Table 1. 3,4-Dihydroxy-L-phenylalanine (dopa) requirement and time course of tyrosinase reaction in skin of 5- to 6-day-old hamsters. Each value is the mean of two homogenates and has been corrected for a control, lacking the enzyme, equivalent to 0.015 μmole of ^{3}HOH.

Incubation time (min)	L-Dopa (μmole)	Activity*
60	0	0.018
60	0.05	0.643
60	0.10	0.840
60	0.15	1.00
60	0.20	0.676
30	0.15	0.52
90	0.15	1.69
120	0.15	2.18

* Micromoles of ^{3}HOH per hour per gram of tissue (fresh weight).

Table 2. Comparison of tyrosinase in dorsal and ventral areas of the skin. The areas were separated on the basis of hair pigmentation and homogenized separately.

Age of animal (days)	Activity*	
	Ventral	Dorsal
3	0.106,0.136	0.640,0.660
4	0.138,0.112	0.971,0.770
7	0.144	1.00

* Micromoles of ^{3}HOH per hour per gram of tissue (fresh weight).

moles of ^{3}HOH per hour per milligram of DNA or per gram (wet weight) of skin.

The standard assay mixture contained L-tyrosine-3,5-^{3}H (5 to 7 × 10^6 disintegrations per minute; 1 μmole); 3,4-dihydroxy-L-phenylalanine (L-dopa) (0.15 μmole); sodium phosphate buffer, *p*H 6.8 (25 μmole); and homogenate (0.50 ml) in a total volume of 1.25 ml. The reactions were stopped, ^{3}HOH was isolated, and the radioactivity was counted as described (*8*).

The activity is strictly dependent upon dopa, as with melanoma tyrosinase (*8*), and is proportional to time (Table 1). A similar dependence on dopa has been reported for rat skin tyrosinase (*3, 6*) but apparently not for mouse skin tyrosinase (*2*). The reaction is also proportional to the concentration of enzyme. In a large-scale experiment, dopa-^{3}H was isolated and identified as a product of the reaction by electrophoresis at *p*H 1.9 and chromatography on Dowex 2 (borate) (*9*). Skins from male and female animals aged 1, 2, 4, 6, and 10 days were compared, and no significant sex differences were apparent. The K_m of tyrosine for skin tyrosinase is $2 \times 10^{-4} M$, about the same value found for melanoma tyrosinase (*8*).

Overall there is a seven- to eightfold increase in tyrosinase specific activity and about a threefold increase from day 1 to day 6, with a leveling off at days 5 to 6 (Fig. 1). If the enzyme values are computed on the basis of wet or dry weight of skin, curves of similar shape are obtained. Although not shown in the figure, the specific activity remains close to the maximum until about day 16. By day 22 it falls to the level found for day 1 or 2 and then remains constant at least until day 32. Coleman (*2*) observed a leveling off of increase in specific activity on days 4 to 6 for brown and black mice, but found no activity by day 30. Black rats (*3*) exhibit maximum activity at 5 to 13 days of age and continued low activity at 10 percent of the maximum even on day 69.

The results shown so far are for whole body skin. Although ventral skin (white) clearly has enzyme, it is only about 15 percent that of dorsal skin (pigmented) (Table 2). Experiments with mixtures of dorsal and ventral skins showed that there was no inhibitor in ventral skin.

In contrast to the results for white ventral skin, skin from albino hamsters at 1, 2, 3, or 4 days of age (*10*) had no detectable enzymatic activity, even after a 3-hour incubation, and exhibited no inhibition of tyrosinase from Syrian golden hamster skin. I estimate that activity of 4-day-old albino skin is < 0.02 μmole/hr per gram of wet skin, compared to about 0.6 to 7 μmole/hr per gram of skin from a 4-day-old Syrian golden hamster. Gaudin and Fellman (*6*), on the other hand, report activity in adult albino rat skin at about 10 percent of that in adult hooded rats.

