Generation Age of Efficient Milk Cows

Continuing my studies on the subject of the influence of generation age[1], I considered it possible that there might be a similar effect with animals. Upon my request, Mr. HANS EUGSTER, dipl. Ing. agr. ETH, director of the 'Herdebuchstelle des Schweizerischen Braunviehzuchtverbandes', was kind enough to permit me to inspect the efficiency indices of groups of milk-cows descending from different bulls, bulls which he had selected at random.

These indices depend on the excess of the average daily milk quantity after subtraction of the quantum considered as the norm, so that these indices can also be negative. They are further modified in a certain way according to the milk quality.

The results are as follows:

No. of the bull	Generation age (life data)	No. of daughters	Efficiency indices of the daughter cows
1	1 (3)	16	+126
	2 (4)	46	+128
	3 (5)	70	+234
	4 (6)	102	+193
2	1 (3)	125	+156
	2 (4)	156	+184
3	1 (3)	30	+400
	2 (4)	66	+514
	3 (5)	88	+550
4	1 (3)	18	−178
	2 (4)	174	+157
	3 (5)	234	+170
	4 (6)	313	+152
5	1 (3)	23	+304
	2 (4)	30	+306
	3 (5)	44	+435
	4 (6)	83	+317
	5 (7)	107	+350
6	1 (3)	15	+383
	2 (4)	144	+989
	3 (5)	298	+999
	4 (6)	517	+878

It appears that the efficiency indices are in fact distinctly higher in the 3rd generation year of the father bull and begin descending again in the 4th generation year.

However, it is to be kept in mind that the above data could not be statistically completely worked out: for instance, among the cows quoted under a certain year are included those of preceding years. In this way the efficiency numbers indicated are already smoothed in a certain degree so that the real influence of the generation year is obviously even more pronounced than is seen at the first glance from numbers given above.

Thus the hypothesis appears justified that in some general way the 'quality' of descendants depends on the generation age of the male parents. It would be of some interest to apply the 'trinomial tests'[1] to animals also. In this connection it may be mentioned that the particularly 'efficient' bull, bull No. 6, was also generated in the 3rd generation year of his father.

Summary. A connection is made plausible between the efficiency of milk-cows and the generation age of the father bulls.

A. DIETZ-HELMERS

CH-6932 Biogno di Breganzona (Switzerland), 10 June 1975.

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Malaria, Favism and Glucose-6-Phosphate Dehydrogenase Deficiency

The relationship between the balanced polymorphisms involved in the hemoglobinopathies of hemoglobin S, hemoglobin C, β-thalassemia, etc., and a putative protection against malaria has attracted considerable interest[1–3]. Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency has also been advanced as a protection against malaria[4–8]. Two difficulties have been noted with respect to the malaria hypothesis and G-6-PD deficiency, however: 1. There is only poor correlation between the distribution of malaria and G-6-PD deficiencies in many areas. 2. The highest frequencies of G-6-PD deficiencies occur in areas where selection *against* them is high from favism, an acute hemolytic anemia associated with the ingestion of fava beans, *Vicia faba*, or exposure to fava pollen ('Baghdad spring fever'). In this communication we suggest that these two problems are related and actually support the malaria hypothesis. Furthermore, we believe selection has occurred to *retain* favism in certain populations rather than eliminate it. Evidence for malaria protection from G-6-PD deficiency has resembled that for the hemoglobinopathies, but has been more difficult to assemble. Zoogeographic and ecological arguments involving the distribution and frequency of various hemoglobins and the incidence of falciparum malaria have been strong for hemoglobin S but somewhat weaker for hemoglobin C, hemoglobin E, etc.[3,9–12].

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G-6-PD deficiency likewise appears to be geographically correlated with the incidence of malaria in the Mediterranean region and some areas of the Far East[3,13]. However, in parts of New Britian and New Guinea, the correlation is often poor[3,14,15] and this has led KIDSON and GORMAN[16] to question the hypothesis.

KRUATRACHUE et al.[17] found that erythrocytes with low levels of G-6-PD were less frequently infected than were normal cells. LUZZATO et al.[18] found that in the erythrocyte mosaicism occurring in heterozygous females, the parasite rate was from 2 to 80 times higher in normal cells than it was in the deficient erythrocytes. In a study of black Americans in Viet Nam[19], it was found that the incidence of malaria among carriers of the African form of G-6-PD was less than expected (statistically significant at the 95% confidence level).

