α helix. There is, however, a helical configuration for the polypeptide chain not hitherto described which satisfies all the major Pauling, Corey and Branson[4] restrictions and which can be formed using the Corey-Donohue[5] polypeptide chain dimensions. The configuration is obtained when the polypeptide chain is coiled into a helical form in such a way that each amide group is hydrogen bonded to the fourth amide group beyond it along the chain. In this sense, it is intermediate between the α and γ helices in which the amide groups are hydrogen bonded to the third and fifth amide groups beyond them respectively. We have named it, therefore, the π helix.[6] There are approximately 4.4 amino acid residues per turn, with a unit residue translation along the helical axis of approximately 1.14 Å., giving a pitch of about 5 Å. The structure involves some slight distortion (less than 5°) in the intra-chain α carbon bond angle. Angular distortions of the "tetrahedral" carbon bond angle of this order are not unknown[7] and changes of less than 5° should introduce only a small amount of strain energy.

There is a cylindrical hole down the center of the helix, and it has been criticized on this basis.[8] The hole is less wide than that down the center of the γ helix, and it is, therefore, not large enough to accommodate a water molecule. Thus, there is no way of bridging the long intra-chain van der Waals distances across the cavity. Since the original Pauling–Corey restrictions were enunciated, a further stereochemical limitation has been postulated by these workers,[9] which is considered adequate to exclude the γ helix completely. The restriction is concerned with the potential function for orientation about a single bond between the α carbon (tetrahedral) and the peptide nitrogen or peptide carbon. The orientations about the α C–N and α C–C bonds for the π helix have not yet been determined. Calculations of the X-ray form factor and Fourier transforms for the helix are now proceeding (with H. J. Grenville-Wells). They will be published together with a more precise formulation of the helix later.

The π helix is certainly under some slight strain and the α helix represents a lower potential energy minimum for the atoms of the bare chain $(HCCONH)_n$. The π helix can probably not be ruled out, however, on this basis since the potential energy function for the protein chain with attached polyfunctional (R) groups $(RCHCONH)_n$ may well be rather different from that of the bare chain.

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ON THE STABILITY OF SERUM LIPOPROTEINS AND EVIDENCE OF A STABILIZING FACTOR

Sir:

Research into the origin of atherosclerosis has been stimulated by the introduction of ultracentrifugal techniques for the isolation and characterization of serum lipoproteins.[1] Correlations are found[2] between the levels of lipoproteins in the S_f 10–100 classes and the incidence of atherosclerosis in humans [rabbits show a quite analogous behavior[3]]. Perhaps the lipid constituents are deposited in the formation of the atherosclerotic lesions, Other factors are, however, involved since the presence of these lipoproteins is frequently without effect.

Physical chemical studies in this Laboratory on ultracentrifugally isolated lipoprotein fractions from the sera of humans and rabbits have shown that lipoproteins of the S_f 5–30 classes are unstable when dialyzed against buffered saline solutions. Several related changes take place *in vitro* that are due neither to thermal nor biological degradation:

(1) The S_f value (s) gradually decreases. By controlled dialysis progressively lower S_f values are obtained, ultimately even leading to sedimentation. With a homogeneous fraction of given initial S_f value this instability results in a decrease in lipoprotein within the initial S_i range and in an increase in sedimenting material.

(2) The ultracentrifugal pattern spreads, indicating increased heterogeneity.

(3) Lipid material is released from the lipoprotein and in a low centrifugal field forms a layer on the surface of the solution. Also, the turbidity of the dialyzed fraction may increase. With S_f 5–9 lipoproteins this is not readily apparent to the eye, but higher S_f fractions become markedly turbid.

These changes do not occur when the lipoproteins are dialyzed against saline solution to which a concentrate obtained from bovine or rabbit serum has been added. It appears that a stabilizing factor is contained in the isolated material. In Fig. 1 the sedimentation behavior obtained in a typical dialysis experiment is reproduced.

These findings lead us to postulate an equilibrium between a lipoprotein complex and its components (protein, lipid and stabilizing factor), although we have been unable so far to reconstitute the complex in our *in vitro* experiments.

This equilibrium permits one to speculate on a possible mechanism for the deposition of lipid within the arterial wall. If the concentration of the stabilizing factor were insufficient, or were destroyed by metabolic or excretory pathways, lipoprotein in the region of the arterial wall would degradate by a loosening of its lipid components.

(1) J. Gofman, F. Lindgren, H. Elliott, W. Mantz, J. Hewitt, B. Strisower, V. Herring, and T. Lyon, *Science*, **111**, 166 (1950).

