in the nodal membrane of the toad nerve fiber (*3*). The demonstration of this phenomenon in the normal squid axon suggests that the mechanism of production of action potential is very different from what has hitherto been generally accepted.

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Role of Proline in Polypeptide Chain Configuration of Proteins

Optical rotation can be used to detect the presence of helical configurations of the polypeptide chains in proteins, to determine screw sense, and to estimate the extent of helical regions (*1*). Moffitt's theory (*1a*) of rotatory dispersion for helical macromolecules has been successfully applied to synthetic polypeptides (*2*) and to proteins (*2, 3*), and at the present time it may be applied empirically to estimate α-helix content. Globular proteins have been shown to have relatively low (*2*) and fibrous proteins to have relatively high (*3*) helix content. We have examined the amino acid composition of proteins which belong to the keratin-myosin-epidermin-fibrinogen class (KMEF proteins) to account for the wide variation in amount of α-helix present; in this report we demonstrate a striking correlation between proline content and extent of helical configuration.

Table 1 lists data obtained by proline determinations using the method of Troll and Lindsley (*4*). Helix content is based on rotatory dispersion measurements reported previously (*3*). We assume for this discussion that light meromyosin fraction I is 100-percent helical and that the helices have a single sense of twist. Fragmentation of myosin by tryptic digestion into light meromyosin and heavy meromyosin corresponds to a fractionation into one component relatively poor and one relatively rich in proline. Furthermore, the light meromyosin may be separated by ethanol into two fractions differing in proline content, one of which (light meromyosin fraction I) remains soluble after treatment with ethanol concentrations of 50 percent (volume by volume) or higher and represents about 25 percent of the intact myosin by weight (*5*). In each case, the higher the α-helix content, the lower the amount of proline present.

These results suggest that proline interferes with the formation of the α-helical configuration. By adopting a very simple model for the α-proteins consisting of helical and nonhelical regions, one may estimate the disordering effect of a single proline residue, assuming that the proline is distributed statistically. Table 1 shows that, on the average, each proline residue is associated with 15 to 20 residues (hence several helical turns) not participating in the right-handed α-helical configuration in aqueous solution. In nonaqueous solution, this effect may be decreased (*2*). These observations are supported by model building from which it is seen that the pyrrolidines do not fit well into a right-handed α-helix.

Available data on proteins other than the KMEF series show a generally similar correlation of proline and helix content. On the basis of dispersion studies (*2, 3*), one may take the specific rotation of native proteins in aqueous solution as inversely proportional to the α-helix content. Thus, a fully-coiled right-handed α-helix may be characterized by $[\alpha]_D \cong 0°$ (*2*) and nonhelical chains by $[\alpha]_D \cong -100°$ (*6*). Globular proteins have rotations $[\alpha]_D \cong -30°$ to $-60°$, corresponding to low helix content of about 30 to 40 percent (*2*). Tristram's compilations (*7*) show that these proteins contain from 3 to 8 percent proline, corresponding to values expected from the data given above on the KMEF series. Exceptions such as lysozyme, insulin, and avidin having less than 2 percent proline might be accounted for by sulfur or phosphorus cross-linkages which may interfere with α-helix formation. It should be noted that such cross-linkages, as well as side-chain interactions, may stabilize or disrupt helical configurations (*8*). Thus the KMEF proteins discussed above, having few if any sulfur cross-linkages (fibrinogen excepted), provide simpler systems for this correlation than do the "globular" proteins (*9*).

Proline content higher than about 8 percent would be expected to cause almost complete absence of the α-helix, provided that the proline residues do not exist as "blocks" in the polypeptide chain. The rotation of casein $[\alpha]_D \cong -100°$ (*10*) supports this idea, and rotatory dispersion data on casein and the prolamines should be of considerable interest (*11*). Collagen has an exceptionally high pyrrolidine content (about 25-percent), but rather than assuming a nonhelical configuration, the polypeptide chains in collagen comprise a cable of three left-handed helices, each similar to the poly-L-proline configuration (*12*). Solutions of collagen have rotations of the order of $[\alpha]_D \cong -350°$ (*13*), comparable to rotations found for poly-L-proline (*14*). Thus native proteins exhibit at least two kinds of helical configuration, depending on the amount of proline present, and the two classes of fibrous proteins, the collagen and KMEF, may be described respectively as pyrrolidine-rich and pyrrolidine-poor (*15*).

Table 1. Helix content and proline concentration of KMEF proteins.

Protein	Wt. % helix	Wt. % proline	No. of nonhelical residues per proline residue
Light meromyosin fraction I	100	0.22	
Tropomyosin	94	0.35	15
Paramyosin	91	0.21	36
Light meromyosin	74	0.97	23
Myosin	56	2.08	18
Heavy meromyosin	45	2.87	16
Fibrinogen	32	3.84	15

In summary, we suggest the generalization (i) that less than 3 percent proline distributed statistically in a chain permits more than 50 percent α-helix, (ii) that about 8 percent proline deforms the backbone into a random coil, and (iii) that very high proline may favor a poly-L-proline type helix.