LIVINGSTONE[3,10] has called attention to seeming anomalies in the distribution, frequency, and severity of symptoms of G-6-PD deficiency in various geographic areas. In the absence of specific complications resulting from drugs or diet, G-6-PD deficiency differs from the more severe hemoglobinopathies in having relatively high homozygote and hemizygote (the gene is sexlinked) fitnesses. Thus selection *against* this condition is relatively relaxed in certain areas such as Africa, eastern Asia, and the western Pacific. In contrast, there can be strong selection against homo- and hemi-zygotes in some cultures because of the incidence of favism. This acute hemolytic anemia occurs in certain carriers of the Mediterranean variant of the enzyme following ingestion of fava beans, a dietary staple in the region. In Sardinia, for example, there are 5 cases of favism per 1000 per year, 10–40% of which are fatal[20]. Ironically, it is in the Mediterranean and other Near Eastern populations afflicted by favism that the highest frequencies of G-6-PD deficiency are found, sometimes over 50% in Kurdish Jews, and some desert oasis and Nile Delta populations[3]. In these populations, as well as elswehere in the Mediterranean, e.g., Greece[21], Sardinia[22,23], Cyprus[24], etc., there is generally good correlation between the frequencies of G-6-PD deficiences and malaria[3]. LIVINGSTONE has called attention to this problem([3], pp. 64 and 67), particularly by stating: 'The close correlation of the G-6-PD deficiency and malaria in the Mediterranean area contrasts with the findings from New Guinea and the Pacific. There would seem to be a relationship to the cultivation of the fava bean in all the areas of the Mediterranean where the correlation is close' ([3], p. 67).

Thus we have the apparent paradox: Over most of the world G-6-PD deficiencies are not very deleterious but they are not very abundant, either. In the one area of the world where favism provides strong selection against deficients, we find the highest frequencies. LIVINGSTONE[3] suggested that strong selection against G-6-PD deficiency in homo- and hemi-zygotes by favism with concomitant strong selection against 'normals' by malaria would allow gene frequencies to approach equilibrium values more quickly. However, it is unlikely that in the presence of strong malarial selection observed values would differ very much from equilibrium in any event. Furthermore, as LIVINGSTONE later admits, the strong selection against G-6-PD deficients should lower the equilibrium values, in contrast to the observed high frequencies in favistic populations.

There must be a compensating increased fitness of the heterozygote in the Mediterranean region to offset the negative selection from favism. We suggest that the fava bean is actively involved in this selection and that, rather than being deleterious, 'favism' in the broad sense of the word is responsible for the high frequencies of G-6-PD deficients. Erythrocytes with low G-6-PD levels harbor fewer malaria parasites than normal[17]. The stress imposed on the erythrocyte by hemolytic favism may act synergistically with the already unfavorable G-6-PD levels to eliminate parasitized erythrocytes. The carriers of the abnormal variant of G-6-PD would be at a selective advantage with respect to malaria.

The mechanism of the action of fava beans in unknown[25]. It appears that in addition to the sex-linked G-6-PD deficiency there is an autosomal locus which is involved in favism since not all G-6-PD deficients develop favism upon exposure. ROTH and FRUMIN[26] showed the involvement of a serum factor in the hemolysis. It may be coincidental, but in addition to being susceptible to favism, G-6-PD deficients may suffer similar hemolysis from antimalarials such as primaquine, etc.

There appears to be one further analogy between the hemoglobinopathies and G-6-PD deficiencies. There is some evidence that the relatively mild hemoglobinopathies were replaced (or are now being replaced) by the more radical hemoglobin S. LIVINGSTONE[27] states: 'Natural selection seems to favor the most abnormal of the alleles at these loci'. This is because the Hb_SHb_A heterozygote is more effective against malaria just as the Hb_SHb_S homozygote is less viable. The use of the fava bean as a food item has done the same thing with respect to G-6-PD deficiency: A relatively mild abnormality has been enhanced with greater mortality of the homozygote but enhanced protection of the heterozygote. The basis of the balanced polymorphism is strengthened.

Although we have stressed the possible parallelism between the favism-G-6-PD deficiency effect and hemoglobinopathies, our suggested mechanism could work in exoerythrocytic stages of the life cycle such as the recurrent infection of the parenchyma cells of the liver by *Plasmodium vivax*, a more important selective agent in the Mediterranean[10].

The spotty distribution of G-6-PD deficients in the south-western Pacific and elsewhere in the Far East is consonant with the above interpretation. In the absence of the enhancement of protection against malaria by favism, pressures from migration, mutation, and/or genetic drift could be responsible for the erratic frequencies. However, it is also possible that some analogue of the

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fava bean is present in certain local diets leading to very high fitness of the G-6-PD homozygote. Careful surveillance of local diets for such a possibility would be of interest.

Summary. Although glucose-6-phosphate dehydrogenase deficient individuals may suffer (sometimes fatally) from favism, a high incidence of this trait occurs in many Mediterranean populations. This apparent paradox is explained on the basis of a synergistic interaction between favism and G-6-PD deficiency that provides increased protection against malaria compared to that of the G-6-PD deficiency alone. This relationship is analogous to that between various hemoglobins and malaria in that there is selection for a more severe trait if it provides more protection against malaria.

J. E. Huheey and D. L. Martin

Department of Chemistry, University of Maryland, College Park (Maryland 20742, USA), 15 May 1972.

