mice against poliomyelitis virus intracerebrally was several thousand times the amount which was effective for *in vitro* neutralization. 2. The prophylactic effect of gamma globulin was maximal with a dose of 4 ml/kg intraperitoneally when given 4 days before inoculation of virus intracerebrally; at 7 to 14 days protection had apparently disappeared.

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Electrophoretic Plasma Protein Patterns in Families with Hemophilia.* (20343)

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The appearance of an electrophoretic anomaly in a group of hemorrhagic diseases has been described in an earlier paper(1). This anomaly, which was called α_x-globulin, represents one of the few instances in which the electrophoretic patterns are typical for a given disease or for a certain group of diseases. The detection of this anomaly was made possible by the thorough morphologic study of the electrophoretic patterns(2) rather than by

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the customary determination of the relative concentrations of the individual protein components. The anomaly is more pronounced in the rising boundaries than in the descending boundaries. It possesses all of the following characteristics: 1. The appearance of a sharply pointed peak (α_x) which migrates somewhat faster than the usual α_2-globulin peak; the mobility of this α_x-globulin in the rising boundaries ranges between 4.3 and 4.6 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1}, in veronal citrate buffer of pH 8.6 and ionic strength $\mu = 0.1$. 2. The disappearance of the usual α_2- and/or α_3-globulin peaks, *i.e.*, there is no peak perceptible between the α_x- and the β-globulin. 3. The pronounced asymmetric shape of the α_x-peak with a very steep slope towards the albumin, but with only a gradual decline towards the β-globulin. Although it cannot be ascertained whether the α_x-globulin peak actually represents a particular type of protein absent in normal and in most pathological plasma, or merely an electrophoretic artifact, it is possible to calculate from this peak a relative concentration of protein. Accordingly, the α_x-globulin, whenever it appears, represents between 6 and 15% of the total plasma protein. To date, in a population in which blood diseases are represented in an unusually high proportion, the α_x-anomaly has been found in 102 cases out of a total of 440 individuals studied by electrophoresis. With very few exceptions, this electrophoretic characteristic occurs only in patients with certain hemorrhagic and certain allergic diseases. This anomaly is found with great constancy in hemophilia, in idiopathic thrombocytopenia of the acute variety(3), and in Henoch-Schönlein purpura which is probably on an allergic basis.

In the present paper the α_x-anomaly is shown to occur also in the plasma of members of hemophilic families although they may not be bleeders themselves. A relationship between the presence of an α_x-peak and the prothrombin utilization in the plasma of these individuals is described. The prothrombin utilization which is known to be high in normal blood but low in hemophilic blood, is usually high in mixtures of known hemophilic with normal blood. When the blood of a known hemophiliac is mixed with the blood of a member of a hemophilic family having an α_x-peak, however, a low prothrombin utilization may be observed.

Methods. Electrophoretic experiments were carried out as described in a companion paper(2). *Test for the detection of deficiency of anti-hemophilic protein (prothrombin utilization)*: The plasma of a known hemophiliac is first obtained. Blood is added to 1/10 volume of 0.2 M sodium citrate and centrifuged at 2000 r.p.m. for 10 minutes. Plasma from the individual who is being investigated is collected similarly. Equal volumes of known hemophilic plasma and plasma under investi-

TABLE I. Prothrombin Utilization in Members of Hemophilic Families.

Name	Sex	Age	Family relationship	Bleeder	Prothrombin time (sec.) of clotted mixture of plasma under investigation and known hemophilic plasma	α_x-globulin
			Family M.			
Mary M.	♀	56	Mother	—	59	—
Sally M.	♀	20	Children of Mrs. Mary M.	—	55	—
Thomas M.	♂	18		--	13	±*
Martin M.	♂	15		+	9	+
Jack M.	♂	11		—	12	+
Nancy M.	♀	10		—	14	+
Patricia M.	♀	9		—	15	±*
			Family S.			
Mary S.	♀	42	Mother	—	69	—
William S.	♂	12	Children of Mrs. Mary S.	+	15	+
Joan S.	♀	10		—	22	+
Gerald S.	♂	7		+	11	+

* ± designates a doubtful α_x-globulin.

