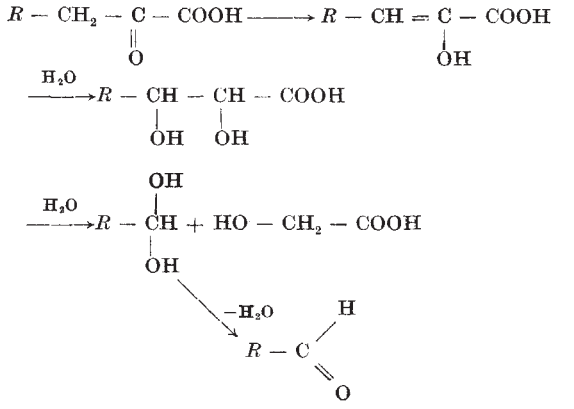
7·68 ± 0·08 which was in agreement with the value 7·66 calculated from the literature[4].

The following mechanism is tentatively proposed for the alkaline conversion of 4-hydroxyphenylpyruvic acid to 4-hydroxybenzaldehyde.



Support for an intermediate chromophorically similar to tyrosine was obtained following paper chromatography of 4-hydroxyphenylpyruvic acid in water/ethylmethylketone/diethylamine (77 : 921 : 2)[5] and elution with phosphate buffer, *p*H 7·7. This treatment for some unknown reason slowed down the effect of subsequent alkali, and thus allowed the detection of transient maxima at 240 mμ and 290–295 mμ similar to the absorption of tyrosine at *p*H > 12 (Fig. 4).

It seemed possible that the alkali-induced replacement of a pyruvic acid side-chain by an aldehyde group was a more general reaction. Phenylpyruvic acid eluted from paper chromatograms and treated with alkali (*p*H > 12), first developed an absorption maximum at 320 mμ, which then declined in intensity to an insignificant level. Subsequent adjustment to *p*H < 2 did not result in the changes characteristic of phenylpyruvic acid. In general, the spectral changes seemed consistent with a slow conversion to benzaldehyde, but this was not investigated further. Kaper and Veldstra[6] reported that chromatography of indolepyruvic acid in alkaline solvents gave indolealdehyde as one product of breakdown. It would therefore appear that in the general handling and chromatography of pyruvic acid derivatives of these types, it is as well to avoid high *p*H particularly since the conversion of 4-hydroxyphenylpyruvic acid to 4-hydroxybenzaldehyde was apparent even at *p*H10·0.

In lignin chemistry oxidation with nitrobenzene in alkali was introduced[7] as a degradative procedure by

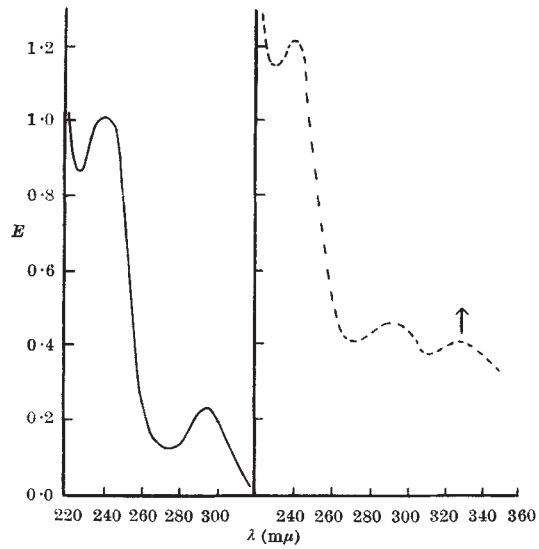



Fig. 4. The absorption at a transient stage in the alkaline conversion of 4-hydroxyphenylpyruvic acid to 4-hydroxybenzaldehyde compared with the spectrum of DL-tyrosine, *p*H > 12 (see text). —, Approx. $10^{-4}$ *M* DL-tyrosine

which various aromatic aldehydes were liberated, presumably from the lignin. The origin of one of these aldehydes, 4-hydroxybenzaldehyde, gave some concern, and it has been shown[8,9] that protein tyrosine may account for most of this compound released by the nitrobenzene oxidation of buckwheat. Since 4-hydroxyphenylpyruvic acid is considered to be a lignin precursor in grasses[9], the release of 4-hydroxybenzaldehyde from the former using alkali alone may be of particular interest.

