bohydrate is then calculated as glucose from the following relation:

$$\text{mg. of glucose in sample} = \frac{\text{mm}^3\ CO_2\ \text{produced}}{248\ \text{mm}^3}$$

No determination of a correction factor from standard glucose solutions is necessary.

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CONVERSION OF GLOBULAR TO ORIENTED FIBROUS PROTEINS

IT was reported recently[1] that several globular proteins can be changed into a fibrous form by heating them in the presence of water and subjecting them to high shear stress. As evidenced by x-ray diffraction, the converted protein molecules have substantially the same spatial arrangement as the molecules of the natural fibrous protein, β-keratin, as it occurs in feathers and stretched wool and hair. To the list of globular proteins that can thus be made fibrous in the molecular sense may now be added gliadin, the mixed proteins of blood serum[2] and the globulins of tobacco and pumpkin seed.[2]

Conversion from the globular to the fibrous form has also been effected by soaking protein filaments in aqueous solutions of various reagents, followed by stretching, both treatments being carried out at room temperature.[3,4] Ovalbumin, lactoglobulin, casein and pumpkin seed globulin have been converted from the globular to the oriented fibrous form in this way. Ovalbumin has been particularly easy to unfold and orient. β-keratin diffraction patterns have been given by ovalbumin filaments stretched at room temperature after being treated with such aqueous solutions as the following: 75 per cent. methanol, 75 per cent. ethanol, 75 per cent. isopropanol, 50 per cent. t-butanol, saturated benzyl alcohol, 5 per cent. phenol, 75 per cent. formamide, 50 per cent. urethane, 50 per cent. pyridine, saturated aniline, 75 per cent. acetaldehyde, 90 per cent. acetone, saturated methyl ethyl ketone, 50 per cent. ethylene glycol monoethyl ether, 75 per cent. dioxane, 10 per cent. chloral hydrate, 10 per cent. silver nitrate, saturated cerous nitrate, 50 per cent. cupric nitrate, 10 per cent. trichloroacetic acid, 25 per cent. sulfuric acid, 20 per cent. hydrochloric acid, 17 per cent. nitric acid, 10 per cent. toluene sulfonic acid and 5 per cent. sodium hydroxide. Ovalbumin filaments soaked in acetic anhydride, glacial acetic acid, 98–100 per cent. formic acid, 65 per cent. lithium bromide, 50 per cent. ammonium thiocyanate, or saturated aqueous solutions of either urea or guanidine hydrochloride, and then in water were stretched to give the β-keratin structure.

It should be mentioned that the "egg-white" pattern of Astbury, Dickinson and Bailey,[3] characterized by the 9.8 Å reflection on the equator and the 4.7 Å reflection on the meridian, has been obtained also from chemically treated ovalbumin.[1]

The concentration of the reagent is not critical, and the time of soaking required to render the filaments stretchable ranges from a few minutes to several hours. For example, filaments soaked in saturated ammonium thiocyanate for two minutes and then in water for three minutes were stretched to a draw ratio (ratio of final to initial length) of 4.9 and gave the β-keratin pattern. Soaking in saturated aniline did not produce good stretching characteristics until after about five hours. Roughly, a draw ratio of five is required to give good orientation. If internal friction is small, the high shear required for orientation is not developed on stretching, and despite a large draw ratio the specimen gives only an amorphous diffraction pattern. Conversely, if cohesion and internal friction are large, a draw ratio of only two or three will give good fiber patterns. Ordinarily, filaments are most easily stretched while moist, but certain ovalbumin preparations, particularly those treated with phenol solution, may be stretched readily when air-dry. The phenol-treated ovalbumin filaments were also remarkable in that they exhibited the typical characteristics[5] of cold drawing, that is, on application of tensile stress they "necked down" and with continued stretching the necked-down, oriented section grew at the expense of the larger, unoriented sections.

Chemical treatment increases the degree of ordering of the peptide chains. Strong diffraction rings at approximately 4.7 and 9.8 Å sharpen, and fainter rings are resolved from diffuse halos.[6] As many as five diffraction rings have been obtained from ovalbumin treated with aqueous methanol, formamide or urethane. The rings from the formamide-treated ovalbumin occurred at 10.7, 4.7, 3.7, 2.2 and 2.0 Å. As a rule, preparations showing the largest number of diffraction rings and the sharpest rings were most easily oriented and gave fiber patterns containing the greatest

[1] F. R. Senti, C. R. Eddy and G. C. Nutting, *Jour. Am. Chem. Soc.*, 65: 2473, 1943.

[2] We should like to thank Sharp and Dohme, Inc., for the serum proteins, and H. B. Vickery, of the Connecticut Agricultural Experiment Station, for the pumpkin seed globulin.

[3] W. T. Astbury, S. Dickinson and K. Bailey, *Biochem. Jour.*, 29: 2351, 1935.

