Note

1. It has been suggested by G. W. Patterson of the Moore School of Electrical Engineering that the usefulness of the proposed system of names would be very much enhanced if the system were extended by adding means for expressing powers of hi. He suggests bihi, and so forth, for this purpose. While the author is largely in agreement with this point of view he has preferred to allow extension of the system to await demonstrated need. Appreciation is due Professor Patterson for critical review of the proposal and for a number of useful suggestions.

25 June 1958

Some Genetical Implications of Physical Studies of Human Haptoglobins

Genetical differences obtained with human and cattle serum proteins have been demonstrated by the technique of starch gel electrophoresis (*1, 2*). These proteins in man, which are known as haptoglobins, migrate with the α_2-globulins and have the specific property of binding hemoglobin (*1*). Family studies are usually compatible with the hypothesis that their synthesis is controlled by two autosomal genes with incomplete dominance (*3*). It has been proposed that the three genotypes should be designated Hp^1/Hp^1, Hp^2/Hp^1, and Hp^2/Hp^2 (*4*).

The electrophoretic differences which first enabled classification of human sera into the three groups are complex and are shown diagrammatically in Fig. 1, column 2. The serum corresponding to group 1–1 contains a protein with a mobility in the fast α_2 fraction which, when combined with hemoglobin, stains with benzidine, whereas that designated group 2–2 has three bands of slower mobility which also stain with benzidine when combined with hemoglobin. Although the heterozygote, group 2–1, also shows a weak band corresponding in position to the fast α_2-globulins, the three slower bands differ from those in the group 2–2 serum in position and relative intensity. An even greater degree of complexity is suggested by the frequent presence of additional hemoglobin binding proteins in trace amounts.

Electrophoretic separation of hemolyzed serum by conventional starch zone electrophoresis also enables a distinct differentiation of the three groups to be made. When this method is used, the red band of the haptoglobin-hemoglobin complex can be seen against the white starch background. The diagrams in Fig. 1, column 3, show that the most rapidly migrating red band is found in group 1–1, whereas the slowest corresponds to group 2–2. The heterozygote does not separate into two discrete colored zones, as might have been anticipated, but migrates as a single band of intermediate mobility. These observations are in striking contrast to many of the hemoglobinopathies in which the heterozygote usually reveals a mixture of the two components found in the homozygotes.

To investigate these proteins further, samples of the haptoglobin-hemoglobin complex of the three genetic groups were purified in the following manner. Fifteen to twenty milliliters of serum free from hemolysis were separated by starch-zone electrophoresis (barbital buffer, *p*H 8.6, $\Gamma/2$, 0.1). The α_2-globulin peak was eluted and concentrated, and hemoglobin C was added in sufficient quantity to ensure saturation of the hemoglobin-binding capacity of the haptoglobin. The mixture of α_2-globulin and hemoglobin C was separated on a second starch block (barbital buffer, *p*H 8.6, $\Gamma/2$, 0.05). The presence of hemoglobin C resulted in sufficient slowing of the haptoglobin-hemoglobin complex to allow it to be seen as a red band behind the other α_2-proteins and ahead of the excess free hemoglobin C. Hemoglobin A, which migrates more rapidly, causes less slowing of the haptoglobin complex and therefore results in poorer separation of the haptoglobins from the remainder of the α_2-globulins. Purified preparations of haptoglobin-hemoglobin complex from each of the three hemoglobin types were then studied in the ultracentrifuge (Fig. 1, column 4).