Although the increase in specific activity is only threefold over the first 6 days of life, the total tyrosinase activity per animal body skin increases from about 0.03 μmole/hr to about 0.6 μmole/hr, a 20-fold increase in total enzyme content during this period. An estimate of activity of 0.8 to 1.0 μmole/hr per gram of wet skin for 5- to 6-day-old hamsters may be compared to an activity of 10 to 20 μmole/hr per gram of hamster melanoma (*8*). The maximum specific activity for young black rats by the melanin assay can be calculated from the data of Chen and Chavin (*3*) to be 0.07 μmole/hr per gram of wet skin, about 10 percent of the maximum activity for hamster skin. The difference may be due to both method and species differences. Gaudin and Fellman (*6*) report a maximum activity of only 0.5 to 0.7 nanomole/hr per gram of wet skin for adult hooded rats, with an assay similar to the one used here. However, these workers employed only 0.05 μmole of tyrosine in the incubation, which was considerably lower than saturating amounts.

SEYMOUR H. POMERANTZ
Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore 21201

References and Notes

1. M. Foster, *J. Exp. Zool.* **117**, 211 (1951); *Proc. Soc. Exp. Biol. Med.* **79**, 713 (1952).
2. D. L. Coleman, *Arch. Biochem. Biophys.* **69**, 562 (1962); K-H. Kim and T. T. Tchen, *Biochim. Biophys. Acta* **59**, 569 (1962); Y. M. Chen and W. Chavin, *Proc. Soc. Exp. Biol. Med.* **121**, 497 (1966); *Experientia* **23**, 917 (1967); *ibid.* **24**, 332 (1968).
3. Y. M. Chen and W. Chavins, *J. Invest. Dermatol.* **50**, 289 (1968).
4. S. H. Pomerantz, *Biochem. Biophys. Res. Commun.* **16**, 188 (1964).
5. P. F. Hall and K. Okazaki, *Biochemistry* **5**, 1202 (1966).
6. D. Gaudin and J. H. Fellman, *Biochim. Biophys. Acta* **141**, 64 (1967).
7. W. C. Schneider, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 3, p. 680.
8. S. H. Pomerantz, *J. Biol. Chem.* **241**, 161 (1966).
9. ——— and M. C. Warner, *ibid.* **242**, 5308 (1967).
10. Adult albino hamsters of this type (also obtained from Lakeview Hamster Colony) have pink eyes, but a small amount of pigment on the tips of the ears.
11. Supported by grant CA-07093 from the National Institutes of Health. I thank L. Chuang for technical assistance.

9 January 1969

Glucose-6-Phosphate Dehydrogenase Deficient Red Cells: Resistance to Infection by Malarial Parasites

Abstract. *Erythrocyte mosaicism occurs in females heterozygous for glucose-6-phosphate dehydrogenase deficiency. In blood from female children with acute* Plasmodium falciparum *malaria the parasite rate was 2 to 80 times higher in normal than in deficient erythrocytes. This may be the mechanism whereby the gene for glucose-6-phosphate dehydrogenase deficiency confers selective advantage against malaria to heterozygous females, and thus may have attained the polymorphic frequency occurring in populations living in areas with endemic malaria.*

Balanced polymorphism is a theoretically well-characterized situation in Mendelian populations (*1, 2*). However, there are very few examples, in which the mechanism has been clarified whereby a heterozygote is at ad-