[4] K. J. Palmer and J. A. Galvin, *Jour. Am. Chem. Soc.*, 65: 2187, 1943.

[5] W. H. Carothers and J. W. Hill, *Jour. Am. Chem. Soc.*, 54: 1579, 1932.

[6] G. L. Clark and J. H. Shenk, *Radiology*, 28: 58, 144, 1937, observed sharpening of the 4.7 and 9.8 Å diffraction rings of ovalbumin and hemoglobin precipitated from dilute solution by trichloroacetic acid, ethanol, acetone or formalin. M. Spiegel-Adolph and G. C. Henny, *Jour. Phys. Chem.*, 46: 58, 1942, observed sharpening of the pattern of pseudoglobulin denatured by ethanol.

detail. The nine forms found in the fiber pattern of β-keratin and heated ovalbumin[1] have been found also in chemically treated ovalbumin. The chemicals used were by no means equally effective in converting the globular protein to the fibrous form. However, the degree of crystallinity and orientation produced by treatment with aqueous formamide, urethane or aliphatic alcohols was at least equal to that obtained after heat treatment. Judging from the sharpness and length of the diffraction arcs, the best of the converted protein preparations were equal to the natural fiber, raw silk, in both crystallinity and orientation.

Filaments of the more soluble proteins were prepared by mixing the powdered material with approximately half its weight of water and extruding the mixture through a die in an arbor press. One per cent. of sodium chloride was added to the mixture of pumpkin seed globulin and water to make it readily extrudable. Casein filaments were extruded from a briefly heated casein-water mixture. This heating produced no detectable change in the x-ray diffraction pattern.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A CLOSED CIRCUIT APPARATUS FOR THE MEASUREMENT OF RESPIRATORY METABOLISM

SEVERAL devices for calorimetry of small animals have been described.[1, 2, 3, 4] Metabolic studies frequently require the determination of the exchange of respiratory gases for large numbers of animals. Because of certain disadvantages in some of these methods we have devised an apparatus shown in Figs. 1 and 2.

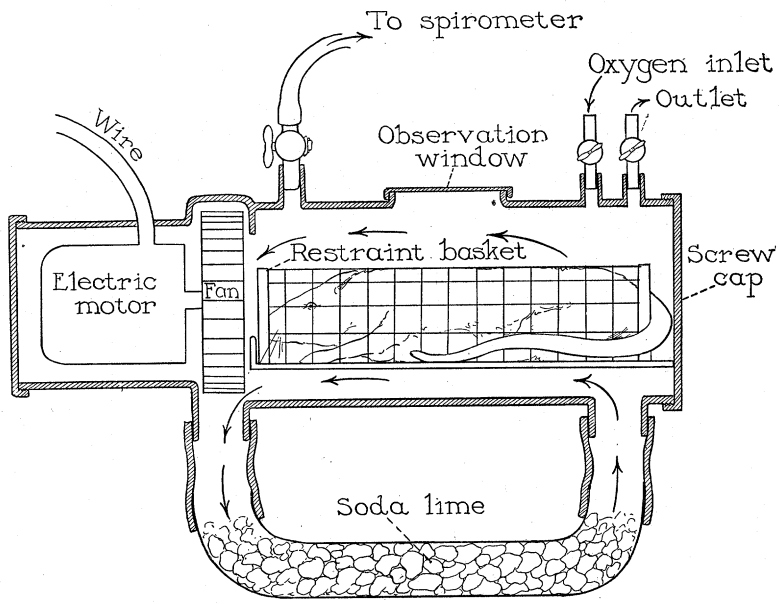

FIG. 1. Cross section of metabolic chamber showing rat in place in restraint basket.

The chamber is constructed of heavy brass tubing, 4 inches (10 cm) in diameter and 9 inches (23 cm) in length. A screw cap is fitted to one end and a smaller brass tube 3 inches (7.6 cm) long and 3¼ inches (8.3 cm) in diameter is riveted to the other end and closed by a screw cap. The smaller tube contains a 6-volt electric motor and fan assembly from an automotive windshield defrosting unit. This centrifugal fan circulates the gases through a 1-inch (2.5 cm) glass tube which is filled with soda lime and attached by rubber connections to the bottom of the main chamber.

Three outlets equipped with stopcocks lead from the upper part of the chamber; one is for the inflow of oxygen, one is a simple outlet or vent and the third one connects the respiratory chamber with a small, brass, Krogh spirometer measuring 5½ by 3 by 2¼

[1] F. G. Benedict and Grace MacLeod, *Jour. Nutrition*, 1: 343–366, 1929.
[2] M. L. Tainter and D. A. Rytand, *Proc. Soc. Exper. Biol. and Med.*, 32: 361–363, 1934.
[3] E. L. Schwabe and F. R. Griffith, Jr., *Jour. Nutrition*, 15: 187–198, 1938.
[4] W. H. Newton, *Jour. Physiol.*, 89: 421–428, 1937.

[7] One of four Regional Research Laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.