Colloidal Gold Granules as Markers for Cell Surface Receptors in the Scanning Electron Microscope

Several techniques have been developed recently in order to visualize cell surface receptor sites by high resolution scanning electron microscopy (SEM). The peroxidase-diaminobenzidine reaction product (easily detected by TEM) is quite difficult to distinguish from surface differentations[1], whereas immunolatex spheres[2,3] and haemocyanin molecules[4] appear to be better suited as markers for SEM observations. However, the preparation of these immunospecific labels is laborious. We report here a simple method colloidal gold granules as visible markers, a technique which has already given satisfactory results in TEM[5–8].

Experimental. Homogeneous populations of colloidal gold granules of different particle sizes can be easily prepared[9]. The granules carry a net negative charge and can be coated by addition of proteins such as Concanavalin A or antibodies, the binding being probably of non-covalent nature. However, protein-coated gold granules

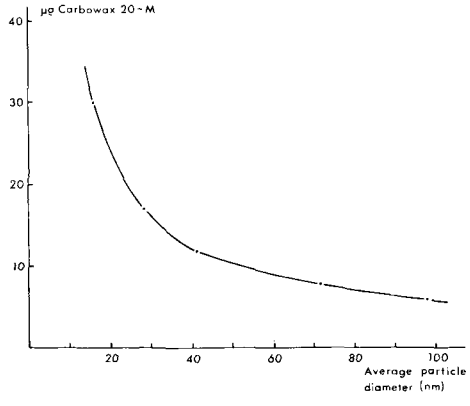

Fig. 1. Stabilization of colloidal gold by polyethylene glycol. A series of monodisperse gold sols of increasing particle diameter (16–97.5 nm as determined by TEM) was prepared[9] and neutralized to pH 7.0 by addition of 0.2 *M* K_2CO_3 (pH-paper, not pH-meter as the electrode is readily contaminated with the colloid). Colloidal gold (5 ml) was mixed with 1 ml water containing increasing amount of Carbowax 20-M (Union Carbide Chemicals Co.). After 1 min, a 10% NaCl solution (1 ml) was added. The absorbance was measured after 5 min at 525 to 540 nm (maximum of the uncoated colloid). A S-shaped curve was constructed from which the minimum amount of Carbowax necessary to fully stabilize the colloid against flocculation was determined.

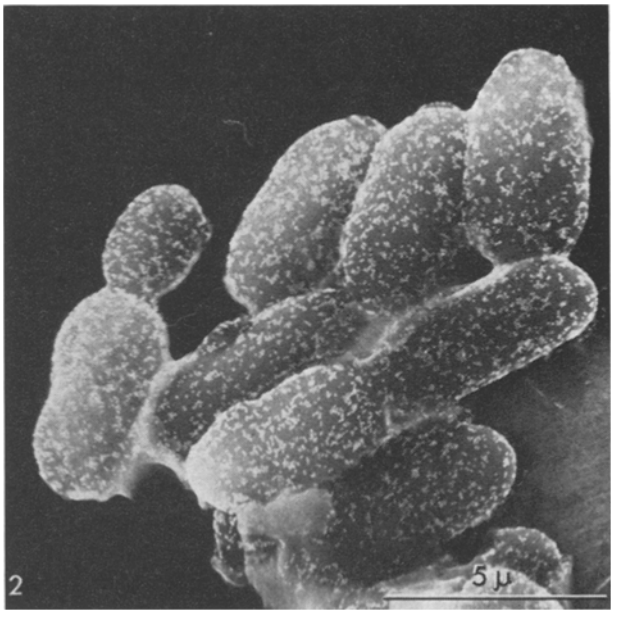

Fig. 2. A group of *C. utilis* cells marked with the total anti-*C. utilis* antiserum adsorbed to 60 nm colloidal gold particles. The antiserum was dialyzed overnight at 5 °C against 5 m*M* NaCl (pH 7.0). The optimal amount of protein to stabilize the colloid against flocculation by NaCl was determined as described in the legend of Figure 1, using the antiserum instead of Carbowax 20-M. The antiserum (1.8 mg) was added, with stirring, to colloidal gold (200 ml, sol B of ref.[9]). After 1 min, a 1% Carbowax solution in water (2 ml) was added and the colloid neutralized to pH 7.0 with 0.2 *M* K_2CO_3. The colloid was centrifuged at 10'000 rpm for 30 min at 5 °C. The supernatant was sucked off and the pellet redispersed in 20 ml *Tris*-buffered saline (pH 7.5), containing 0.2 mg/ml Carbowax 20-M. The yeast cell suspension was incubated in the same buffer at 25 °C for 1 h with an excess of the colloid. The suspension was centrifuged at low speed and washed with the buffer. The yeast cells were rapidly dehydrated in increasing concentrations of ethanol (2–3 min) and finely dispersed on a conductive copper adhesive, mounted for SEM aluminium stubs. The preparations were examined without metal coating in a Cambridge S4-10 Stereoscan, at an accelerating voltage of 30 kV at a tilt angle of 45°.

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