shown that the expectation of a good result is increased if the patient is young, is in sinus rhythm, has no calcification of the valve, and has had a perfect valvotomy; also if the result of the first operation has been good and if a long period separates the two valvotomies.

The follow-up has shown that with the passage of time there has been the same tendency to deterioration after the second operation as after the first. This has occurred despite the use of the transventricular dilator in almost all the cases, although it has not yet been seen in primary cases done by the same method. This observation supports the view that mitral stenosis is a progressive disease and that though surgery may slow the process it cannot halt it. This should not allow the possibility of a third valvotomy to be forgotten, and it may well be that many patients who have had good results from second valvotomies followed by deterioration will require yet another operation.

It may be concluded that while the results of second operations for mitral stenosis are not as good as those of the first the operative mortality is the same, and almost half the patients are much improved. The operation of choice at present seems to be blind transventricular valvotomy, and this should be recommended to all patients with restenosis of the mitral valve.

Summary

The results of 100 second operations for mitral stenosis are analysed: 48 were regarded as "excellent" or "good," 34 as "fair" or "poor." There were 12 late deaths, and the operative mortality was 5.

The factors responsible for the unsatisfactory results have been studied. Advancing age, increasing heart size, atrial fibrillation, and calcification of the valve all proved to be slightly adverse factors.

Mitral incompetence was common but seldom had an adverse influence on the result.

Deterioration appeared to be more rapid after the second operation than after the first.

The results of second operations, although not as good as those of primary ones, more than justify them in the treatment of restenosis of the mitral valve.

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Thalassaemia, Glucose-6-Phosphate Dehydrogenase Deficiency, Sickling, and Malarial Endemicity in Greece: A Study of Five Areas*

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For the last few years the concept that a balanced polymorphism maintains high frequencies of the genes for abnormal haemoglobins, thalassaemia, and glucose-6-phosphate dehydrogenase deficiency has been the stimulus for intensive studies, and many important contributions have clarified some aspects of this problem; these studies have been reviewed by Allison (1961), Motulsky and Campbell-Kraut (1961), and Neel (1962). The main effort of scientists has been concentrated on the exploration of the polymorphisms concerning the abnormal genes for sickling, for haemoglobin C, and for glucose-6-phosphate dehydrogenase deficiency (G.-6-P.D.d).

The indications that haemoglobin S and G-6-P.D.d. genes confer on their carriers considerable genetic resistance against *Plasmodium falciparum* malaria (Allison, 1954; Motulsky, 1960; Allison and Clyde, 1961) provided grounds for the hypothesis that malarial endemicity is the main factor maintaining a balanced polymorphism of these two genes. These positive findings have led to broad acceptance of the malarial theory for thalassaemia also. This last hypothesis, introduced by Haldane (1949) and favoured by the similar geographical distribution of malaria and thalassaemia in the Mediterranean basin, has been tested practically only once (Carcassi *et al.*, 1957). That study was carried out in Sardinia; and its results agree well with the malarial hypothesis; however, the need for similar studies in other thalassaemic areas is evident.

The present investigation was undertaken to test the malarial hypothesis by studying thalassaemia, G.-6-P.D.d., and Hb S frequencies in certain areas of Greece, which in the past were either non-malarious or moderately to severely malarious. Four out of the six areas discussed here were chosen because in each there had been several zones of different malarial endemicity in close proximity. In addition, thalassaemia-trait frequencies between older and younger age-groups were compared in a part of the sample.

