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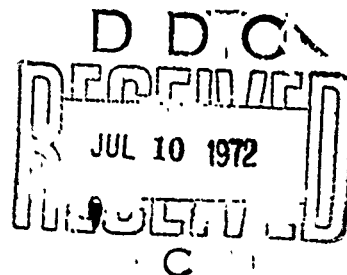
SURVEYS OF THALASSEMIA AND  
OTHER BLOOD DEFECTS IN SPAIN

by

J. B. BATEMAN

June 1972

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13. ABSTRACT Drs. A. Pellicer and A. Casado of the Institute of Genetics and Anthropology, Center for Biological Research, Madrid, have accumulated extensive data on the incidence of thalassemia trait and G6PD deficiency in Spain, with particular reference to the former incidence of malaria. They find no correlation, in the case of thalassemia trait. The G6PD deficiency gene they believe to be uniformly distributed in Spain. They have failed to find any cases of sickle cell trait despite the presence of an African negroid component in segments of the population. The studies are being extended to acatalasemia: three cases of presumably heterozygous hypocatalasemia have been found. The paper also contains outlines of the research programs of the other sections of the Institute of Genetics and Anthropology, and the Departments of Genetics and of Anthropology in the "original" University of Barcelona.			
Key words: Thalassemia; Sickle cell trait; Malaria; Spain; Favism; Glucose-6-phosphate dehydrogenase; Acatalasemia			

TECHNICAL REPORT

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OTHER BLOOD DEFECTS IN SPAIN

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J.B. Bateman

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I. INTRODUCTION

Enquiring about sickle cell anemia in Spain and related matters during a visit to the Biological Research Center, I was introduced to Professor Eugenio Ortiz de Vega, Vice-Director of the Institute and Chief of the Section on Animal Cytogenetics, who in turn led me to Dr. Angel Pellicer

and his co-worker Dr. Angela Casado. Supported only by one technician and working in very cramped quarters, they have accumulated a great mass of data on thalassemia and glucose-6-phosphate dehydrogenase deficiency, though not--strangely enough--on the sickle cell trait, which they have sought but not found. In the following, some of their publications are summarized, with additional details here and there gleaned from the rather unsatisfactory exchange in broken English and still more broken Spanish.

## II. THE INSTITUTE OF GENETICS AND ANTHROPOLOGY, BIOLOGICAL RESEARCH CENTER, MADRID

The Biological Research Center (Centro de Investigaciones Biologicas) will be described elsewhere. The Institute of Genetics and Anthropology is one of seven constituent institutes, itself split into departments and sections:

### Anthropology Department

Human Somatology  
Human Genetics

### Dynamic Genetics Department

Population Genetics  
Development Genetics

### Cytogenetics Department

Plant Cytogenetics  
Animal Cytogenetics

The personnel strength of the Institute amounts to 12 professional scientists in various categories, 24 fellows and doctoral candidates, and 16 for administrative and technical support. The 29 research themes listed in ref (2) and in some cases summarized in ref (1) cover a rather wide range, including, for example:

### Anthropology

Historical and prehistoric anthropology  
Genetic polymorphism  
Dermatoglyphs  
Anthropology of the newborn  
Cytology of malignant neoplasms  
and oncostatic processes  
Thalassemia, G6PD deficiency,  
acatalasemia

### Dynamic Genetics

Origin and maintenance of genetic variability and its role in adaptive phenomena  
Evaluation of genetic load in Drosophila populations  
Analysis of interactions between chromosomes II and III in experimental populations subjected to rapid selection  
Mechanisms of mitotic "crossing over"  
Mode of action of morphogenetic wing mutants

### Cytogenetics

Cytogenetic studies of the genus Digitalis including action of chemical agents  
Various studies of plant cytology and karyology  
Polymorphism in natural populations  
Cytogenetics of osteopathies and dystrophies  
Sex chromosomes and delinquency

### III. THALASSEMIA, GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY, SICKLE CELL TRAIT, FAVISM AND MALARIA MORTALITY IN SPAIN

The work of Dr. Pellicer since 1965 and more recently that of his former doctoral student Miss Casado has been guided by the suggestion, originating apparently with W.B.S. Haldane in 1949, that the superior resistance of thalassemics to malaria would confer a selective advantage. It might be expected, therefore, that the frequency of heterozygotes for thalassemia (thalassemia trait) would tend to be higher than elsewhere in regions where malaria is endemic. A similar conjecture might apply to the frequency of occurrence of glucose-6-phosphate dehydrogenase (G6PD) deficiency, which is known to be particularly common in malarial regions in and around the Mediterranean.

