of acetone was added to 5 cc. of well-mixed whole milk to obtain the filtrate. The results of four studies conducted on separate days are summarized in Table I. All samples were from different cows. Atmospheric temperatures at which the samples were exposed were never less than 60°F. nor higher than 72°F.

The mean loss of riboflavin in sixty minutes was 0.69 γ. The standard error of this loss based on the variation in loss from series to series was 0.055 γ. Observed losses of riboflavin at exposure intervals of 30, 90, 120 and 210 minutes were, respectively, 28, 50, 66 and 72%.

Control samples of milks used in the study showed no loss of riboflavin when stored in the dark at room temperature for twenty-four hours, nor did any loss occur when they were stored in a refrigerator for seven days. In order to determine whether an initial exposure to sunlight would stimulate a more rapid decrease when samples were subsequently placed in the dark under refrigeration, three samples (1.54 γ/cc.) were placed in the refrigerator after one-half, one, three and five hours of exposure. After forty-eight hours, the additional loss which had taken place was extremely small, averaging 0.016 γ, and in no case exceeding 0.07 γ.

It is evident that the destruction of riboflavin in milk by sunlight proceeds at a very rapid rate, even at ordinary temperatures.

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THE LINKAGE OF CORPUSCULAR PROTEIN MOLECULES. I. A FIBROUS MODIFICATION OF INSULIN

Sir:

When 1.5 to 2.0% solutions of insulin hydrochloride (Lilly, amorphous) at *p*H 2.0 to 2.5 are heated for thirty minutes at 100°, thixotropic gels are formed which exhibit static double refraction.[1,2] Dilution to 0.5% produces a viscous solution showing intense flow double refraction.

Electron micrographs of this material reveal the presence of uniform fibrils several microns in length, having uniform widths of approximately 200 Å. (an asymmetry well above 100).

Insulin gels, containing 2% protein, have been frozen and thawed many times in a solid carbon dioxide–alcohol mixture. During this treatment the viscosity gradually decreases to low values. Flow double refraction replaces static double refraction and after 10 cycles even the former decreases greatly in magnitude. A second heat treatment produces a thixotropic gel showing static double refraction. This indicates that segments of fibrils produced by the freezing-thawing treatment unite in a directional manner.

After standing for twenty-four to forty-eight hours at *p*H 11.0–11.5 and 0°, a dispersion of insulin fibrils loses its characteristic double refraction and viscosity. The solution thus produced resembles an equivalent solution of native insulin so far as the methods mentioned have demonstrated.[3] Protein may be recovered after such a "reversal" and dissolved to form a 2% solution at *p*H 2.0. Heat treatment again produces a thixotropic gel showing static double refraction. This cycle has been repeated several times.

Prolonged heating of insulin in acid solution or at reactions well below *p*H 2.0 produces sphaerocrystalline aggregations of insulin fibrils termed the "heat precipitate of insulin" by du Vigneaud, Geiling and Eddy.[4] These authors have shown that this material, dissolved in dilute alkali and immediately acidified, exhibits 80% of the biological activity of the original insulin. Since the heat precipitate of insulin is now shown to consist of aggregations of insulin fibrils, it is considered that insulin fibrils show such activity after reversal by alkali whether or not they have formed the "heat precipitate."

Insulin fibrils are considered to be constructed of corpuscular molecules which have been deformed only slightly in the process. Jensen[5] has suggested that the insulin molecules present in the heat precipitate are linked by covalent bonds. Unpublished chemical evidence shows that insulin fibrils may be formed after the sulfur has been split off by alkali and after ammonia has been liberated by heat. This type of evidence indicates that fibril formation may take place after the removal of those groupings which are expected to take part in covalent bond formation. Therefore it is proposed that insulin molecules are linked mainly through the secondary valence forces resulting from the approximation of nonpolar side chains. Two surface concentrations of these chains on opposite portions of the insulin molecule would account for the bifunctional nature exhibited in fiber formation.

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(2) D. F. Waugh, *Am. Jour. Phys.*, **133**, p. 484 (1941).

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(5) H. Jensen, *Science*, **75**, 614 (1932).