Material and Methods

This study was carried out in 1961 and 1962. A total of 2,143 subjects were studied for thalassaemia trait; of these, 1,474 were also examined for G.-6-P.D.d. and 1,021 for Hb S. Five areas were screened: Karditsa, Elasson, Corfu, Petromagoula, and Serifos (see Map). The characterization of the malarial endemicity in each area before 1945 was based on older data (Livadas and Sphangos, 1940) and on information obtained through the local public health centres. This information was checked and found accurate by Dr. G. Belios, Professor

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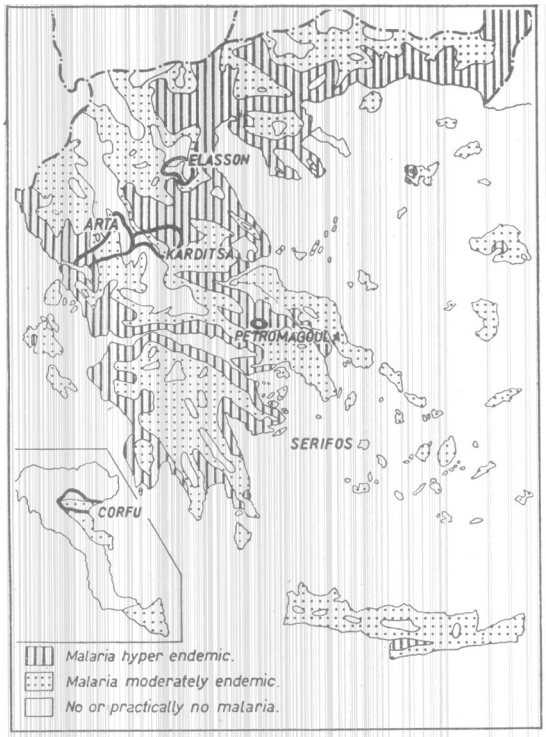
of Malariology and Tropical Diseases, Athens School of Hygiene, whom we thank for his helpful collaboration. On the basis of these data malarial endemicity in the examined areas has been classified as follows: no or practically no malarial endemicity in the past, malaria 0; moderate malarial endemicity in the past, malaria +/++; severe malarial endemicity in the past, malaria +++.

From each individual 5 to 10 ml. of venous blood was drawn. Thalassaemia was studied by a combination of haematological and biochemical methods (Malamos, Fessas, and Stamatoyannopoulos, 1962). The haematological criteria for the diagnosis of thalassaemia trait were: (*a*) alterations in the erythrocyte morphology, (*b*) increased osmotic resistance, and (*c*) low mean corpuscular haemoglobin. The biochemical criteria were: (*a*) increase in the percentage of haemoglobin A_2 above 3.5%, (*b*) increase of haemoglobin F above 3.5%, (*c*) the presence of haemoglobin Pylos, and (*d*) the presence of haemoglobin Bart's or of occasional red cells with the erythrocytic inclusions of haemoglobin H. For the diagnosis of the trait at least two of the haematological and one of the biochemical criteria were required.



Distribution of malaria in Greece. (By courtesy of the Department of Malariology and Tropical Diseases, Athens School of Hygiene.)

For the diagnosis of Hb S both paper and agar electrophoresis were applied. For G.-6-P.D. activity the test of Motulsky and Campbell-Kraut (1961) was used. In addition, in the sample of Corfu were studied: (*a*) the degree of relationships between the families of the semi-mountainous village and those of the lowland villages, (*b*) the ABO blood groups, and (*c*) the haptoglobin types by starch gel electrophoresis.

Findings

The findings obtained in the five areas are shown in Table I.

Karditsa (South-western Thessaly)

From the high schools of the town Karditsa 428 male pupils aged 12 to 17 were examined. They originated from the following sections of this area: (1) The highland section—altitude above 801 m.; malaria 0; about 20,000 inhabitants; the section is represented in the sample by 122 schoolboys. (2) The semi-mountainous section—altitude 301–800 m.; malaria +/++, particularly at the regions of lower altitude; about 20,000 inhabitants; 98 schoolboys of the sample come from this section. (3) The lowland section—altitude 100 to 300 m.; malaria +++; about 90,000 inhabitants; 208 schoolboys from this section are included in the sample.