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Interaction of Stigmasterol and 2,4-Dinitrophenol in the Growth of Tetrahymena piriformis

Stigmasterol has been reported as a growth factor for several organisms, including the guinea pig (*1*), *Paramecium aurelia* (*2*), *Paramecium multimicronucleatum* (*3*), *and Stylonychia* (*4*). Extensive studies have been carried out on the mode of action of stigmasterol (antistiffness factor) in the metabolism of the guinea pig (*1*). These investigations revealed a marked reduction of anaerobic glycolysis in the tissues of deficient animals—a condition corrected by the addition of the antistiffness factor or adenosine triphosphate (ATP). Van Wagtendonk concluded that the steroid deficiency led to an altered phosphate metabolism, possibly a defect in the mechanism for the generation or transport of high-energy phosphate groupings. We believe that this hypothesis is strengthened by studies on the ciliated protozoan, *Tetrahymena piriformis.*

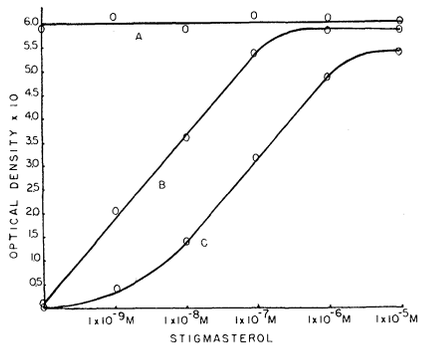

Fig. 1. Effect of DNP and stigmasterol on the growth of *Tetrahymena piriformis, W. Tetrahymena* were cultured in Kidder's medium A by means of the techniques and procedures developed by him for axenic culture (*11*). All points represent the optical density (×10) of cultures grown at 25°C for 96 hours, as measured in a Lumetron colorimeter at 650 mμ. Each experiment was repeated 5 times. Curve *A* represents stigmasterol; curve *B,* stigmasterol + DNP ($5 \times 10^{-5} M$); and curve *C,* stigmasterol + DNP ($1 \times 10^{-4} M$).

Tetrahymena has no exogenous nutritional steroid requirement but synthesizes a steroidlike compound, the configuration of which is not as yet known (*5*). However, the presence of this compound is capable of maintaining the growth of another protozoan, *Paramecium aurelia* (*6*), which has an absolute steroid growth requirement. The molecular configuration necessary for biological activity has been well established for *Paramecium,* and stigmasterol is one of the most effective sterols in promoting the growth of this organism (*7*). The *Tetrahymena* steroid is equivalent to stigmasterol in growth-promoting activity (*6*); thus, it seems possible that the *Tetrahymena* steroid is a member of the stigmasterol group.

In *Tetrahymena,* certain growth inhibitors, both steroidal and nonsteroidal in nature, induce a steroid requirement, which can be satisfied by stigmasterol (*6*)—further evidence of a possible relationship between the *Tetrahymena* steroid and stigmasterol.

Among the nonsteroid growth inhibitors, the most interesting is 2,4-dinitrophenol (DNP). The reversal of DNP growth inhibition in *Tetrahymena* in the presence of stigmasterol is shown in Fig. 1.

These growth studies indicate a close relationship between DNP and stigmasterol in the metabolism of *Tetrahymena.* The linearity of the reversal of DNP growth inhibition may indicate a competitive phenomenon. Further, since DNP is known to be an effective uncoupling agent of oxidation and phosphorylation (*8*) and an activator of adenosine triphosphatase (*9*), we believe that this study in *Tetrahymena* (*10*) not only indicates a mode of action of stigmasterol similar to that suggested for the guinea pig but greatly strengthens such an interpretation. While the studies with the antistiffness factor in the guinea pig indicated an influence of this compound on anaerobic glycolysis, this interpretation may be too limited and perhaps should be enlarged to include aerobic mechanisms. Further *in vivo* and *in vitro* experiments are being conducted to test this hypothesis.

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Occurrence of trans Fatty Acids in Human Tissue

Except for small amounts of *trans* fatty acids in animal fats, dietary fats are composed of unsaturated fatty acids of *cis* geometric configuration. In 1928, Bertram (*1*) found small amounts of *trans* Δ 11-octadecenoic acid in ox, sheep, and butterfat; more recently (*2*), the presence of 4- to 11-percent *trans* fatty acids has been reported in deer, ox, and sheep depot fats. Although *trans* fatty acids do not seem to be normally present in nonruminants, they are found in the depot fats of rats which have been fed *trans* fatty acids (*3*).

Considerable amounts of *trans* fatty acids are formed during the commercial hydrogenation of vegetable oils (*4*); the shortenings and margarines which include these hydrogenated oils have been reported to contain as much as 23 to 42 percent of *trans* fatty acids (*5*). Furthermore, the isomers formed during selective hydrogenation are composed of a complex mixture of both geometric and positional isomers (*6*). The consumption of such fats would presumably lead to the deposition of *trans* fatty acids in depot fats.

In the present study, autopsy and biopsy material from 24 human subjects (*7*) was examined for the presence of *trans* fatty acids. The tissues were extracted in a Soxhlet apparatus for 24 hours with acetone and petroleum ether (Skellysolve F) as solvents, the extracts were dried over anhydrous sodium sulfate and filtered, and the solvent was removed under vacuum. The amounts of *trans* isomers in the lipid extracts were determined by the Jackson and Callen baseline method (*8*), in which a Beckman IR-2A spectrophotometer was used.