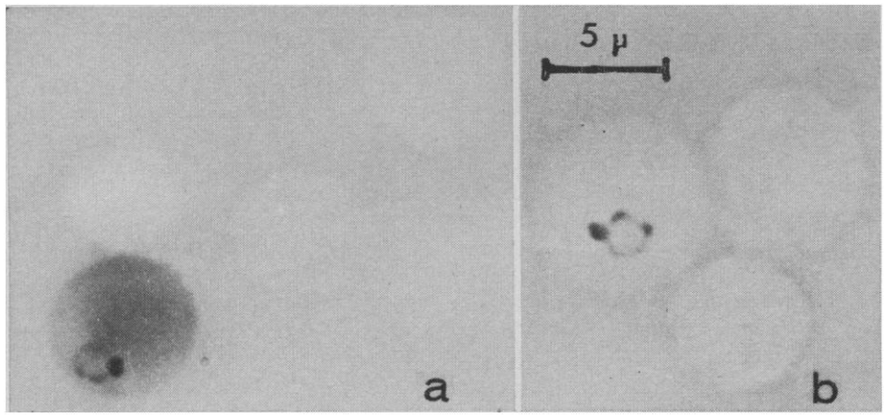

Fig. 1. Photomicrographs of blood smears from children with malaria, processed by the test for localization of glucose-6-phosphate dehydrogenase (*10*). After elution, smears were stained with Leishman's stain because by the hemalum-erythrosin method malarial parasites were not adequately revealed. Normal cells (*15*) appear stained; enzyme-deficient cells are unstained. (a) A normal red cell (*15*) containing a ring of *P. falciparum*, contrasted with three nonparasitized deficient cells. The blood was from case 23 (Table 1). (b) Parasitization does not interfere with the elution technique. An unstained cell contains a ring of *P. falciparum* in a preparation from a totally deficient subject (case 30 in Table 1).

Table 1. Preferential infection by *P. falciparum* of normal erythrocytes as opposed to those deficient in glucose-6-phosphate dehydrogenase. The percentages of eluted (*d*) and uneluted (*n*) cells (*12*) were determined by counting 400 erythrocytes at random, regardless of whether they contained parasites or not. The parasite rate (*R*) in all cells was determined by counting 500 erythrocytes, regardless of whether they were eluted or not. In order to determine the parasite rate in deficient cells (R_d), 200 parasitized cells were counted and the percentage of deficient cells among them was multiplied by R/d. The parasite rate in normal cells (R_n) was similarly determined.

Case	Red cells		Parasite rate in cells (%)			Preferential infection of normal cells ($Q = R_n/R_d$)
	Eluted (%)	Uneluted (%)	All	Deficient	Normal	
			Intermediate (heterozygous) females			
1	25	75	2.0	1.2	2.3	1.9
2	31	69	1.5	0.2	3.4	17
3	35	65	1.0	0.14	1.5	10.7
4	38	62	20	5.3	29	5.5
5	39	61	9.0	3.6	12	3.3
6	40	60	3.3	0.07	5.5	79
7	40	60	1.2	0.17	2.2	12.9
8	41	59	4.8	1.2	7.3	6.1
9	44	56	4.0	0.64	6.6	10.3
10	46	54	5.0	2.9	6.7	2.3
11	47	53	4.5	2.4	6.4	2.7
12	48	52	15.5	7.8	23	2.9
13	54	46	9.0	5.0	13.7	2.7
14	58	42	4.5	0.31	10.2	33
15	62	38	1.2	0.39	2.4	6.2
16	66	34	7.0	2.3	16.5	7.2
17	68	32	2.6	1.4	5.1	3.7
18	72	28	5.5	1.9	14.5	7.6
19	73	27	0.1	0.03	0.28	8.5
20	75	25	0.3	0.11	0.88	8.0
			Deficient (homozygous) females			
21	85	15	8.5	4.2	25	6.0
22	89	11	0.3	0.15	1.55	10.3
23	97	3	4.0	1.4	88	63
24	98.5	1.5	10	8.7	93	10.7
25	99	1	5.0	4.2	80	19
			Deficient (hemizygous) males			
26	83	17	4.5	3.1	11.2	3.6
27	86	14	2	0.16	13.3	83
28	95	5	5.6	5.1	13	2.6
29	99	1	0.6	0.55	6.0	10.9
30	99.5	0.5	11.0	10.8	44	4.1

vantage over both homozygotes (*3*). In man, only one genetic polymorphism, pertaining to the locus for the β-chain of hemoglobin, has been shown conclusively to be stabilized by heterosis in at least some populations (*4*): but even in that case it is not known in what manner the gene involved—the *S* gene—confers an advantage on the heterozygous subjects against malaria, the selective force.