Mean frequencies of thalassaemia trait 14.25%, haemoglobin S 1.4%, and G.-6-P.D.d. 11.22% have been found. In addition a haemoglobin Pylos heterozygote and a carrier of the persistent haemoglobin F gene were observed.

The frequency of thalassaemia trait from 11.5% in the malaria 0 section and 6.1% in the malaria +/++ section increases to 19.7% in the malaria +++ section. The last-mentioned frequency is one of the highest frequencies observed in Greece until now. The frequency in the malaria +++ section was found significant ($P<0.01$) when compared with the combined frequencies of the other two sections, while the difference between the malaria 0 and malaria +/++ sections was not significant ($P>0.05$).

The prevalence of G.-6-P.D.d. was 3.3% in the malaria 0 section, 4.5% in the malaria +/++ section, and 19.8% in the malaria +++ section. The striking increase in the frequencies in the malaria +++ section was found significant ($P<0.001$) when compared with either the non-malarious or the moderately malarious sections.

No case of Hb S was found in the malaria 0 section, while only one among 98 individuals was observed in the malaria +/++ and 5 among 208 in the malaria +++ section. The small frequencies of Hb S do not permit a statistical comparison among the sections.

TABLE I.—*Distribution of Thalassaemia, G.-6-P.D.d., and Hb S in Relation to Malarial Endemicity in Six Areas of Greece*

Malarial Endemicity	Karditsa			Elasson			Corfu			Petromagoula			Serifos			Arta†			Total		
	Examined	Abnormal		Examined	Abnormal		Examined	Abnormal		Examined	Abnormal		Examined	Abnormal		Examined	Abnormal		Examined	Abnormal	
		No.	%		No.	%		No.	%		No.	%		No.	%		No.	%		No.	%
Thalassaemia																					
0	122	14	11·5	78	8	10·3	146	23	15·75				167	23	13·8	176	17	9·7	689	85	12·34
+/++	98	6	6·1	165	13	7·9	976	128	13·1							94	10	10·6	1,333	157	11·78
+++	208	41	19·7							183	23	12·6				171	26	15·2	562	90	16·01
G-6-P.D. Deficiency																					
0	122	4	3·3	55	2	3·6	116	1	0·86							176	5	2·8	469	12	2·56
+/++	88	4	4·5	139	11	7·9	772	48	6·22							94	4	4·3	1,093	67	6·13
+++	182	36	19·8							200	29	14·5*				171	28	16·4	553	93	16·82
Haemoglobin S																					
0	122	0	0	78	0	0							167	0	0	176	0	0	543	0	0
+/++	98	1	1·0	165	0	0										94	0	0	357	1	0·28
+++	208	5	2·5							183	37	20·2				171	11	6·4	562	53	9·43

* Choremis *et al.* (1962). † 1963.

Elasson (North-eastern Thessaly)

From the high school of the town Elasson 243 male schoolboys aged 12 to 17 were examined. These came from the following sections of the area: (1) The highland section—altitude above 801 m.; malaria 0; about 15,000 inhabitants; 78 schoolboys were examined. (2) The semi-mountainous section—altitude 400 to 800 m.; malaria +/++; about 35,000 inhabitants; 165 schoolboys of the sample belong to this section.

The mean frequencies were: thalassaemia trait 8.6%, G.-6-P.D.d. 6.7%, Hb S 0%. There were also two Hb Pylos heterozygotes. The frequency of thalassaemia trait fell from 10.3% in the section with malaria 0 to 7.9% in the section with malaria +/++, while G.-6-P.D.d. increased from 3.6% to 7.9%. These differences are not significant.

