added dropwise while the mixture was stirred; stirring was continued for 4 hours. The mixture was next allowed to stand for 6 hours, then filtered through a Büchner funnel and the precipitate washed with 700 cc of water. The filtrate was concentrated at 40° C. *in vacuo* to a volume of approximately 200 cc and its glycogen precipitated by the addition of an equal volume of 95 per cent. alcohol. The precipitate was permitted to settle, then filtered and washed with alcohol. Further purification was effected by twice redissolving in water and reprecipitating from alcohol. Finally it was dried *in vacuo* at 40° to constant weight. This phosphorylated glycogen contained 0.43 per cent. phosphorus and 0.57 per cent. calcium.

The phosphorylation was repeated 7 times by the above method, with a consequent increase in its phosphorus content each time. After the seventh operation, the phosphorylated glycogen contained 1.73 per cent. phosphorus and 2.66 per cent. calcium. Its specific rotation $(\alpha)_D$, after being dried *in vacuo* at 40° to constant weight, was +174°.

The phosphorylated glycogen was soluble in water and the presence of ionic calcium was demonstrated by its precipitation upon the addition of ammonium oxalate. No test for phosphate was obtained with ammonium molybdate, even after acidifying and boiling with dilute nitric acid for a few minutes. Phosphoric acid was split off, however, by treating the phosphorylated glycogen with a few cc of hydrogen peroxide and several drops of nitric acid containing a trace of ferric nitrate, after the manner of Neuberg and Mandel.[2]

The phosphorylated derivatives obtained by enzymatic hydrolysis of the phosphorylated glycogen will be described elsewhere.

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A PRELIMINARY REPORT ON THE SPECIFICITY OF KERATINS[1]

ALTHOUGH species specificity is a general attribute of proteins, serological species differences of keratins have been generally accepted as either poorly defined or not demonstrable.

The experiments of Krusius,[2] in which he employed antiformin for the preparation of keratin, led to hydrolysis and alterations in the protein-molecule which may account for the lack of specificity of keratins from different species. Krusius himself realized this possibility.

Recently, Goddard and Michaelis[3] observed that keratin owes its peculiar resistance against dissolving agents to the di-sulfide bonds in their original positions, which are mainly responsible for the pattern of the structure of keratin and also its physical properties. These observers were able to split these di-sulfide groups by reducing agents in such a manner as to leave intact the chemical composition and avoiding hydrolytic splitting. The reduced protein obtained was called "kerateine" and it behaved more like an ordinary protein than native keratin, both with respect to solubility and behavior toward proteolytic enzymes.

The immunological investigations to be described here were primarily directed toward the study of the antigenic power and specificity of oxidized and reduced keratins prepared by the method of Goddard and Michaelis.

Keratins were prepared from human hair, wool and chicken feathers. Elementary chemical analysis revealed that the compounds are closely related. The total nitrogen, sulfur, cystine and isoelectric points are essentially identical in all the preparations employed.

In brief, the results of these studies disclosed that species specificity is an individual characteristic of the keratins employed and that the specificity observed is dependent on the redox state of the sulfhydryl groups in the protein molecule.

In cross-precipitation reactions overlapping was encountered, and especially in low dilutions of the antigens; but in the very dilute antigens, *i.e.*, ($\pm$ 1:25,000), the antiserums gave specific precipitates in the presence of their homologous antigens.

Of greater significance is the finding that species specificity was obtained only when the reduced keratin (kerateine) was allowed to react with the antiserum prepared by the injection of the homologous reduced keratin; while marked overlapping occurred when oxidized keratin (metakeratin) and the parent protein (75 per cent. oxidized) were employed as antigens.

The same phenomenon was observed when oxidized keratin was allowed to react with its homologous antiserum. This indicates that not only are the keratins species specific, but that immunological differences are detectable in a single keratin preparation depending on the state of oxidation or reduction of the protein employed.

It would seem, as data upon the basic amino acids content of proteins accumulate, that recognition must be given to the view that there exists a central basic nucleus characteristic for any one biological type of

[2] C. Neuberg and J. A. Mandel, *Biochem. Z.*, 71: 196, 1915.

[1] From the Institute of Pathology, Western Reserve University and the University Hospitals, Cleveland, Ohio. Aided by a grant-in-aid, Division Medical Sciences, National Research Council.

[2] Fr. F. Krusius, *Arch. f. Augenheilkunde*, Supplement, 67: 47, 1910.