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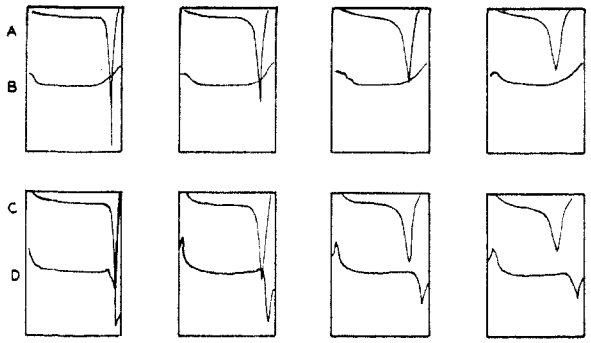

Fig. 1—Ultracentrifugal flotation patterns of a rabbit lipoprotein fraction before and after dialysis: A, original isolated lipoprotein; C, a portion of the original after dialysis for one week at 0–4° against a large volume of saline solution (density, 1.063) which contained 0.05% of material extracted from bovine serum; D, a second portion of the original after an identical dialysis against saline alone. The degradative changes are partially complete in D. Continued dialysis leads to extensive degradation as illustrated in B, whereas, the stabilized portion continues to be unchanged. (B refers to a different original fraction than that in A, C, and D, but the result shown is typical of all fractions.) Runs were made with a Spinco Ultracentrifuge using the double cell technique. Conditions: 22–26°; speed, 52,640 rpm.; medium density, 1,063; inclined slit angle, 45°; exposures, from left to right, at 8 min. intervals; acceleration time, 7 min.; first exposure, 4 min. after reaching maximum speed. The menisci are on the left of each pattern, thus the lipoprotein components are moving from right to left against the centrifugal field.

At one stage there would appear a complex of lower lipid content, of reduced size and changed character, for example, S_f 5–9 lipoprotein. This concept emphasizes that the pathogenesis of the atheromatous lesion is the result, not of the presence of "abnormal" lipoproteins, but of the errant breakdown of lipoproteins in general.

The stabilizing material is obtained as follows. The serum from fresh bovine blood is lyophilized and then extracted several times with glacial acetic acid. The combined extract is diluted with five parts of ether. Concentrated hydrochloric acid is added until no further precipitate forms. The tannish precipitate is filtered, washed with ether, dried, extracted with water, and finally lyophilized. Approximately 12 g. of white solid material is obtained from 4 l. of serum and as little as 0.05% added to saline will completely stabilize lipoproteins in our dialysis experiments. Heparin, as well as a number of other substances tested, does not possess stabilizing ability. This is of interest in view of the profound *in vivo* effect of heparin upon the lipoprotein picture and poses the question as to what relation our stabilizing factor may have to the "clearing factor" of Anfinsen,[4] to the "active principle" of Graham,[5] or to the "cofactor" of Snellman.[6]

Work is continuing toward the further isolation and identification of the factor. Also the bearing these findings may have upon experimental atherosclerosis in the rabbit is being investigated.

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(4) C. B. Anfinsen, E. Boyle and R. K. Brown, *Science*, **115**, 583 (1952).

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(7) G. D. Searle and Company Research Fellow in Chemistry.

CHLOROPHYLL PHOTOSENSITIZED POLYMERIZATION AND FREE RADICAL INTERMEDIATES IN PHOTOSYNTHESIS

Sir:

We are interested in the question whether and what free radical intermediates occur in the process of photosynthesis and chlorophyll photosensitized reactions. We are also interested in efficient methods of photosensitized polymerization and their reaction kinetics. With these aims in view, photochemical experiments were carried out in the presence of a vinyl monomer (methyl methacrylate) and (a) chlorophyll in various solvents, (b) isolated chloroplast suspensions and (c) photosynthesizing algae. In the latter case some evidence suggesting free radical intermediates has been obtained but further investigation, including tracer experiments with $C^{14}O_2$, is in progress. Results with isolated chloroplast suspensions have been negative so far. Some more detail is cited of results observed with chlorophyll. The now generally adopted standard method developed by Zcheile and Comar was used for its preparation from spinach leaves. After we assured ourselves that pure chlorophyll a and chlorophyll b lead to similar results, we used a purified mixture of the two (although even crude spinach extracts were active). The solutions were deoxygenated by passing a stream of nitrogen through the mixture before irradiation. Ultraviolet and most of the visible light were filtered off so that practically only red light passed through the reaction mixture. The final concentration of chlorophyll was approximately 0.2 g./l. ($\sim 2 \times 10^{-4}$ *M*). While in preliminary tests a number of monomers were applied, most experiments were carried out with methyl methacrylate.

This is a brief summary of results: in pure methyl methacrylate as well as in solutions of 10% methyl methacrylate in ethyl alcohol (polymer insoluble) and in pyridine, polymerization was observed in the absence of oxygen. The rate increased with time due to catalysis by dead polymer. Quantum yields in the initial stages were considerably below unity. Average molecular weights were of the order of magnitude of 10^5. No polymerization was obtained when acetone or benzene were used as solvents (in the absence of other substances). Ferrous sulfate and ferrocyanides were found to inhibit polymerization. On the other hand, the rate of polymerization could be enormously increased in the presence of some organic reducing substances such as ascorbic acid and thiourea in pyridine and alcohol solutions and also in pure methacrylate. When all blank tests were taken into account, it was found that the quantum yield of monomer consumption in the system methyl methacrylate–chlorophyll ($\sim 2 \times 10^{-4}$ *M*)–ascorbic