We now present evidence for the mechanism whereby another genetic trait—deficiency of glucose-6-phosphate dehydrogenase—can confer to heterozygous carriers increased resistance against malarial infection. We found that in infected heterozygous female children [genetic mosaics for glucose-6-phosphate dehydrogenase deficiency (*5*)], the probability of finding parasites is 2 to 80 times greater in normal cells than in those deficient in glucose-6-phosphate dehydrogenase. We think it likely that the marked preference of the parasite for "normal" cells confers increased resistance to malaria to heterozygous females. This fact alone could account for the geographical correlation between high frequency of the gene for glucose-6-phosphate dehydrogenase deficiency and high malarial endemicity (*6*), first pointed out by Motulsky (*7*) and by Allison and Clyde (*8*).

Blood was obtained from 1- to 5-year-old children having acute malaria (*Plasmodium falciparum*) and tested for localization of glucose-6-phosphate dehydrogenase by the technique of methemoglobin elution (*9*, *10*). This technique allows one to distinguish by microscopic observation between cells that have a normal amount of glucose-6-phosphate dehydrogenase from cells with a low level of this enzyme, since only the normal ones resist elution and thus appear stained (Fig. 1a).

In subjects with normal glucose-6-phosphate dehydrogenase activity (*11*) over 95 percent of the cells were stained, and virtually all parasites were inside stained cells. The results obtained in intermediate and deficient (*11*) subjects are listed in Table 1. Without exception, the percentage of parasitized cells among erythrocytes with a normal amount of glucose-6-phosphate dehydrogenase (*12–14*) is greater than that among the enzyme-deficient erythrocytes. The ratio between the parasite rates in the two types of cells can be used as an index of the preferential infection of non-

deficient erythrocytes. If the parasites had no preference, the value of this index (Q in Table 1) would be 1; instead, one sees that Q ranges from 1.89 to 83.

In heterozygous intermediate females, the red cell mosaicism manifested in the methemoglobin elution test is usually accepted as being the expression of mosaicism for the X-linked gene for glucose-6-phosphate dehydrogenase (*5, 10*). The tendency of *P. falciparum* to invade the normal erythrocytes reflects its preference for the cells in which the gene for deficiency has been inactivated. In each subject the normal cells act as control for the deficient cells. In deficient subjects (whether hemizygous males or homozygous females) a minor proportion of cells may resist elution in the procedure we used (*10*). These are presumably the youngest red cells, with sufficient enzyme activity for efficient reduction of methemoglobin (*15, 16*). In these subjects too we found a large excess of parasites in normal cells. In the extreme (case 27), although only 14 percent of all cells were uneluted, 93 percent of the parasites were in them. Thus, the plasmodium prefers cells that have a sufficiently high level of glucose-6-phosphate dehydrogenase, whether by virtue of their genetic structure or because of their young age. A noteworthy implication is that, in glucose-6-phosphate dehydrogenase deficient subjects, the parasites tend to invade the youngest cells. This may or may not apply to other subjects (*17*).

Several possible artifacts have been excluded in these experiments. (i) The presence of the parasite in a red cell does not interfere with elution (Fig. 1b). In some subjects the parasite rate in deficient cells can be quite high, but in each case it is less than in normal cells, even when these are very few (cases 24, 30). (ii) There is no significant loss of parasitized cells during the incubation time required by the performance of the test: thus, parasite counts made before and after incubation agreed within ± 5 percent.