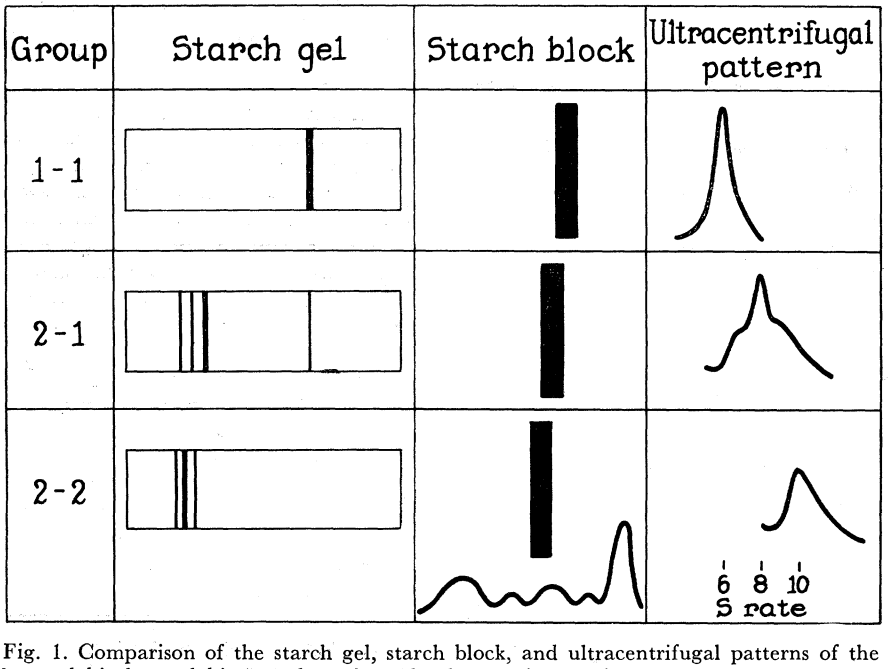

Fig. 1. Comparison of the starch gel, starch block, and ultracentrifugal patterns of the haptoglobin-hemoglobin complexes from the three main genetic groups.

Each of five specimens isolated from the sera of two individuals of group 1–1 had a single protein peak with an approximate sedimentation (s) rate of 6 *S* (5.4 to 6.3), whereas the predominant peak in six samples from three homozygotes corresponding to group 2–2 sedimented with an s rate of approximately 10 *S* (9.4 to 10.7). Because of dependence on concentration, these would correspond to $s^0_{20,w}$ values of approximately 6 and 11 *S* and are similar to the values reported by Jayle *et al.* (*5*) for two types of haptoglobin-hemoglobin complexes. In the heterozygote, however, each of eight fractions isolated from three individuals contained a major component with an s rate of about 8 *S* (7.6 to 8.8) and an approximate $s^0_{20,w}$ of 9 *S*. In addition, there were two minor components with s rates essentially similar to those present in the homozygotes. No material with an s rate of 8 *S* was detectable in either homozygote. Electrophoretically isolated α_2-globulins from two individuals of groups 1–1 and 2–2 were mixed in varying proportions, and hemoglobin C was added to saturate the haptoglobins present. The haptoglobin-hemoglobin complex of this "synthetic heterozygote" was then isolated and studied in the ultracentrifuge. Two distinct peaks with s rates of approximately 6 and 10 *S* were observed. No peak of intermediate sedimentation rate, such as was seen in the "true heterozygote," could be seen.

The presence of three different haptoglobin groups was also reflected by the distinct differences in the ultracentrifugal behavior of the whole α_2-globulin fractions, with and without added hemoglobin (*6*).

It is apparent that the two genes at the haptoglobin locus have given rise to three, and possibly more, closely related proteins some of which can be distinguished ultracentrifugally. One of these appears to be confined to the heterozygote. These findings in man bear some

similarity to the hybrid substance described by Irwin which occurs in doves (*7*), and, more particularly, to the extensive observations of Cohen on some of the red cell antigens in the rabbit in which genetic interaction has been amply demonstrated (*8*). These results may also be relevant to the problem of heterotic vigor and illustrate how this phenomenon might on occasion be due to the presence, in the heterozygote, of a substance not possessed by either homozygote (*9, 10*).

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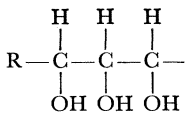
10. These studies were supported in part by a grant-in-aid (A-1542) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

31 March 1958

Isotope Effect in Oxidation of D-Mannitol-2-C^{14} by Acetobacter suboxydans

Previous reports from this laboratory have described the preparation of D-mannitol-1-C^{14}, its oxidation by *Acetabacter suboxydans*, and the carbon-14 assay of the resulting D-fructose-1,6-C^{14} (*1–3*). Because the two halves of the mannitol molecule are stereomerically identical, oxidation by *A. suboxydans* can take place at either carbon 2 or carbon 5. It was pointed out that "if oxidation took place without either an isotope effect or breakdown of the D-mannitol and resynthesis of the fragments, the derived D-fructose would be labeled equally and exclusively at carbons 1 and 6" (*3*). The early work showed that, within the error of measurement, D-fructose-1,6-C^{14} was, indeed, labeled equally and exclusively at carbons 1 and 6.

Oxidation of glycitols by *A. suboxydans* is specific for the group



where R is either H or certain other substituents. Presumably, the enzyme responsible for the oxidation forms an intermediate complex that involves the three carbon atoms indicated. The presence or absence of an isotope effect in the oxidation of a labeled D-mannitol should provide information concerning the rate-determining step, and hence, the mechanism of reaction.