Corfu (Ionian Islands)

In seven villages of an area in the northern part of the island, with 5,120 inhabitants, 1,122 subjects were selected for this study—that is, from 20 to 25% of the individuals of each village. Only schoolboys aged 6 to 12 and males aged 25 to 28 and 40 to 75 were examined, for comparison of the incidence of thalassaemia trait between the older and younger age-groups. Approximately 95% of the individuals belonging to these age-groups were examined. The area consists of two sections: (1) The semi-mountainous section—altitude 500 m., represented by one village (Sokraki); malaria 0; 146 subjects were examined. (2) The lowland section—altitude 0 to 100 m., represented by six villages (Ano and Kato Korakiana, Scripero, Aghios Markos, Doukades, Cardelades); malaria +/++; 976 inhabitants were examined.

The frequency of thalassaemia trait in the sample was 13.5% and G.-6-P.D.d. 5.5%. One Hb Pylos heterozygote was also found. Haemoglobin S is absent in this area.

The incidence of thalassaemia trait in the non-malarious semi-mountainous village (Sokraki) was slightly higher than in the six moderately malarious villages (15.75% and 13.1% respectively). Inversely, the malarious villages had clearly higher frequencies of G.-6-P.D.d. than Sokraki (6.22% and 0.86% respectively). The difference in the frequencies of G.-6-P.D.d. was found significant at the 5% level.

The two sections had practically the same frequencies of the haptoglobin types (Table II), while a significant difference ($P<0.001$) in the distribution of blood groups (Table III) was observed. The differences in the distribution of blood groups among the lowland villages were not significant ($P>0.5$). Of the 11 big families of Sokraki none was related to families of the lowlands. Only 5 out of 151 smaller families of this village had one parent originating from the malarious area.

TABLE II.—*Corfu: Percentage Distribution of Haptoglobin Phenotypes*

Villages	No. Examined	1-1	2-1	2-2	0
Semi-mountainous ..	82	9·76	50·00	40·24	—
Lowland	570	10·88	46·14	42·10	0·88
Unselected Greek average*	1,000	11·94	47·64	39·64	0·78

* From Mitropoulos (1963).

TABLE III.—*Corfu: ABO Blood-groups Distribution*

Villages	Examined	Phenotype Frequencies				Allele Frequencies		
		O	A	B	AB	O	A	B
Semi-mountainous ..	133	62·41	33·83	3·01	0·75	79·0	19·1	1·9
Lowland ..	874	43·59	35·58	15·22	5·61	66·02	23·32	11·03
Unselected Greek average*	39,020	43·7	38·4	13·1	4·8	66·21	24·55	9·24

* From Constantoulis and Paidoussis (1958).

The distribution of thalassaemia trait in five age-groups of the lowland sample is shown in Table IV. The lowest incidence (10.2%) was observed in the group of children. The mean frequency in individuals aged 41 to 75 was 14.2%. The difference between these two groups was not significant ($P>0.1$).

TABLE IV.—*Corfu-Lowland Section: Distribution of Thalassaemia Trait in Age-groups*

Age-groups	Examined	Thalassaemia Trait Carriers
61–75	221	36 (16·3%)
51–60	229	27 (11·8%)
41–50	231	34 (14·7%)
41–75	681	97 (14·2%)
Children	245	25 (10·2%)

Petromagoula (Central Greece)

Malaria +++. This area, a known focus of Hb S presenting also a high frequency of G.-6-P.D.d. (Choremis *et al.*, 1951, 1962), had not been studied for thalassaemia. A total of 183 schoolboys aged 6 to 12 were examined.

Hb S was found in 20.2% of the sample and thalassaemia trait in 12.6%. Among the 23 thalassaemia-trait carriers, five were typical cases of α-thalassaemia. Another three children with microcytosis, abnormalities of red-cell morphology, low Hb A_2, and no Hb F were probably also carriers of α-thalassaemia. However, these cases were excluded, as the diagnosis of α-thalassaemia was somewhat uncertain.

Serifos (Cyclades Islands)

Malaria was practically absent in the past (malaria 0). Thalassaemia-trait frequency was studied among 167 schoolboys aged 6 to 12, in parallel with a search for the persistent Hb F gene in the six villages of this island.