Dr. Pellicer's work in Spain began, of necessity, too late, because malaria eradication procedures were started long ago, so that the most recent epidemiological data on malaria uninfluenced by such procedures are those of 1919. In the meantime, eradication is said to have become virtually complete.

The series of papers from Pellicer's laboratory represents the gradual extension of his studies to larger numbers of cases and broader geographic coverage, without any essential change in laboratory techniques. He was concerned throughout with the asymptomatic blood picture, not with the clinical manifestation of thalassemia major, more than 41 cases of which had been reported in Spain since 1942. The thalassemic trait has been identified by the appearance of morphological abnormalities in the erythrocytes, by enhanced osmotic resistance, and by the presence of increased proportions of hemoglobins A<sub>2</sub> and F when determined by electrophoresis on gelatinized cellulose acetate at pH 7.4 and subsequent processing. For G6PD an accepted methemoglobin reduction test was used, and evidence of sickling was sought by incubation in sodium metabisulphite solution. The assembled laboratory data, combined with the available information about the people from whom the blood was taken, were then sorted out for comparison with malaria mortality figures for 1919 published in 1923 in a book by G. Pittaluga (Calpe, Madrid).

It may be said at once that the growth in volume of the available data did not necessitate any radical change in Pellicer's conclusions, but it did close certain loopholes, as a brief account of his papers in order of appearance will show.

A small study made in 1965 dealt with a sample group of people in Madrid, 80% of whom were "immigrants" (i.e. Spaniards settling in Madrid, presumably). In (3), the paper published in 1967 results from a "random" group of 6610 persons in Madrid were published. Of these, 80, or 1.21%, showed the thalassemia trait and this figure was adopted as the national average for Spain. The majority of cases were diagnosed as  $\beta$ -thalassemia, with elevated hemoglobin A<sub>2</sub> and/or F, while a few, showing morphological evidence but uncharacteristic hemoglobin values, were presumed to be  $\alpha$ - or  $\delta$ -thalassemia. A breakdown of data on these 6610 persons into 13 regions of supposed origin failed to show any correlation with the 1919 malaria mortality figures, a finding that differed from that reported by Siniscalco for Sardinians. In another paper (4) Pellicer selected a group of immigrants to Madrid from two provinces where the malaria mortality had been particularly high in 1919 (30 - 50 per 100,000): among these the thalassemia percentages were 1.09 among 457 persons from Toledo, and 1.23 among 565 from Haen, neither being significantly different from 1.21, the assumed national average. This confirmed the

previous failure to find the sort of correlation that would confirm Haldane's hypothesis and that has been reported by others. This does not constitute a valid rejection of the hypothesis, however, because population migrations, particularly those caused by the 1936-39 civil war,

"diminish(es) the significance of the birthplace declared by the individuals sampled, since their ancestors may have come from other regions with different intensities of malaria." (3, page 698).

The actual frequencies in terms of supposed place of origin were comparable to those of Italian cities, but the amplitude was greater: 0.4 to 1.7% in Italy, 0 to 3.3% in Spain.

The next investigation removed some of the uncertainties of origin by leaving Madrid and entering the province of Huelva in the extreme southwest, bordering on Portugal, where circumstances favored study of a population long exposed to malaria:

1. A mild climate favorable for mosquitoes, with marshes in the south and deserted mines and stagnant water in the hills.
2. A stable population from ancient times: the biblical Tharsis, ethnically identifiable.
3. The presence of a negroid element from the immigration (and importation) of African negroes in the 15th century, whose gradual mixing would be expected to have diffused the sickle cell trait in the general population.
4. The second highest 1919 malaria mortality rate: 105 per 100,000.

