in the sequence of amyloid X. Amyloid X is also homologous with the other two proteins, although it does differ from them in at least two of the first 24 positions.

Amyloids VIII-b and X are, therefore, portions of κ-type light chains of the $V_{\kappa I}$ variable region subgroup. The molecular weight of an intact light polypeptide chain is approximately 22,500. The molecular weights of 7,500 for amyloid X and 18,300 for amyloid VIII-b, as determined by both SDS polyacrylamide gel electrophoresis and on calibrated Sephadex G-100 columns (*6*), indicate that these proteins are not complete light chains. Since both proteins start with the appropriate amino-terminal sequence, it is possible that these represent light chain fragments with varying amounts of the constant portion degraded. Alternatively, amyloid may consist of light polypeptide chains having internal deletions of varying lengths affecting variable regions, constant regions, or both, such as those reported in proteins from patients with "heavy chain disease" (*13*).

The losses on purification and the yield on sequence analysis (when compared with known homogeneous light chains) indicate that the proteins sequenced are the major components of amyloid and are not minor contaminants. Since only 30 percent of the protein content of the fractionated amyloid fibril concentrate is lost in purification and since sequence analysis and disc-gel electrophoresis give evidence of the presence of a single monomeric component in the final preparation, the possibility that nonspecifically absorbed homogeneous immunoglobulins accounted for 70 percent of the amyloid fibril concentrate would appear highly unlikely. The major protein component of these amyloid preparations is, therefore, a portion of an immunoglobulin polypeptide chain.

Observations and speculations concerning the possible relation between "antibodies" and amyloid deposits were made initially by Magnus-Levy (*14*) and Apitz (*15*). Experimental support for these speculations was later provided by the studies of Vasquez and Dixon (*16*) and Mellors and Ortega (*17*). Osserman *et al.* (*18*) analyzed the urines and serums of a large series of amyloid patients, and using more modern concepts of immunoglobulin structure they concluded that gamma globulins, particularly light chains, were directly involved in the amyloid infiltrates. Our data prove that in at least some cases, the major protein component of the fibrils of amyloid deposits are portions of light polypeptide chains.

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9 February 1971

Plasmodium berghei: Enhanced Protective Immunity after Vaccination of White Rats Born of Immune Mothers

Abstract. *Young white rats born of immune mothers had a significantly higher level of immunity to* Plasmodium berghei *after immunization with a nonliving antigen than either unvaccinated littermates or vaccinated rats born of normal nonimmune mothers.*

Partial protective immunity to *Plasmodium berghei* can be conferred by passive transmission of maternal antibody (*1*) and by artificial active immunization with a nonliving antigen (*2*). The combined effect of these two factors has not been studied before. Data presented here suggest that vaccination of white rats born of immune mothers confers a significantly higher level of protective immunity than vaccination of rats born of normal mothers.

An inbred strain of Wistar white rats and the NYU-2 strain of *P. berghei* constituted the host-parasite system in my investigation. An initial study on the relation of age and weight to pathophysiology revealed that, in this system, 50 to 80 percent of rats weighing 75 g (about 28 days old) to 100 g (about 34 days old) would die of acute infection characterized by high parasitemia and hepatorenal damage. In my study, animals were vaccinated when they weighed 50 g (about 21 days old), and the challenging infective inoculum was given at 75 g. This procedure was

Table 1. Mortality rates, average peak parasitemias, and prepatent periods after challenge with *Plasmodium berghei* in vaccinated and unvaccinated young white rats born of immune and normal nonimmune mothers.

Group		Mortality (%)	Average peak parasitemia (% infected erythrocytes)	Average prepatent period (days)
Description	No.			
Unvaccinated progeny of normal mothers	51	70	50 ± 13.6	1.2
Unvaccinated progeny of immune mothers	47	19	32 ± 14.1	3.2
Vaccinated progeny of normal mothers	40	40	41 ± 12.2	1.3
Vaccinated progeny of immune mothers	35	0	8 ± 5.3	4.2

adopted in order to use animals old enough to be immunologically responsive but young enough to be highly susceptible.

Antigen was prepared by saponization of heavily infected heparinized rat blood according to the method of Zuckerman *et al.* (*3*). The liberated, washed parasites were crushed in a Hughes-Colab press, and the cell-free extract was obtained by centrifugation at 15,000*g*. Protein content as determined by Biuret analysis ranged from 1.3 to 2.1 g per 100 ml of extract.