The frequency of thalassaemia trait was 13.8%. In addition there were five cases of persistent Hb F gene, all from the same village, in contrast to the uniform distribution of thalassaemia in the six villages of the island. Hb S was absent.

Arta

This area has been examined in collaboration with the Department of Paediatrics, University of Athens, and the Department of Malariology, Athens School of Hygiene (Choremis *et al.*, 1963). The data are included in Table I.

Summary of Findings

The findings obtained in these six areas can be summarized as follows:

Thalassaemia.—In three areas (Karditsa, Elasson, and Corfu), neighbouring sections of which presented different degrees of malarial endemicity, a slight fall in the frequencies from the malaria 0 to the malarious +/++ sections was observed; in Arta, the incidence of thalassaemia in the corresponding sections was unaltered. A mean thalassaemia-trait frequency of 12.34% was found in five sections with malaria 0 (the highlands of Karditsa, Elasson, and Arta, the semi-mountainous section of Corfu, and the island Serifos), while the mean frequency was 11.78% in four moderately malarious sections (the semi-mountainous sections of Karditsa, Elasson, and Arta, and the lowlands of Corfu). The increase in the mean frequency of thalassaemia to 16.01% in the three severely malarious sections (the lowlands of Karditsa and Arta, and Petromagoula) was not found statistically significant ($P>0.05$) when compared with the mean frequency of the non-malarious ones.

It was significant at the 5% level when compared with the moderately malarious areas.

G.-6-P.D.d.—In all areas a parallel distribution in G.-6-P.D.d. frequency and malarial endemicity was observed. The mean frequency of 2.56% in the non-malarious sections increased to 6.13% in the moderately malarious ($P<0.01$). A striking prevalence was observed in the severely malarious sections (mean frequency 16.82%), highly significant ($P<0.001$) when compared with the prevalence in either the non-malarious or the moderately malarious sections.

Haemoglobin S.—This abnormality was absent in four non-malarious sections and practically absent in three moderately malarious. The sample of Corfu was not searched because it was known that Hb S does not appear in this island. Only in the three severely malarious areas was Hb S observed in low (Karditsa), moderate (Arta), or very high (Petromagoula) frequencies.

Discussion

Factors such as ethnic dissimilarities, population mixture, and genetic drift in isolates are frequent sources of variation in population studies. These factors had been taken into account when the areas for the present investigation were selected for study and also during the sampling. In only one area was there a possibility of modification in the trait frequencies because of mixture. This was the area of Corfu in which the possibility of admixture between the lowlands and the semi-mountainous village Sokraki had to be considered. The difference in blood groups and the absence of family relationships between the two sections do not support significant mixing.

In all examined areas the findings in G.-6-P.D.d. fit well with the malaria theory: G.-6-P.D.d. in the highly malarious areas was six times more frequent than in the non-malarious areas. These results are similar to those obtained by an analogous study in Sardinia (Bernini *et al.*, 1960). As for the Hb S gene, the findings of the present study support previous observations of a focal distribution and restriction of this gene to certain of the highly malarious areas of Greece (Choremis and Zannos, 1957; Fessas, 1959).

The geographical distribution of thalassaemia was not found very closely related to malarial endemicity. Similar frequencies have been observed in non-malarious and moderately malarious areas. The higher frequencies in the severely malarious areas are not of such magnitude as to suggest a significant protection of the thalassaemic heterozygote against malaria. However, it should be pointed out that in the severely malarious areas the Hb S gene is also present at varying levels of frequency; the simultaneous presence of this gene is expected to influence the frequencies of the thalassaemia gene, and thus to distort the comparisons between some areas; these two genes when occurring in the same population would tend to be mutually exclusive, since their association results in more or less serious disease and consequently in a relatively higher loss of the rarer gene. Therefore it is unlikely that the frequencies of β-thalassaemia gene will increase in areas where Hb S occurs in high frequencies.