The mechanism underlying the predilection of the parasites for normal erythrocytes is not clear. At least three possibilities can be considered. (i) Failure of infection—where the parasite recognizes, perhaps on the basis of a difference in the membrane (*18*), normal from deficient cells and prefers not to enter the latter. (ii) Abortive infection—where the parasite enters either type of cell indifferently, but it fails to thrive in the deficient cells perhaps because of their impaired reductive potential (*7*). (iii) Suicidal infection—where the parasites can develop in deficient erythrocytes, but upon parasitization these are rapidly removed from circulation with destruction of the erythrocyte and death of the parasite (*19*). If the mechanism of preference is clarified, it may become possible to interpret the wide variability of the Q values in Table 1, which we cannot yet explain. There was no correlation between the parasite concentration (which ranged from 5×10^3 to 4×10^5 parasites per microliter of blood) and the value of Q.

Our data indicate that in heterozygous females only one-half of the erythrocyte population, on the average, is readily available for parasitization. In that mortality from malaria is closely related to parasite concentration in the blood (*20*), it is likely that this mechanism confers a direct advantage in viability to the heterozygous females in an environment with endemic malaria. This conclusion is not dependent on the specific mechanism that causes decreased rate of parasitization of deficient cells. Whether infection fails, or aborts, or leads to death of the plasmodium, its successful propagation is drastically impaired, and the chance of survival of the human host is proportionately increased. Since parasitization can occur in deficient cells, our data do not predict whether a similar situation applies in enzyme deficient hemizygous males or homozygous females. It is possible that no preferential survival does exist in this group, who are also exposed to the risk of fatal hemolysis (*21, 22*), and this would explain why data comparing parasite rate or parasite density in normal and deficient subjects, usually males (*8, 23*), have been inconclusive.

That, for polymorphism at a sex-linked locus to reach equilibrium, a greater fitness of the heterozygote female is sufficient has been established (*24*). Allison and Clyde (*8*) had already envisaged this possibility for the case of glucose-6-phosphate dehydrogenase deficiency, and Livingstone (*25*) calculated a frequency of 0.16 for the responsible gene at equilibrium; the observed gene frequency in Nigeria is about 0.2 (*26*). Thus, it appears that in this case selection specifically favors the heterozygote female, because genetic mosaicism enables her to enjoy the benefits of having two types of red cells at the same time (*27*). This peculiar situation, unknown for any autosomal locus, has made it possible to rationalize, at the cellular level, the basis of the selective advantage and to provide direct experimental support to the hypothesis (*7, 8*) —grounded thus far mostly on geographical data (*28*)—that malaria is a major factor in maintaining polymorphism at the glucose-6-phosphate dehydrogenase locus.

LUCIO LUZZATTO
ESSIEN A. USANGA
SHUNMUGAM REDDY
Sub-Department of Haematology, University College Hospital, Ibadan, Nigeria

