the starch gels indicated that the J component comprised more than 50 percent of the hemoglobin present. Blood samples from an additional 36 subjects among the 1923 studied showed evidence of a fast component apparently identical with J; however, we think additional blood samples from these subjects should be examined before a final decision is made concerning its identity.

The subjects do not represent a random population sample chosen specifically for a survey of abnormal hemoglobin incidence; nevertheless, they do provide a small sampling from northeastern Thailand. In almost all instances only one member of a family group is included. The size of the sample precludes reliable estimates concerning the incidence of J_{Korat} in various parts of northeastern Thailand; however, its occurrence in approximately 0.5 percent of the entire sample is noteworthy. Our results suggest that the incidence of hemoglobin J_{Korat} may be shown in future detailed studies to be appreciable in some portions of Thailand. It appears quite likely that, next to hemoglobin E, J_{Korat} is the most frequent anomalous hemoglobin among normal Thais. It also appears possible that considerable heterogeneity will be found within the Thai people with respect to the incidence of J_{Korat}.

The occurrence of particular anomalous hemoglobins in several ethnic groups may prove to be of some ethnological importance. Therefore it is of interest to compare the structural relationship of hemoglobin J_{Korat} with that of the J-type hemoglobins reported previously. Following Thorup's initial report of hemoglobin J in an American Negro (*3*), other reports have appeared concerning hemoglobin J in Negroes (*11–13*), European Caucasians (*14–16*), Algerians (*17*), Gujerati Indians (*18*), tribesmen from northwestern Pakistan (*19*), Indonesians (*20*), Chinese (*21*), and others of obviously mixed ancestry (*22*). Clearly, not all of the hemoglobins J are identical; some are alpha-chain anomalies and others are beta-chain anomalies (*11–16; 23*); two of them, $J_{Baltimore}$ and J_{Oxford}, have established structures. The structure for hemoglobin $J_{Baltimore}$, found in an American Negro family by Weatherall (*13*) and in an English Caucasian family by Holman *et al.* (*15*), was found by Baglioni and Weatherall (*12*) to be $\alpha_2{}^{A}\beta_2{}^{16Asp}$. The same structure was found independently by Holman *et al.* (*15*) in their English family. Hemoglobin $N_{New\ Haven\text{-}2}$, from a French Caucasian family (*24*), also has a structure identical with that of $J_{Baltimore}$. Liddell *et al.* (*16*) found that J_{Oxford} has an analogous replacement of glycine by aspartic acid at position 15 of the alpha chain: $\alpha_2{}^{15\ Asp}\beta_2{}^{A}$; the same structure was reported (*25*) for hemoglobin $I_{Interlaken}$.

Although its precise structural anomaly has not been established, hemoglobin J_{Korat} is different from both $J_{Baltimore}$ and J_{Oxford}; our preliminary work (*26*) indicates that the anomaly in J_{Korat} resides in the sequence encompassing positions 41 to 59 of the beta chain (tryptic peptide β T5), where an aspartic acid replaces either phenylalanine or glycine. The same region of the beta chain is also affected (*26*) in hemoglobin $J_{Meinung}$, a J hemoglobin found in a Hakkanese Chinese family in Taiwan (*27*).

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Sterols and Temperature Tolerance in the Fungus Pythium

Abstract. *A fungus of* Pythium *species survives high and low temperatures longer when a suitable sterol is added to the growth medium.*

Certain sterols are required for sexual reproduction of pythiaceous fungi (*1*) and can stimulate their growth (*2*). Evidence now indicates that there is yet another role for sterols. *Pythium* sp. PRL 2142 (*3*) survives high temperatures longer when provided with a suitable sterol (cholesterol, β-sitosterol, and others) than it does when such a sterol is not available. Somewhat comparable effects were noted at the low temperature as well. The temperature at which this organism dies was affected not only by the presence of a sterol (that is, the sterol affects the nutritional state) but also by the speed with which the organism was heated to the critical temperature.

Agar cultures of the *Pythium*, with and without cholesterol, were presumed killed when kept several hours at

47°C. But examination of the plates several days after their return to 25°C revealed that the fungus had resumed growth at the points where sterol had been applied, but not elsewhere.

To confirm this observation, Difco potato-dextrose agar plates were inoculated, near the edge, with fungus and incubated at 30°C for 16 hours until the colonies were 45 to 50 mm in diameter. On the advancing edge of the colony, toward the center of the plate, a few crystals of cholesterol or β-sitosterol were placed. The plates were incubated at 30°C for 2 hours and then placed in test chambers in pairs (one of the pair with, the other without, sterol) and bottom-to-bottom, with thermocouples taped between the plates at the sites of sterol application. Temperatures were recorded on an eight-point Brown potentiometer during the test periods. Following each test period the plates were returned to 25° or 30°C, and the extent of subsequent growth was recorded periodically.

