the third. A negative test in the presence of hyperglobulinemia may be explained by the fact that high globulin concentration is due to an increase in the concentration of the less readily precipitated pseudoglobulin I and II rather than to an increase in the euglobulin fraction. On the other hand, a positive test in the presence of a normal total globulin concentration, may have been caused by a relative increase in the euglobulin fraction. A more detailed discussion of these problems is reserved for another paper.[10]

From the results as presented above it can be concluded that the 40 mm CO_2 test generally is positive, if the globulin concentration of the serum is increased beyond 3% and that the reaction is in accord with the Takata-Ara, Formol-gel, and Bauer tests. As a test for hyperglobulinemia the 40 mm CO_2 test has the advantage of simplicity and speed.

10212 P

Specificity of Kerateine Derivatives.

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In a previous communication,[1] it was shown that species specificity is an individual characteristic of the keratins. The suggestion was offered that the redox state and spatial arrangement of the amino acids cystine and cysteine may be intimately connected with the specificity of these proteins.

In this article it will be shown that the substitution of the hydrogen in the –SH groups of reduced keratin (kerateine) with organic halogen compounds gives the protein a new immunological character dependent on the introduced "determinant group."

Since keratins contain a very high percentage of di-sulfide sulfur (10 to 15% cystine) and are readily reduced to sulphydryl proteins by alkaline thioglycolate, a large number of substituent groups may be introduced into their molecules by reaction with simple organic halogen compounds according to the following formula:

$$A — SH + R — X \rightarrow A — S — R + HX$$

Such a reaction occurs readily in a mildly alkaline environment,[2]

[10] Greene, C. H., and Naumann, H. N., to be published shortly.

[1] Pillemer, L., and Ecker, E. E., *Science*, 1938, **88**, 16.

[2] Goddard, D. R., and Michaelis, L., *J. Biol. Chem.*, 1935, **112**, 361.

and this has been applied to proteins by Mirsky and Anson,[3] and Goddard and Schubert.[4] The method involves a mild treatment of the protein and has the added advantage of being carried out at a pH of 8.

Although Michaelis and Schubert[5] have observed that organic halogens react with amino groups, there was no demonstrable substitution of the amino groups under the conditions employed in these experiments, while complete or near complete substitution of the sulphydryl groups was accomplished.

The derivatives studied were prepared from wool and feather kerateine by reaction with iodo-acetic acid, alpha bromo-propionic acid, alpha bromo-n-butyric acid, alpha bromo-iso-butyric acid, alpha bromo-n-valeric acid, alpha bromo-iso-valeric acid, alpha bromo-n-caproic acid, alpha bromo-ethyl-benzene, and benzyl chloride.

The nomenclature designated by Goddard and Michaelis[2] was adopted, *i. e.*, by reaction with iodo-acetate, alpha-carboxy-methyl kerateine was obtained. The methods used in the preparation of the compounds and the analytical data will be detailed in a forthcoming paper.

The chemical analysis of the derived proteins revealed that the percentages of total nitrogen and sulfur contained in these compounds are similar to the values obtained in the case of the parent protein (kerateine). The kerateine derivatives differ in their isoelectric point and solubilities depending on the nature of the substituted group. In general, it may be stated that nearly all of the available –SH groups were substituted, while no detectable substitution of the amino groups occurred.

The antiserums to the keratin derivatives were prepared by immunization of rabbits with the substituted kerateines from feathers, while the derived proteins from wool kerateines were employed as test antigens. This was done in order to reduce the possibility of interactions between the parent proteins and their antiserums.

The precipitin reactions showed that the most marked precipitations occur at the interface of the proteins and their homologous antiserums.

The derived proteins containing the shorter aliphatic groups, *i. e.*, alpha carboxy-methyl kerateine and alpha carboxy-ethyl kerateine, gave the most specific reactions, while the range of activity broadens when the length of the side chain is increased in the deter-

[3] Mirsky, A. E., and Anson, M. L., *J. Gen. Physiol.*, 1935, **18**, 308.

[4] Goddard, D. R., and Schubert, M. P., *Biochem. J.*, 1935, **29**, 1009.

[5] Michaelis, L., and Schubert, M. P., *J. Biol. Chem.*, 1934, **106**, 331.

minant groups. Some cross reactions were often encountered between compounds like alpha carboxy-amyl kerateine and alpha carboxy-n-butyl kerateine. A striking specificity was exhibited in the case of 2 compounds with benzene rings in their side chains. Benzyl kerateine was easily differentiated from compounds containing aliphatic side chains as "determinants."

These results compare favorably with those obtained by Landsteiner and van der Scheer[6] in their studies on antigens containing azo-components with aliphatic side chains.

With the exception of the azo-method introduced by Landsteiner the possibility of the introduction of determinant groups into proteins has been limited.

It has been generally assumed that the various methods employed affected the benzene ring, tyrosine being regarded as playing a major rôle in the determination of the specificity of proteins. The observations of Hopkins and Wormall,[7] in which the reaction of the free amino groups of proteins with phenyl-iso-cyanates was used to introduce new determinant groups, indicated that a protein may be altered immunologically by a process not affecting the benzene ring.

From the studies presented here it is therefore also evident that substitution of the –SH group of kerateines by simple chemical compounds influences the serological behavior as well as the chemical characteristics of these derived proteins.

10213 P

Influence of Balloon Distention of Duodeno-Jejunal Loops on Volume of Combined Digestive Secretions.*

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In the course of certain studies which were designed to disclose whether acute intestinal obstruction is associated with the production of an increased flow of the digestive secretions, Swindt and

[6] Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1934, **59**, 751.

[7] Hopkins, S. J., and Wormall, A., *Biochem. J.*, 1933, **27**, 740.

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