mixture melted at 112–114°. A mixture melting point with the derivative from a known sample of 1,5-diphenyl-1-pentene, m.p. 113.5–115°, came at 113–114.5°.

The *trans*-1-amino-2,6-diphenylpiperidine can be isomerized to the *cis* isomer in 15% yield with lithium aluminum hydride in refluxing ether for 24 hours. Similarly, reduction of the *trans*-1-nitroso-2,6-diphenylpiperidine with lithium aluminum hydride in ether for 12 hours gave 18% of the *cis* amino compound.

It would appear that this novel oxidation is a useful one for specific types of ring closures in cyclic systems and represents a new type of elimination process. Other examples and modifications of this new reaction and its mechanism will be described in more detail at a later date.

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RECEIVED FEBRUARY 6, 1957

THE SUB-FRACTIONATION OF HUMAN GAMMA-GLOBULIN IN A CONTINUOUSLY DEVELOPING *p*H GRADIENT[1]

Sir:

Human gamma globulins migrate homogeneously in an electrophoretic field. However, other physico-chemical fractionation techniques including ultracentrifugation, convection-electrophoresis and low temperature-ethanol fractionation have established the heterogeneity of this material. Thus Cann and Kirkwood[2] utilized differences of isoelectric points of gamma globulins and afforded separation by means of electrophoresis-convection.

Kolin's technique[3] which utilizes a combination of *p*H and conductivity gradients has been used for the separation and identification of human hemoglobins.[4] The technique described by Kolin, however, proved unsatisfactory for the separation of gamma globulins.

In the present study, a rapid method for the preparative fractionation of human gamma globulins has been developed. A very even gradient with narrow limits of *p*H but with considerable spread was produced by the action of an electric potential on a weak buffer. Empirically, the following system afforded good separation: negative electrode, low density buffer (*p*H 3.5), high density buffer (*p*H 4.5), low density buffer (*p*H 4.0), positive electrode.

The high density buffer (*p*H 4.5) was introduced in the bottom of the U-tube of a Kolin isoelectrophoresis cell. The low density buffer of *p*H 3.5 was layered on one side in contact with the negative electrode, while the second low density buffer of *p*H 4.0 was layered on the other side in contact with the positive electrode. Then 0.15 ml. of the globulin solution[5] was introduced via a tuberculin syringe through an inlet in the bottom of the U-tube into the high density buffer, coming to rest between the high density buffer (*p*H 4.5) and low density buffer (*p*H 4.0). A potential of 200 volts between the electrodes was applied for 10 minutes creating an expanded *p*H gradient. The protein solution showing only faint signs of heterogeneity was removed by micro-pipet. Gamma-globulin solution (0.15 ml.) was introduced and again exposed to the same potential gradient of 200 volts. The preformed *p*H gradient in the system continued to develop further and the top fraction (I) separated very clearly within a few minutes, being removed after nine minutes by micro-pipet. Fraction I contained 76 μg. of protein. Seven minutes later a second band (II) separated clearly and this contained 47 μg. of protein. After seven more minutes fractions III and IV became clearly defined; these contained 25 and 121 μg. of protein respectively. Average nitrogen values were derived from over 40 experiments on the basis of pooled samples of 2 to 3 runs each.

Figure 1 illustrates the extent of separation obtained with the system employed. The center

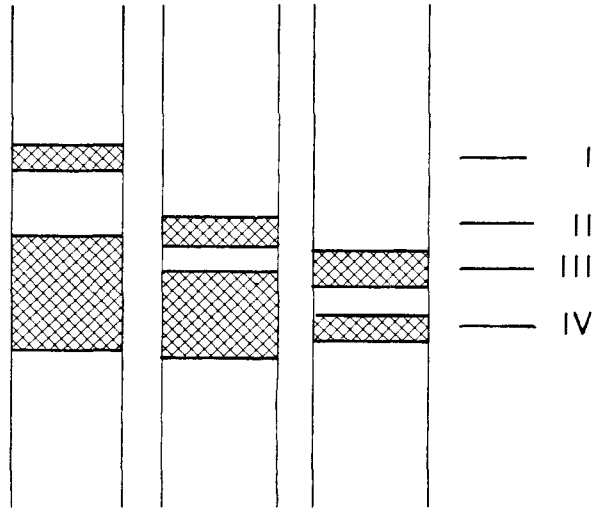

Fig. 1.

part of the isoelectrophoresis cell is photographed. The globulin fractions are made visible by dark-field illumination. The picture on the left shows the separation of fraction I. In the middle picture separation of fraction II can be seen, and in the picture on the right separation of fractions III and IV is depicted.

Detailed data on the individual fractions will be reported separately.

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FURTHER INTERMEDIATES IN THE BIOSYNTHESIS OF INOSINIC ACID *de novo*[1]

Sir:

A previous report[2] has described the isolation and characterization of a new ribotide which is an

(1) This investigation was supported by a grant from the USPHS, National Heart Institute, H 2271.

(2) J. R. Cann and J. G. Kirkwood, *Cold Spring Harbor Symposia, Quant. Biol.*, **14**, 9 (1950).

(3) A. Kolin, *J. Chem. Phys.*, **23**, 407 (1955).

(4) A. H. Tuttle, *J. Lab. Clin. Med.*, **47**, 811 (1956).

(5) Squibb Poliomyelitis Immune Globulin, Human, Lot 305-1.

(1) This work has been supported by grants-in-aid from the National Cancer Institute, National Institutes of Health, United States Public Health Service, and the National Science Foundation.

(2) L. N. Lukens and J. M. Buchanan, *Federation Proc.*, **15**, 305 (1956).