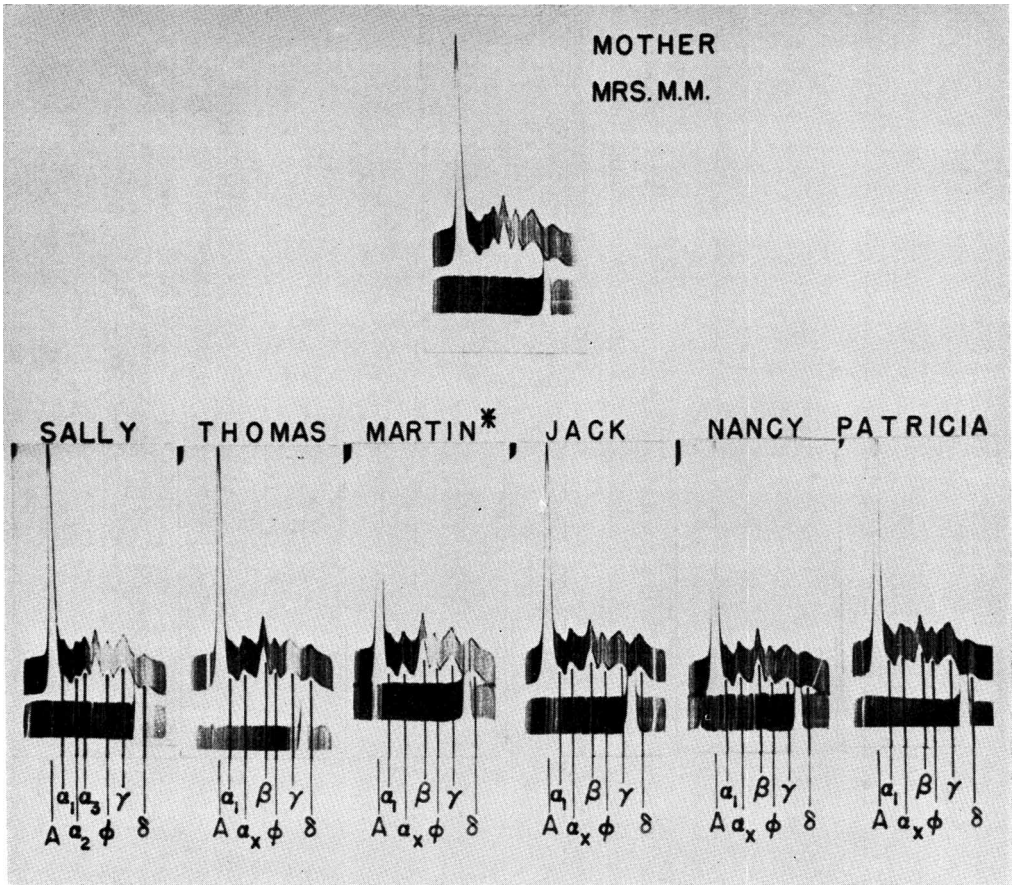

FIG. 1. Electrophoretic plasma protein patterns of Family M. * designates a bleeder. A = albumin; α_1, α_2, α_3, β, and γ = the corresponding globulins; α_x = α_x-anomaly; ϕ = fibrinogen; and δ = remainder of δ-salt anomaly; the lower lines represent the beginning gradients at time t = 0.

gation are mixed. The mixture is then recalcified by the addition of 1/10 volume of 0.2 M calcium chloride and, one hour after completion of the clotting, the serum is separated and its prothrombin activity determined according to the method described by Stefanini and Crosby(4).

Results. A total of 14 individuals with active hemophilia have been studied. An α_x-globulin anomaly was present in the plasma of all 14 individuals.