The sample can be described as follows: using data given in ref (5):

Males	269	Females	258	Total	527
Lowland	426	Highland	101	"	"
"Negro" cities	298	"Non-negro"	229	"	"
Negroid physical features	14				

Age: 3 to 67

and the results in round figures:

	<u>Lowlands</u>		<u>Highlands</u>
	percent		
$\beta$ -thalassemia	1.9	=	1.0
G6PD <sup>-</sup>	0.7		0
Sickle cell	0		0
Favism	0		0

There is nothing here to suggest any correlation of thalassemia trait or G6PD<sup>-</sup> deficiency with malaria. More surprising, perhaps, is the complete absence of sickle cell trait and favism. There is no evidence for selection, under malarial pressure, of a sickle cell trait presumably imported from Africa five centuries ago. The absence of favism is surprising because the fava bean is a popular food in Huelva. I am reminded of the frequent occurrence of favism in Iran where, I am told, it can be caused simply by the presence of the growing plant (6). This has led Professor Frances Aylward of the University of Reading to suppose that different varieties of the bean may differ in their properties: Those grown in Huelva might be worth examining.

Pellicer's latest paper with Dr. Casaco (7) extends the field studies to three provinces with a formerly high incidence of malaria and two with none at all, and the results are presented and discussed in conjunction with those of the earlier investigations:

Thalassemia Trait, G6PD Deficiency  
and 1919 Malaria Mortality  
in Spain

Data abstracted and simplified from Pellicer and Casado, ref (7), Tables 1 and 2 and Fig. 1.

M = malaria mortality per 100,000  
T = thalassemia trait, %  
G = G6PD<sup>-</sup>, %

	M	T	G
Biscay	0	0	1.11
Corunna	0	3.23	0.32
Madrid	1-25	1.21	0.75
Jaén	26-50	1.22	?
Toledo	26-50	1.09	?
Murcia	51-100	1.34	0.48
Badajoz	101-150	3.15	0.51
Huelva	101-150	1.70	0.74
Cáceres	150 +	1.59	1.53



With regard to thalassemia, the data are rather striking, because in addition to the lack of any defensible quantitative correlation with malaria incidence, one notices the qualitative fact of a high frequency of thalassemia incidence in an area--Corunna--where

"there is not the slightest historical hint of the existence of malaria."  
(Ref (7), page 302.)

The thalassemia trait was probably introduced into the Celtic population of Galicia when a Mediterranean group, the Ligures, invaded and settled around 800 - 600 B.C. The Basques, with neither thalassemia nor malaria, have been segregated and inbred to a large degree since the neolithic age, as a token of which they still retain the doubtful advantage of the world frequency record for the blood group factor Rh(d). But any argument that this double negative supports the Haldane hypothesis is itself rejected by the case of the Galicians.

The proper conclusion seems to be that malarial selection cannot be demonstrated in the genetically heterogeneous stocks available in Spain, either because of heterogeneity and topographical instability per se or because of more fundamental difficulties in finding the proper circumstances for a test. The authors refer, for example, to calculations by A. Baserga reported in a N.Y. Academy of Sciences panel discussion showing that the survival advantage conferred by the thalassemia trait would amount to only one case in a thousand in 50 generations, so that quite exceptional stability would be required for its detection.

G6PD deficiency is widespread and the authors consider that the gene is uniformly distributed throughout the country--but one recalls that none was found in the hills of Huelva among 101 persons tested (p. 6). Perhaps I may summarize these investigations as follows:

(1) The thalassemia trait is found in varying frequencies throughout Spain except in the Basque province of Biscay. It occurs in Corunna where there has never been any malaria, and in comparable frequencies in areas where malaria has existed. There is no correlation between present thalassemia trait and former malaria mortality, nor is it certain that any such correlation should be expected on the Haldane hypothesis under the conditions existing in Spain.

(2) The thalassemia trait found in Spain is usually characterized by high levels of hemoglobin A<sub>2</sub> and/or F. Hemoglobin H has not been found.\*

(3) G6PD deficiency is fairly uniformly distributed throughout Spain at frequencies comparable to those found in Italy, though it was not found in the hilly country in the province of Huelva (101 samples).

(4) Sickle cell trait has not been detected.

(5) Favism has not been reported in the province of Huelva where the fava bean is a favorite food.

#### IV. ACATALASEMIA

Dr. Pellicer has been interested in reports of the incidence of acatalasemia, which from 1948 until its detection in Switzerland was thought to arise from an error of metabolism peculiar to the Japanese. Although only two Swiss cases were found among 18,459 examined, the exclusiveness to Japan was thus disproved. Dr. Pellicer is struck by the fact that whereas in Japan the condition is associated with ulcerous stomatitis leading sometimes to gangrene, no such symptoms were reported in the Swiss cases. Because of the anthropological interest of the matter and the possible relationship, hitherto unsuspected, to pathological manifestations, he thought it important to investigate the possible occurrence of acatalasemia in Spain. In a preliminary communication (8) announcing this decision he reported that in three of 1200 individuals examined, the blood catalase activity was only one half of the normal value. This hypocatalasemia he supposes to represent the heterozygous condition, and he is seeking the cooperation of the dental faculty in obtaining samples from cases of ulcerous stomatitis, perhaps with severe complications, with the possibility of establishing a correlation with homozygous acatalasemia.