References and Notes

1. R. A. Fisher, *The Genetical Theory of Natural Selection* (Clarendon Press, Oxford, 1930).
2. E. B. Spiess, Ed., *Animal Population Genetics* (Little, Brown, Boston, 1962).
3. D. Apirion and D. Zohary, *Genetics* **46**, 393 (1961).
4. A. C. Allison, in *Abnormal Hemoglobins in Africa*, J. H. P. Jonxis, Ed. (Blackwell, Oxford, 1965), p. 365.
5. E. Beutler, M. Yeh, V. F. Fairbanks, *Proc. Nat. Acad. Sci. U.S.* **48**, 9 (1962); R. G. Davidson, H. M. Nitowski, B. Childs, *ibid.* **50**, 481 (1963); V. F. Fairbanks and L. Lampe, *Blood* **31**, 589 (1968).
6. *World Health Organization Technical Report No. 366* (WHO, Geneva, Switzerland, 1967).
7. A. G. Motulsky, *Hum. Biol.* **32**, 28 (1960).
8. A. C. Allison, *Nature* **186**, 531 (1960); ———, and D. F. Clyde, *Brit. Med. J.* **1961-I**, 1346 (1961).
9. G. Sansone, A. Rasore-Quartino, G. Veneziano, *Pathologica* **55**, 371 (1963); O. Tonz and E. Rossi, *Nature* **202**, 606 (1964).
10. J. C. Gall, G. J. Brewer, R. J. Dern, *Amer. J. Hum. Genet.* **17**, 359 (1965).
11. Here, and in the following, *normal* refers to nondeficient homozygous females and hemizygous males; *intermediate* to the female genotype heterozygous for deficiency; *deficient* to deficient homozygous females and hemizygous males.
12. The variability in the expression of enzyme deficiency (measured by the ratio n/d) in the heterozygous females has been observed by others [R. T. Gross, R. E. Hurwitz, P. A. Marks, *J. Clin. Invest.* **37**, 1176 (1958); R. Davidson, B. Childs, M. Siniscalco, *Ann. Hum. Genet.* **28**, 61 (1964); G. J. Brewer, J. C. Gall, M. Honeyman, H. Gershowitz, D. C. Shreffler, R. J. Dern, C. Hames, *Biochem. Genet.* **1**, 41 (1967)]. It is attributable to a variable ratio between numbers of cells in which normal and deficient X-chromosomes undergo inactivation (*13, 14*).
13. W. E. Nance, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 415 (1964).
14. A. G. Motulsky, in *Abnormal Hemoglobins in Africa*, J. H. P. Jonxis, Ed. (Blackwell, Oxford, 1965), p. 143.
15. J. C. Gall and G. J. Brewer [*Clin. Res.* **14**, 309 (1966)] have shown that such red cells resisting elution have a quantitatively normal glucose-6-phosphate dehydrogenase activity, although they come from genetically deficient subjects in whom the enzyme is qualitatively abnormal [L. Luzzatto, and N. C. Allan, *Biochem. Biophys. Res. Commun.* **21**, 547 (1965)]. These cells for convenience are referred to as normal.
16. P. A. Marks, A. B. Johnson, E. Hirschberg, *Proc. Nat. Acad. Sci. U.S.* **44**, 529 (1958); A. S. Beitt, T. W. Smith, J. H. Jandl, *New Engl. J. Med.* **275**, 397 (1966).
17. It has been observed by R. L. Ladda [*Mili-*