In all areas the thalassaemia carriers were of the β-chain variety and only occasional cases belonged to the α-chain thalassaemia group, the incidence of the latter falling within the limit obtained in unselected Greek populations (Malamos *et al.*, 1962). The elevation in the prevalence of the rare α-thalassaemia ($Hb^{Th}\alpha$) gene from 0.002 (the mean frequency of the unselected Greek population) to 0.014 observed in Petromagoula is a further phenomenon of interest. It is known, and has been substantiated by our own observations, that α-thalassaemia does not interact with either β-thalassaemia (Fessas, 1962) or the Hb S gene (Lehmann, 1962); therefore carriers of an $Hb^{Th}\alpha$ gene in association with either of these β-chain abnormalities are not at disadvantage, and the presence of Hb S or $Hb^{Th}\beta$ genes in a population will not decrease the frequency of the $Hb^{Th}\alpha$ gene. In case the frequencies of Hb S and $Hb^{Th}\beta$ genes cannot rise further because of being already in equilibrium to the malarial pressure, the rare $Hb^{Th}\alpha$ gene may be allowed to increase.

A similar increase of α-thalassaemia has been observed in a study of two villages in Arta, where also high frequencies of Hb S and $Hb^{Th}\beta$ genes coexisted. It may also be of interest that of the three α-thalassaemia cases detected among 745 individuals studied by Barnicot *et al.* (1963) in Greece, two were found among the 102 persons of Chalkidiki; therefore, in this known focus of Hb S (S 23.5%, thalassaemia 3.9%) the α-thalassaemia occurs at a frequency of 2%, which compares well with the situation in Petromagoula. These findings suggest that α-thalassaemia may also confer some protection against malaria but clearly less than the protection offered by the β-chain abnormalities under discussion. Such a protection may be operating in areas where frequencies up to 5% of α-thalassaemia have been found, as in south-eastern Asia (Lie-Injo Luan Eng, 1962).

Two interesting findings of the present investigation were (1) the not strictly parallel geographical distribution of G.-6-P.D.d. and thalassaemia genes, and (2) the relatively high frequencies of thalassaemia in the non-malarious areas. G.-6-P.D.d. distribution was found to be in good agreement with the "malarial theory." However, the distribution of thalassaemia obtained in the present study appears to be in disagreement with the other evidence linking high thalassaemia frequencies to malarial endemicity. This evidence can be stated as follows: the distribution of thalassaemia in large parts of the world coincides with that of *P. falciparum* malaria, and practically no cases of thalassaemia are observed in areas removed from the malarial belt. The highest frequencies of thalassaemia have been observed in highly malarious areas. Studies conducted in Sardinia have shown low frequencies of thalassaemia and G.-6-P.D.d. in non-malarious highland villages, in contrast to the high ones observed in the malarious lowlands (Carcassi *et al.*, 1957, Bernini *et al.*, 1960). The latter parallel distribution of the two genes has been attributed to the action of the same selective factor: malaria (Motulsky and Campbell-Kraut, 1961). Therefore a more detailed discussion of our findings concerning the distribution of thalassaemia is necessary.

First, the thalassaemia frequencies in the non-malarious areas could be attributed to genetic drift; although this hypothesis cannot be excluded, it is rather improbable that such a phenomenon occurred in all the non-malarious areas of the present study.

Second, if it is accepted that thalassaemia protects against malaria, then an extensive migration of malarious populations towards the non-malarious areas a few generations ago could be responsible for the present thalassaemia frequencies in the latter. In this case one wonders why the migrated population had in all instances such low frequencies of G.-6-P.D.d. The migration might have happened at a time when G.-6-P.D.d. frequencies in the malarious areas were not high. This last hypothesis presupposes a more recent appearance of the G.-6-P.D.d. gene in the Greek population; however, favism and G.-6-P.D.d. in Greece do not have the focal distribution of a "new" gene. There are also some indications that favism was known in ancient Greece. On the other hand, the difference in the frequencies of thalassaemia in the malarious and non-malarious populations being small, the hypothetical migration must have taken place in rather recent times. No historical evidence supports such a hypothesis, and this is a further reason for rejecting the explanation of thalassaemia frequencies in non-malarious areas by recent migration.