This work was supported by a grant from the Australian National Health and Medical Research Council. The analyses were carried out by the Australian Microanalytical Service, Commonwealth Scientific and Industrial Research Organization, Melbourne.

[1] Saul, J. A., and Trikojus, V. M., *Biochem. J.*, **42**, 80 (1948).
[2] Acerbo, S. N., Schubert, W. J., and Nord, F. F., *J. Amer. Chem. Soc.*, **80**, 1990 (1958).
[3] Knox, W. E., and Pitt, B. M., *J. Biol. Chem.*, **225**, 675 (1956).
[4] Heilbron, J., and Bunbury, H. M. (eds.), "Dictionary of Organic Compounds", **2**, 231 (Eyre and Spottiswoode, London, 1946).
[5] Reio, L., *J. Chrom.*, **1**, 338 (1958).
[6] Kaper, J. M., and Veldstra, H., *Biochim. Biophys. Acta*, **30**, 401 (1958).
[7] Freudenberg, K., Lautsch, W., and Engler, K., *Ber.*, **73**, 167 (1940).
[8] Stone, J. E., Blundell, M. J., and Tanner, K. G., *Can. J. Chem.* **29**, 734 (1951).
[9] Brown, S. A., Wright, D., and Neish, A. C., *Canad. J. Biochem. and Physiol.*, **37**, 25 (1959).

# GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN RED BLOOD CELLS OF EAST AFRICANS

By Dr. A. C. ALLISON

National Institute for Medical Research, Mill Hill, London, N.W.7

MANY investigations, reviewed by Beutler[1], have shown that about 11 per cent of American Negro males have an intrinsic red blood cell abnormality. A striking feature of the abnormality—and perhaps the primary genetically controlled defect—is low activity of the enzyme glucose-6-phosphate dehydrogenase, which is responsible for the first and rate-controlling step in the hexose monophosphate shunt metabolic pathway in red cells[2]. The defect appears to be inherited as a sex-linked character with full expression in hemizygous males and homozygous females and partial expression in heterozygous

Table 1

| Tribe | Locality | Males tested | Enzyme deficiency | Females tested | Enzyme deficiency | Hemizygote = gene frequency |
|---|---|---|---|---|---|---|
| Giriama | Malindi | 101 | 17 | 65 | 10 | 16·8 |
| Bondei | Muheza | 121 | 33 | 124 | 24 | 27·3 |
| Sambaa | Tanga district | 29 | 6 | 33 | 5 | 20·7 |
| Digo | Tanga district | 26 | 6 | 33 | 6 | 23·1 |
| Zigua | Tanga district | 26 | 6 | 29 | 4 | 23·1 |
| Kikuyu | Nairobi and Naivasha district | 70 | 2 | 73 | 2 | 2·9 |
| Masai | Kajiado | 59 | 1 | 12 | 0 | 1·7 |
| Luo | Nairobi and Kisumu district | 50 | 14 | 27 | 4 | 28·0 |
| Ganda | Kampala | 40 | 6 | 46 | 5 | 15·0 |

females[3]. Persons with the defect are liable to develop severe hæmolysis on exposure to therapeutic doses of the antimalarial 8-aminoquinolines (primaquine, pamaquine, etc.) and certain other drugs, and also on ingestion of broad beans (*Vicia faba*).

The trait is known to be common in many Mediterranean populations, including Sephardic Jews, and also in many South-East Asian populations[1,4]. The only published indications of its presence among continental Africans are isolated reports of hæmolysis induced by pamaquine in a South African, a Nyasaland and an East African Bantu[5,6]. The development of a relatively simple screening test for red cell glucose-6-phosphate dehydrogenase deficiency[4] has made more extensive surveys of the distribution of the trait possible. This report embodies the results of tests on 964 East Africans belonging to 9 different tribes. The test was used as described[4] except that all volumes were reduced to one-half and incubations were carried out in 1-cm. diameter tubes.