tary Med. **131**, 993 (1966)] that trophozoites of *P. berghei* were mostly detected in the reticulocytes of infected mice or in their precursors, seldom in mature erythrocytes.
18. D. Danon, Ch. Sheba, B. Ramot, *Blood* **17**, 229 (1961).
19. This last possiblity would seem consistent with the finding by K. Devakul, L. Garby, T. Harinasuta [*Ann. Trop. Med. Parasitol.* **60**, 432 (1966)] that, in subjects deficient in glucose-6-phosphate dehydrogenase, old red cells are destroyed preferentially upon infection with *P. falciparum*.
20. J. Vandepitte and J. Delaisse, *Ann. Soc. Belge Med. Trop.* **37**, 703 (1957); J. W. Field, *Trans. Roy. Soc. Trop. Med. Hyg.* **43**, 33 (1949).
21. Neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency [F. Panizon, *Lancet* **1960-II**, 1093 (1960); S. A. Doxiadis, P. Fessas, T. Valaes, *ibid.* **1961-I**, 297 (1961)] was thought to be mild in the African type of deficiency [A⁻ according to the nomenclature of (*6*)]; see (*14*) and S. E. Levin, R. W. Charlton, I. Freiman, *J. Pediatrics* **65**, 757 (1964). It has become clear in Nigeria that this enzyme deficiency can underlie the development of kernicterus in newborns [A. E. Ifekwunigwe, L. Luzzatto, *Lancet* **1966-I**, 667 (1966)] and that it represents a major factor in causing this condition [R. G. Hendrickse, in *Abnormal Hemoglobins in Africa*, J. H. P. Jonxis, Ed. (Blackwell, Oxford, 1965), p. 208; and personal communication].
22. Acute hemolysis in subjects with glucose-6-phosphate dehydrogenase deficiency can occur not only in response to a number of drugs [(listed in (*6*)] but to other conditions as well; see E. R. Burka, Z. Weaver, III, P. A. Marks, *Ann. Int. Med.* **64**, 817 (1966).
23. R. Harris and H. M. Gilles, *Ann. Hum. Genet.* **25**, 199 (1961); M. Kruatrachue, P. Charoenlarp, T. Chongsuphajaisiddhi, C. Harinasuta, *Lancet* **1962-II**, 1183 (1962); I. M. Porter, S. H. Boyer, E. J. Watson-Williams, A. Adam, A. Szeinberg, M. Siniscalco, *ibid.* **1964-I**, 895 (1964); R. D. Powell and G. J. Brewer, *Amer. J. Trop. Med.* **14**, 358 (1965); G. M. Edington and E. J. Watson-Williams, *Abnormal Hemoglobins in Africa*, J. H. P. Jonxis, Ed. (Blackwell, Oxford, 1965), p. 393; H. M. Gilles, K. A. Fletcher, R. G. Hendrickse, R. Lindner, S. Reddy, N. Allan, *Lancet* **1967-I**, 138 (1967); R. G. Hendrickse, A. H. Hasan, S. Reddy, A. R. Cooke, L. S. Olumide, A. Akinunmi, International Congress of Tropical Medicine and Malaria, 8th, Teheran (1968); M. Kruatrachue, N. Sadudee, B. Siripanach, *ibid.*
24. J. H. Bennett, *Nature* **180**, 1363 (1957); S. P. H. Mandel, *ibid.* **183**, 1347 (1959); P. A. Parsons, *Heredity* **16**, 103 (1961).
25. See model V in F. B. Livingstone, *Amer. J. Hum. Genet.* **16**, 435 (1964); this model assumes fitness of 1.1 for the heterozygous female, 1.0 for the normal male and female, and 0.9 for deficient male and female.
26. H. M. Gilles and B. G. Taylor, *Ann. Trop. Med. Parasitol.* **55**, 64 (1961); L. Luzzatto, N. C. Allan, A. De Flora, *Biochem. J.* **97**, 19P (1965).
27. R. L. Teplitz and E. Beutler, *Blood* **27**, 258 (1966).
28. The concordance between high incidence of glucose-6-phosphate dehydrogenase deficiency and areas with past or present endemic malaria is evident on a worldwide basis (*6*). It has also stood critical tests where ethnically similar population groups are found in malarious and nonmalarious sites within a small area, as in Sardinia [M. Siniscalco, L. Bernini, G. Filippi, B. Latte, P. Meera Khan, S. Piomelli, M. Rattazzi, *World Health Org. Bull.* **34**, 379 (1966)], in Greece [G. Stamatoyannopoulos, A. Panayotopoulos, A. G. Motulsky, *Amer. J. Hum. Genet.* **18**, 296 (1966)], and in Thailand [G. Flatz, S. Sringam, *Lancet* **1963-II**, 1248 (1963)].
29. We thank Dr. A. C. Allison for stimulating this work; Prof. G. M. Edington and H. M. Gilles for encouragement; Dr. D. Schlessinger and Dr. D. Apirion for helpful suggestions; Professors A. O. Lucas, J. Beetlestone, and R. G. Hendrickse for reviewing the manuscript; and I. O. Olanipekun for technical assistance. Financial support was given by the World Health Organization.