In a more extensive study of a possible isotope effect, both 1-C^{14}- and 2-C^{14}-D-mannitol have been oxidized by *A. suboxydans*. The distribution of carbon-14 in the resulting D-fructose-1,6-C^{14} and D-fructose-2,5-C^{14} has been determined by several chemical methods, selected to avoid isotope effects in the analysis.

The results, summarized in Table 1, confirm the earlier radioactivity analysis of D-fructose-1,6-C^{14}; no isotope effect was detected. However, a small disproportionation in the distribution of carbon-14 was found for D-fructose-2,5-C^{14}. This indicates that, in the oxidation of D-mannitol-2-C^{14} by *A. suboxydans*, there is a small isotope effect, and oxidation is slightly less rapid at carbon 2 (C^{14}) than at carbon 5 (C^{12}).

Because of the extensive use of C^{14}-labeled products in biochemical studies, the demonstration of an isotope effect in a biological oxidation is particularly significant. Although the effect is small, it shows the need for caution in the use of biological oxidations for determining the distribution of carbon-14 in carbohydrates.

All of the compounds listed in Table 1 were recrystallized to constant radioactivity, and samples were assayed in solution (*4*).

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Table 1. Radioactivity analysis of D-fructose-1,6-C^{14} and D-fructose-2,5-C^{14}.

Compound assayed	Carbon atoms of original D-fructose	Radioactivity after successive recrystallizations (μc/mmole)	Av. % of original radioactivity
Analysis of D-fructose-1,6-C^{14}			
D-Fructose-1,6-C^{14}	1, 2, 3, 4, 5, 6	2.62*	100
Potassium D-arabonate	2, 3, 4, 5, 6	1.32*	50.4
"D-Glucose" phenylosotriazole†	1, 2, 3, 4, 5, 6	4.35, 4.33, 4.34	100
4-Formyl-2-phenylosotriazole‡	1, 2, 3	2.15, 2.17, 2.16	49.8
Dimedon-formaldehyde compound§	6	2.17, 2.18	50.1
Analysis of D-fructose-2,5-C^{14}			
D-Fructose 2,5-C^{14}	1, 2, 3, 4, 5, 6	1.93, 1.96, 1.93	100
"D-Glucose" phenylosotriazole†	1, 2, 3, 4, 5, 6	1.94, 1.90, 1.91	
4-Formyl-2-phenylosotriazole‡	1, 2, 3	0.93, 0.94, 0.93	48.4
Erythritol	3, 4, 5, 6	0.97, 0.98, 0.99	51.4
Erythritol tetrabenzoate	3, 4, 5, 6	1.01, 1.00, 0.99	
Dimedon-formaldehyde compound§	6	none	

* Value taken from Frush and Isbell (*3*). ‡ D-*arabino-Hexose* phenylosotriazole. ‡ Phenyl-2*H*-1,2,3-triazole-4-carboxaldehyde. § 2,2′-Methylenebis(5,5-dimethyl-1,3-cyclohexanedione).

References and Notes

1. This is part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the Division of Research of the U.S. Atomic Energy Commission.
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7 April 1958

Iodine-131 Fallout in Bovine Fetus

Iodine-131 from radioactive fallout is concentrated in thyroid glands of grazing animals (*1, 2*) and the distribution suggests that I^{131} spreads over the hemisphere in which it is released (*2*). A study of the fallout of I^{131} in the thyroids of fetal animals will serve two purposes: (i) It will be helpful in evaluating the maximum biological accumulation of radioactive fallout; and (ii) it might give useful information regarding iodine distribution during pregnancy.

Gorbman *et al.* (*3*) injected I^{131} into two cows during the ninth month of pregnancy, sacrificed them 24 hours later, and studied the I^{131} distribution. They found that the I^{131} concentrations in the thyroid of the fetuses were 6 to 7 times those of the dams. Autoradiographs showed uniform distribution of radioactivity in fetal glands but nonuniform concentrations in the adult thyroids. Wolff *et al.* (*4*) showed that bovine fetuses began to concentrate iodine at 53 days of gestation and that the amount of iodine increased rapidly after the fifth month.

Barnes *et al.* (*5*) have studied I^{131} distribution in pregnant sheep which were chronically fed the radioisotope. They showed that the near-term fetal thyroid I^{131} concentration was 1 to 2 times that of the dam.

Part of one lobe of the thyroid gland from each of 40 pregnant cows was removed along with the laryngothyroid