Adult female rats (approximately 250 g) infected with *P. berghei* 2 to 4 months earlier were reinoculated with rat blood containing 1×10^6 parasitized erythrocytes and mated 1 week later, to produce offspring of immune mothers. Litters of immune and normal mothers were weaned when they were 50 g and divided into two groups each. One group received 0.2 ml of antigen subcutaneously. The other group remained as unvaccinated controls. All rats were challenged 1 week later, when they weighed approximately 75 g, with 1×10^6 parasitized erythrocytes inoculated intraperitoneally. A thin blood film from each animal was made daily and stained with Giemsa; 200 erythrocytes were examined to calculate the percentage of parasitized to normal erythrocytes. The experiment was repeated four times.

The results of the four experiments are summarized in Table 1. Maternal immunity alone conferred protection in that the mortality rate was significantly lower ($P < .01$) than in the control group from normal mothers. However, the parasitemia was relatively high in many rats born of immune mothers, as reflected by the average peak parasitemia of 32 percent. Maternally derived antibody also tended to delay the onset of patency. The single injection of antigen had relatively little effect on the peak parasitemia or prepatent period in progeny of normal mothers. However, the mortality rates were lower ($P < .02$) than in unimmunized control rats, and presumably the immune response was sufficient to enable a large proportion to survive the infection. The vaccinated progeny of immune mothers exhibited a higher level of protective immunity than their unvaccinated littermates. The mortality rate was significantly lower ($P < .01$) in the vaccinated group, no deaths occurring in any of the vaccinated rats. Moreover, the average peak parasitemia was only one-fourth that of the unvaccinated group born of immune mothers. The prepatent period was prolonged another day, and the peak parasitemia was transient, lasting usually not more than 24 or 48 hours. After crisis, only the occasional parasite could be found in the daily blood films.

In areas of holoendemic malaria, the segment of the population most at risk to the serious consequences of the infection is the young children and infant-toddler group. This special age-related susceptibility has generally been attributed to the relatively ineffective immunologic response of young children to malaria. The results of these experiments indicate that young experimental animals may acquire a high level of protective immunity through the combined effect of maternal immunity and vaccination. Whether this phenomenon holds true for young children is not as yet known, nor have the optimal factors of maternal immunity and immunization that promote the maximal protective immunity been elucidated.

Production of a sterile immunity via the combination of maternal immunity and immunization is probably not feasible nor necessarily desirable. It has been argued (*4*) that the most beneficial effect of protective immunity would be to suppress the primary parasitemia so that it is of low-grade transient character without attendant clinical manifestations. A state of premunition would then ensue with a very scanty parasitemia to maintain long-term functional immunity. This has been the pattern of events noted in these experiments, brought about by the combined agencies of maternally derived immunity and immunization with a nonliving vaccine.

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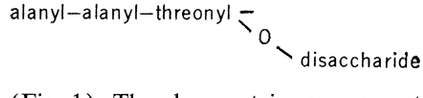
11 February 1971

Glycoproteins as Biological Antifreeze Agents in Antarctic Fishes

Abstract. *The blood serums of Antarctic fishes freeze at −2°C, which is approximately 1°C below the melting points of their serums. This thermal hysteresis is due to the influence of serum glycoproteins. The temperatures of freezing and melting of aqueous solutions of the purified glycoproteins suggest that this thermal hysteresis results from the adsorption of the glycoprotein molecule onto the surface of ice crystals.*

Certain Antarctic notothenid fishes, notably *Trematomus borchgrevinki, T. bernacchii,* and *T. hansoni,* are able to avoid freezing, even though they spend much of their lives in water laden with minute ice crystals (−1.9°C) and may, in fact, rest upon masses of anchor ice (*1–4*). The blood serums of these fishes freeze at approximately −2°C, a temperature at which the fishes die from freezing (*2, 3, 5*). The unusually low temperatures at which the blood serums freeze have been shown to result partly from the presence of a group of glycoproteins (*1, 2, 6*). On a weight basis, these glycoproteins lower the freezing point of the solution as effectively as NaCl, although the glycoprotein molecules are several hundred times larger than NaCl (*1, 6*). These glycoproteins, which occur in the serums of most Antarctic fishes, have been isolated and characterized (*1, 2, 6–8*). They are composed only of alanine, threonine, *N*-acetylgalactosamine, and galactose. These subunits are organized into repeating units with the sequence:



(Fig. 1). The glycoproteins are present in several sizes (molecular weights = 10,500, 17,000, and 21,500), and data from physical studies indicate that they are expanded structures without α-helical or β-structure (*6*). In attempts to elucidate the mechanism by which these glycoproteins so effectively lower the temperature at which ice formation occurs, both physical and chemical approaches have been employed.