Third, the similarity of the thalassaemia gene frequencies in the non-malarious and moderately malarious areas cannot be

explained by accepting an important advantage of the thalassaemic heterozygote against malaria.

Fourth, the same conclusion can be reached from the distribution of Hb S in Greece, all known foci of which correspond to severely malarious areas. Of the three Hb S foci examined by us for thalassaemia frequencies also, in two (Petromagoula and a zone of the lowlands of Arta) the frequency of Hb S was higher than that of thalassaemia. Although it is impossible to know what the situation was in the past, the fact that the foci of Hb S are surrounded by areas with high thalassaemia frequencies suggests that similarly high frequencies of thalassaemia existed in these foci before the appearance of Hb S gene. As the Hb S gene has achieved high frequencies in spite of the presence of thalassaemia, it can be concluded that the latter is less efficient than Hb S in highly malarious areas.

It was hoped that in the comparison of thalassaemia frequencies between age-groups in an area which has been free from malaria for the last 15 years could also serve as a test of the malarial hypothesis: children under 12 would be expected to have less thalassaemia than old people. This comparison was carried out in Corfu, but did not lead to conclusive results. A decreased frequency was found among the children; nevertheless, similarly low frequencies were obtained in the group of men aged 50 to 60. One wonders whether these findings should be attributed to the small sample or to the alternations of the severity of malarial pressure on this area in the past. In a similar study carried out by Montalerti *et al.* (1959), in Ferrara, thalassaemia trait was less frequent in the younger, when the age-groups 0 to 40 years and 41 to 85 years were compared, but the fluctuation of the frequencies within each group was also important.

In two recent papers the "malarial hypothesis" in the case of G.-6-P.D.d. has been challenged. Kruatrachue *et al.* (1962), in Thailand, have not confirmed Allison's observations that parasitaemia by *P. falciparum* is lower in G.-6-P.D.d.-deficient children. Kidson and Gorman (1962), in New Guinea, among five linguistic groups living in areas with holoendemic malaria have found three with high and two with very low G.-6-P.D.d. The distribution of thalassaemia in these lowland linguistic groups was even, while the thalassaemia gene was absent in the highlands. The latter authors have interpreted their findings as supporting the malarial hypothesis in the case of thalassaemia but not in G.-6-P.D.d. The results of these papers, entirely different from those of the present investigation, reveal the complexity of the mechanisms which control the abnormal genes' frequencies, or an inadequacy of the methods used for the study of the complicated problem of balanced polymorphism (Harris, 1962; Allison, 1963).

Nevertheless, it appears that certain conclusions can be reached after evaluation of the data of the present study. (*a*) The increased frequencies of thalassaemia in highly malarious areas indicate that in such areas thalassaemia is of selective value. (*b*) On the other hand, the distribution of thalassaemia in non-malarious areas indicates that malaria may not be the only selective factor. This distribution, as well as the frequencies of Hb S and G.-6-P.D.d. deficiency, suggests that, of the three abnormalities considered as protecting against malaria, thalassaemia is the least efficient.

Summary

The population of five different areas of Greece has been examined for thalassaemia, glucose-6-phosphate dehydrogenase deficiency, and sickling. The frequencies of these three red-cell anomalies in each area have been considered in relation to malarial endemicity. While the distribution of G.-6-P.D. deficiency and haemoglobin S was found to fit well into the "malaria theory," the findings concerning thalassaemia were not clear-cut; they indicate a smaller protection against malaria and the possible presence of additional factors for the maintenance of high frequencies of thalassaemia.

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