23 January 1969

Digitoxin Poisoning: Prevention by Spironolactone

Abstract. *Spironolactone (Aldactone®) protects the rat against the production of myocardial necroses and other manifestations of digitoxin poisoning.*

In the rat, spironolactone, which has been found to block the hypertensive action of aldosterone by competitive inhibition (*1*), minimizes both the cardiovascular lesions characteristic of overdosage with desoxycorticosterone (*2*) and the infarct-like myocardial necroses produced by certain corticoids in combination with bisodium phosphate, excess fat intake, or stress (*3*). In agreement with expectations, spironolactone is also beneficial not only in primary aldosteronism but also in many of the common types of clinical hypertension (*4*).

Independently of these studies it was noted that heavy overdosage with digitoxin produces massive cardiac necroses in the rat and that concurrent oral administration of excess bisodium phosphate and fat facilitates the production of these lesions (*5*).

The aglycones of cardiac glycosides resemble the corticoids in that they possess a steroid nucleus; since, in addition, they have a lactone ring at C-17, they are structurally even more closely related to spironolactone than is desoxycorticosterone or aldosterone. In view of these considerations, it seemed promising to explore the possibility of counteracting digitalis toxicity by a competitive inhibitor of aldosterone such as spironolactone.

Forty female rats from Holtzman Farms (Madison, Wis.), with a mean initial body weight of 100 g (range 90 to 110 g), were divided into four equal groups and treated as outlined in Table 1. Digitoxin (0.5 or 0.25 mg) and bisodium phosphate (1 m*M*), dissolved together in 2 ml of water, and 1 ml of corn oil were given separately by stomach tube twice daily for the production of the cardiopathy. In addition, for prophylaxis, certain groups received spironolactone (10 mg), added to the solution of digitoxin and

Table 1. Prevention of digitoxin poisoning by spironolactone. In addition to the treatments listed, all groups received Na_2HPO_4 and oil as described in the text. The readings include both the animals that succumbed during the experiment and the survivors that were killed on the 5th day.

Group	Treatment		Motor disturbances	Cardiac necroses	Mortality (%)
	Digitoxin (mg)	Spironolactone (mg)			
1	0.5	0	+++	++	100
2	.5	10	+	0	30
3	.25	0	++	+	50
4	.25	10	0	0	0

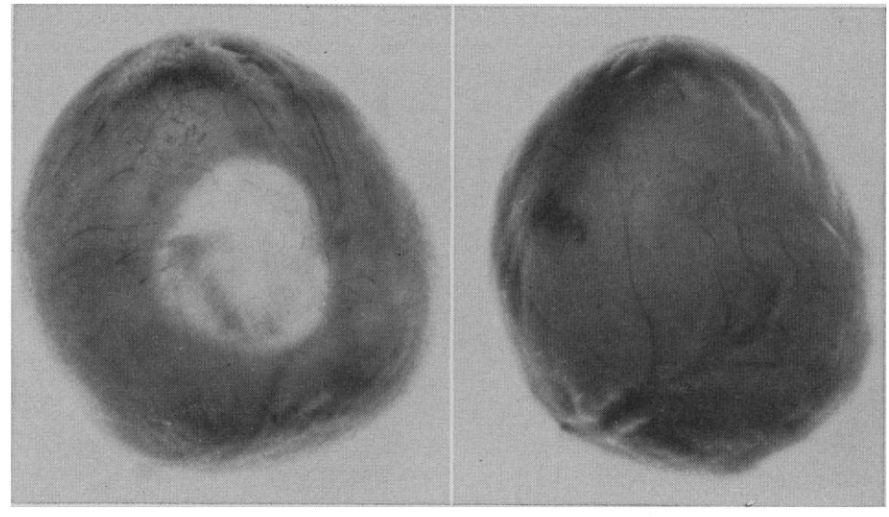

Fig. 1. Prevention of digitalis-induced myocardial necrosis by spironolactone. (Left) Sharply circumscribed circular apical necrosis in the heart of a rat treated with digitoxin (group 1). (Right) Prevention of the necrosis by additional treatment with spironolactone (both hearts are photographed from their apical aspect).