[3] D. R. Goddard and L. Michaelis, *Jour. Biol. Chem.*, 106: 605, 1934.

protein around which the remaining amino acids are united. This view, first advanced by Kossel, was recently substantiated by Block.[4]

Taking into consideration the importance of the terminal amino acids in the experiments of Landsteiner and Van der Scheer,[5] in which peptides were introduced as "determinant groups" it appears plausible that the immunological characters of proteins are determined by the arrangements of amino acids on the surface of the molecule.

If these views are accepted all keratins must possess a peculiarity in chemical structure that characterizes them as keratins, but among these chemically similar substances there must exist a special variant in each species to account for the specificity exhibited by each type of keratin. This may be due to the nature and spatial arrangement of the terminal amino acids, especially cystine and cysteine.

The differences observed between the reactions of oxidized and reduced keratins may possibly depend on the fact that either the—S–S—or—SH groups operate as "determinants," or that the reduction of the—S–S—linkage may produce an inter- or intramolecular rearrangement. An extensive report of these experiments and also on keratin derivatives will appear elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MECHANISM FOR THE AUTOMATIC IRRIGATION OF SAND CULTURES[1]

A VERY satisfactory mechanism for the automatic irrigation of sand in culture experiments can be constructed from stock equipment or from parts that can be readily obtained. The containers for the sand must have an outlet at the bottom through which the culture solution is introduced and withdrawn, and the table on which the containers are placed must be of sufficient height to permit the placing, underneath, of the control mechanism and the carboys which supply the culture solution. With this mechanism any number of sand containers may be simultaneously irrigated and any container or group of containers may be supplied with any desired culture solution. The diagram shows the arrangement of the apparatus.

A vessel (a), made from an inverted one-liter wide-mouthed bottle with the bottom removed, is placed above the table and is adjustable for height. It is connected by a tube (b) to one of the carboys supplying culture solution and by the flexible tube (c) to the top of vessel (d). In vessel (a) tube (b) is brought nearly to the top, and tube (c) is formed into a siphon of 5 mm bore. Vessel (d) is an asphalted metal pan of 7½ inches diameter and of slightly more than 1 liter capacity, to the bottom of which is soldered an iron bar (e) which extends out approximately 18 inches. Through an outlet in the bottom, this vessel is connected by the flexible tube (f) to the top of the same carboy to which vessel (a) is connected. In the tube (f) is inserted a one-way glass valve, which permits flow of solution from the vessel but prevents escape of air from the carboy. The bar (e) is supported by a bearing 7 inches from the vessel (d) and its outer end is attached to the handle of a three-way valve (g). One arm of the valve is connected to the top of the carboy. One arm is attached to a compressed air main (h) carrying 5 pounds of pressure and the remaining arm is unattached. The valve is so arranged that when the vessel (d) is in the "down" position the carboy air line is connected to the unattached arm, and when the vessel (d) is in the "up" position the compressed air main is connected to the carboy air line. A small trip checks the downward

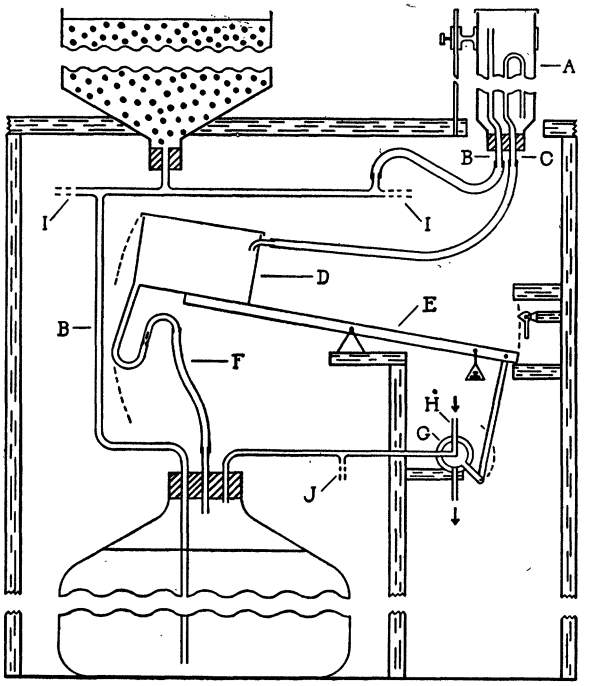

FIG. 1

[4] R. Block, *Jour. Biol. Chem.*, 103: 261, 1933; 105: 455, 1934; and *Proc. Soc. Exp. Biol. and Med.*, 32: 574, 1935.

[5] K. Landsteiner and J. van der Scheer, *Jour. Exp. Med.*, 55: 781, 1932; *ibid.*, 59: 769, 1934.

[1] Contribution No. 557, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.