The organism, without sterol, could resume growth after up to 2 hours at 43° to 44°C. With sterol, growth was stimulated; after exposure for 3 hours at 43° to 44°C or for shorter periods at higher temperatures, growth was resumed only at the points of sterol application. In these experiments the cultures took 1.5 hours to reach the 43°C level. In later work, where cultures were heated to 43°C within 2 minutes, the fungus was barely able to withstand 10 minutes at the test temperature without sterol.

At the low-temperature limits, a series of cultures on the same agar did not survive 48 hours' storage at 1° to 2°C unless provided with a suitable sterol.

The temperatures which the mycelium of this fungus, with and without sterols and other selected nutrients, can survive after heating and cooling at various rates have not been determined.

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Protein Conformations in the Plasma Membrane

Abstract. *Infrared spectroscopy and optical rotatory dispersion have been used to test theories of structure of membrane protein. No evidence has been found to support the view that adjacent to the lipid there is a monolayer of protein in the β-conformation. The extracted protein appears to be a fairly typical globular protein with a low α-helical content.*

The classical Harvey-Danielli model (*1*) for the plasma membrane has formed the basis of discussion of its structure for the last 20 to 30 years. While the model was never intended to explain fully the varied behavior of the membrane, it remains the most acceptable basis for a theory of membrane structure. The state of the protein and the nature of its interaction with the lipid remain, however, almost totally unknown. The proposals of Danielli and his co-workers (*2*) were dependent on the current views on the conformation of proteins at interfaces. They cautiously concluded that proteins "on coming into contact with an oil-water interface, unroll into thin sheets, in a reversible manner" and that the membrane consisted of "a continuous film of lipoidal molecules, of which the two outermost layers are so orientated that the hydrated polar groups are in the oil-water interfaces, with a layer of protein molecules adsorbed on both of these interfaces" (*1*). Globular proteins were then believed to be adsorbed on these layers of "unrolled" or spreading proteins. Later workers have ascribed conformational states—usually the β-conformation—to the layers of protein associated with the lipid; for example, Kavanau (*3*) postulated that they "consist of unfolded and uncoiled fabric proteins in an extended β-conformation, possibly resembling a pleated sheet, with average spacings between the backbones of about 4.7 to 4.9 Å," together with some incompletely unfolded and uncoiled segments which provide regions of potential extensibility (*3a*).

The speculations on the arrangement of protein in the membrane have been greatly influenced by studies on the surface chemistry of proteins which implied that interfacial forces destroy the secondary and tertiary structure of proteins, producing interfacial films of unfolded open-chain molecules lacking any helical regions (*4*). However, infrared spectroscopy, and the measurement of rates of deuterium exchange in monolayers of synthetic polypeptides show that α-helices can exist at interfaces (*5*). Consequently, the whole question of the conformation of the protein in the membrane must be reopened bearing in mind that, since the conformation any protein adopts at any given time is contingent upon its environment, the conformation of membrane protein in vivo requires the presence of its associated lipid. As the techniques available for the study of conformation are mostly confined to substances in true solution or to crystals, the possibilities of a direct approach to membrane-protein conformation are limited.

Infrared spectroscopy is one of the few techniques yielding information on protein conformation that is applicable to the intact membrane, albeit in the form of a dry film. This technique reveals the presence of β-protein in the film by the position of the amide-I and -II bands which in the β state are observed at about 1630 and 1520 cm^{-1} as contrasted with about 1660 and 1540 cm^{-1} given by an α-helix or a random coil (*6*). The infrared spectrum of a film of hemoglobin-free ghosts of ox erythrocytes (*7*), dried in air at 20°C on a barium fluoride plate is shown in Fig. 1*a*. The amide-I band is at about 1660 cm^{-1} with no trace of a component at 1630 cm^{-1}. The amide-II band, while not so reliable for diagnostic purposes, also shows no indication of a β-conformation.

The ghost spectrum may be compared with that of a dry film prepared from a solution of the ghost protein obtained by *n*-butanol treatment of the ghost (*8*) (Fig. 1*b*, solid line). The differences between these two are attributable to the lipid component of the ghosts which can be estimated from a spectrum of the extracted lipid (Fig. 1*b*, broken line). The lipid makes only a small contribution to the spectrum of the ghost except in the region of the CH-stretching frequencies about 2850 cm^{-1}. About 50 percent of the extracted protein can be converted to a state possessing a β-form spectrum by heating with 50 percent ethanol at 70°C for 3 minutes (Fig. 1*c*).

It might be argued that the amount of β-protein required to form a layer on the lipid interface is, relative to other proteins in the membrane, too small to be detected by this method.