Furthermore, the plasma of the members of two hemophilic families which included three of the above mentioned "bleeders" has been studied. The electrophoretic patterns of the plasma of 11 members of these families are shown in Fig. 1 and 2.

The α_x-anomaly is present in the plasma of the 3 "bleeders", W.S., G.S., and M.M., but also in the plasma of 3 other family members, J.S., J.M., and N.M., who are not bleeders, including 2 females, J. S. and N.M. A somewhat doubtful α_x-peak is present in 2 other individuals, T.M., and P.M., in these 2 cases, the peak is believed to be a doubtful α_x-globulin because of the presence of another peak in these patterns with the mobility of the usual α_2-globulins.

The results of the prothrombin utilization (prothrombin time of the clotted mixture of plasma of each person and known hemophilic plasma) are given in Table I. A low prothrombin utilization is indicated by a low prothrombin time; a value below 25 seconds is considered to be typical for active hemophilia. It is obvious that the low prothrombin utiliza-

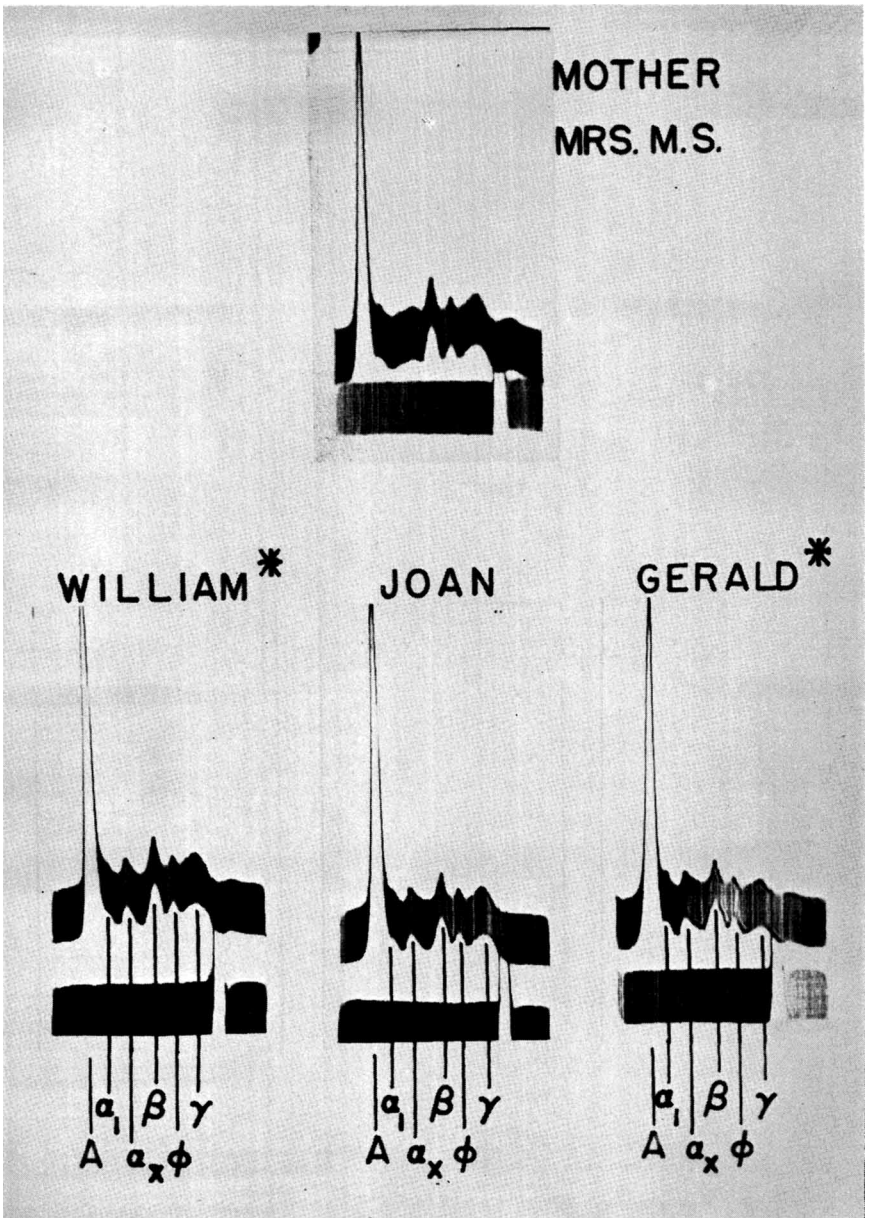

FIG. 2. Electrophoretic plasma protein patterns of Family S. * designates a bleeder. A = albumin; α_1, β and γ = the corresponding globulins; α_x = α_x-anomaly; ϕ = fibrinogen; the lower lines represent the beginning gradients at time t = 0.

tion not only appears in the plasma of the three bleeders, but also occurs in all those non-bleeding family members whose plasmas show an electrophoretic α_x-anomaly.

The appearance of an α_x-globulin has also been found in three nonbleeding members from two other hemophilic families.

Discussion. There is an obvious relationship between the appearance of an α_x-globulin in the electrophoretic patterns and a low prothrombin utilization in the plasma of members of hemophilic families. Both phenomena may be unrelated, however, to active bleeding itself.

From this study it is not possible to state

the degree to which this electrophoretic anomaly is related to the heredity of hemophilia, nor is it possible to predict from the electrophoretic patterns whether a woman will be a carrier of hemophilia or not. From the 7 members of hemophilic families, who are not bleeders themselves but have the electrophoretic anomaly, 3 are females. It is possible that these women will be carriers of the disease. A woman may be a carrier of hemophilia, however, without showing the electrophoretic anomaly. This follows from the observation that the mothers of the two families presented in this paper do not show the α_x-anomaly, although both are believed to be the carrier of hemophilia, the disease being transmitted in both families to male descendants.