The results of the tests are shown in Table 1. Hæmolysates from Western European subjects incubated in the presence of substrate and coenzyme decolorize brilliant cresyl blue in less than 120 min. Hæmolysates from male Africans show a clear bimodal distribution of decoloration times, with the antimode at about 120 min. Spectrophotometric analysis of glucose-6-phosphate dehydrogenase activity[7] in some of the subjects showing decoloration times of longer than 120 min. confirmed that they had significantly lower enzyme activities than normal subjects, and they were therefore classified as hemizygous for the enzyme-deficiency trait. Female Africans show a much less clear distinction between normal and abnormal groups, but those with decoloration times greater than 120 min. were assumed to be heterozygous or homozygous for the enzyme-deficiency trait. Limited family data were consistent with this assumption. The frequency of the abnormal gene, $q$, equals the frequency of hemizygotes in the male population. The expected frequency of heterozygotes and homozygotes in the female population is $2q(1 - q) + q^2$, and the proportion of females showing detectable enzyme deficiency was 41 per cent of that expected, other heterozygotes presumably falling within the normal range of variability in the tests.

The results summarized in Table 1 show that the trait is common in East Africans. However, the distribution is far from uniform. High frequencies of the trait (15–28 per cent) are found among tribes living near the coast and around Lake Victoria, and low frequencies (1·7–2·9 per cent) in the intermediate highland country. The distribution of the trait approximately parallels that of the sickle-cell trait in the same region[8]. All other populations known to have high frequencies of the enzyme-deficiency trait[4] live in regions where malaria is, or was until recently, endemic. Thus the local distribution of the trait in East Africa and the overall distribution are consistent with the interpretation that subjects with deficient glucose-6-phosphate-dehydrogenase activities in red cells are at a selective advantage in malarious environments. There are, in fact, metabolic reasons why malaria parasites might not multiply as well in enzyme-deficient cells as in normal red cells[4]. Recently, evidence has been obtained[9] that young malaria-susceptible East African children of both sexes from a region where *P. falciparum* is holoendemic show lower parasite-counts when enzyme deficient than when red cell enzyme activities are normal.

These results suggest that the trait may be maintained as a sex-linked polymorphism by a balance of selective forces. The hemizygote, and to some extent also the abnormal homozygote and heterozygote, are favoured through protection against malaria, and possibly in other ways. On the other hand, the hemizygote, and perhaps also the abnormal homozygote and in exceptional circumstances the heterozygote, may be at a disadvantage owing to their susceptibility to hæmolysis. The broad bean is not normally eaten by East Africans, and why persons with enzyme deficiency are at a disadvantage there is unknown. If there were no disadvantage, the gene might be expected to have become more common, instead of remaining at the same order of frequency among tribes supposed to have different ethnic origins (for example, the Nilo-Hamitic Luo and the Eastern Bantu Bondei). There is disagreement among theoretical geneticists whether a stable polymorphism at a sex-linked locus can be maintained by advantage of the hemizygote alone or whether the heterozygote must be favoured[10]. The finding that enzyme-deficient female as well as male children have lower malaria parasite counts than children with normal red cell enzymes means that both processes could apply in this particular case.

This investigation was supported by a grant from the Colonial Development and Welfare Fund received at the instance of the Colonial Medical Research Committee. I am indebted for hospitality and help to many East African workers, in particulars Drs. D. B. Wilson and D. F. Clyde, Amani; H. Foy, Nairobi and S. Buck, Kampala.

[1] Beutler, E., *Blood*, **14**, 103 (1959).
[2] Carson, P. E., Flanagan, C. L., Ickes, C. E., and Alving, A. S., *Science*, **124**, 484 (1956).
[3] Childs, B., Zinkham, W., Browne, E. A., Kimbro, E. L., and Torbert, J. V., *Bull. Johns Hopkins Hosp.*, **102**, 21 (1958).
[4] Motulsky, A. G., and Campbell, J. M., *Blood* (in the press).
[5] Mann, W. N., *Trans. Roy. Soc. Trop. Med. Hyg.*, **37**, 151 (1943).
[6] Smith, S., *Trans. Roy. Soc. Trop. Med. Hyg.*, **37**, 155 (1943).
[7] Kornberg, A., and Horecker, B. L., in "Methods in Enzymology", ed. Colowick, S. P., and Kaplan N. O., 323 (Academic Press, New York, 1955).
[8] Allison, A. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, 312 (1954).
[9] Allison, A. C., and Clyde, D. F. (to be published).
[10] Mandel, S. P. H., *Nature*, **183**, 1347 (1959).