Summary. 1. An electrophoretic anomaly, called α_x-globulin, has been found in the plasma of 14 hemophiliacs, *i.e.*, in all of the active bleeders studied. 2. The same anomaly also has been observed in 8 members of hemophilic families who are not active bleeders (over 50% of this category), and who are not afflicted with any of the other diseases in which this anomaly has been found. 3. The electrophoretic anomaly is seen in female as well as in male subjects unlike the bleeding manifestation of hemophilia, found only in males. 4. There is a definite relationship between the prothrombin utilization and the appearance of the α_x-globulin anomaly in the plasma of members of hemophilic families.

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Problems with Spontaneous Ectromelia (Mouse Pox) in a Virus Laboratory.* (20344)

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Since its discovery by Marchal(1), ectromelia virus, the causative agent of mouse-pox, has been recovered from several stocks of laboratory mice in Europe(2). Although mice have been reported in this country with a disease having similar signs, the virus has not been recovered from stock mice in the United States until recently when Trentin described an epizootic in the mouse colony of the Department of Anatomy at Yale(3). The conditions under which he isolated ectromelia virus are outlined in his paper. It was believed that the virus had been eliminated from the colony. However, we have recently observed a severe epizootic among our stock mice and have identified ectromelia virus as the causative agent. This report serves to indicate that the infection as late as February, 1953, was present among laboratory mice in the New Haven area.

The Outbreak. A number of non-specific deaths began to appear in the mouse colony of our laboratory in October and continued through the fall of 1952. During this period 200 pregnant mice arrived each week from 2 dealers. In addition, 200 to 300 were raised in New Haven each week for use when they reached the age of 4 weeks. For several months prior to the outbreak the newborn mice were being employed for isolating Coxsackie viruses from sewage and flies, and the adult mice chiefly for determining neutralizing antibodies against Lansing poliomyelitis virus. The total mouse population at any one time was a few thousand and rarely over 5000.

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