#### V. THE DEPARTMENT OF GENETICS AND THE DEPARTMENT OF ANTHROPOLOGY, FACULTY OF SCIENCE, IN THE "OLD" UNIVERSITY OF BARCELONA

The meeting with Pellicer led me to enquire, when

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\*A note in the official bulletin (1) states that Dr. Casado has found the first instances in Spain of the presence of hemoglobins C and S.

visiting Barcelona, about other Spanish investigations of thalassemia and related matters. No new information was forthcoming but I learned a little about the interests of the departments visited in the "Original" University--a description that I use here to distinguish it from the Universidad Autónoma founded a few years ago, in common with similar institutions in Madrid and Bilbao.

In the Department of Genetics I met a doctoral student, Jaime Baguñá, the department head, Professor Prevosti, being out of town. The department, I gathered, has in the past been rather addicted to Drosophila and even now this fly is being used in an investigation of the neutral or adaptative factors in somatic polymorphism, methods being developed for isolation from Drosophila of such enzymes as the esterases and the alcohol dehydrogenases. At the same time they are trying to move into other fields, including the immunogenetics of the blood groups of indigenous strains of cattle, and the taxonomic immunogenetics of beetles and planarian worms. They have received valuable assistance from Dr. W.H. Stone of the University of Wisconsin. Baguñá himself is interested in planarian regeneration and is shortly to join Waddington's group in the Institute of Animal Genetics, Edinburgh, for work on molluscan embryology, and later possibly to work with E.N. Willmer in Cambridge.

In the Department of Anthropology, Dr. A. Valls is engaged in an elaborate study of the frequency of the non-ABO blood groups hitherto "unknown" in Spain including the X-chromosome-linked Xg series. He is specially interested in the high frequency of inbreeding in Spain in general and in particular in the Balearic Islands and isolated valley communities in the Pyrenees. The study will therefore stress the correlation of blood group distributions with the incidence of endogamy. The blood grouping is being done by a group of four associates, necessary materials being furnished by the Dade Laboratories, Florida. Storage seems to be done manually by punch card. There has been communication with Dr. A.E. Mourant of the Medical Research Council's Serological Population Genetics Laboratory in London and it is to be hoped that the results will become available for inclusion in Dr. Mourant's important compilation of blood group data.

Dr. Valls completed a substantial study of inbreeding frequencies in the Balearics while still a colleague of Dr. Pellicer in Madrid, including in his paper (9) a summary of the ABO system frequencies, which he

found to be similar to those on the adjoining mainland. He makes no further use of these numbers, proceeding instead to a series of computations based on local records of 67,293 marriages, resulting in values of various inbreeding coefficients, related to degrees of endogamy, upon the basis of which comparisons can be made with other communities. Presumably inbred and non-inbred populations will differ in their frequencies of homozygosity for various characteristics, and statistical data of this kind therefore provide a basis for understanding the distribution of blood groups.

#### VI. COMMENT

This paper deals particularly with the work of Dr. A. Pellicer of Madrid on the distribution of thalassemia trait and G6PD deficiency in various parts of Spain. Dr. Pellicer has also looked for the sickle cell trait but has failed to find it, despite evidence for an African negro component in certain areas. He is extending his studies to include a search for cases of homozygous acatalasemia.

Dr. Pellicer and his colleague, Dr. Angela Casado, are obviously highly capable and conscientious research scientists who with limited facilities and with the use of very limited conventional techniques are investigating a subject of considerable anthropological, epidemiological and perhaps medical interest.

It cannot be said that such statistical surveys as these are, in themselves, of great military interest. However, personnel screening for conditions such as the sickle cell, thalassemia and G6PD traits, and probably for other genetically determined traits yet to be recognized, is assuming increasing importance. The latent biological disadvantage they confer may become overt under conditions of stress, in the form of gross and even fatal disturbances of function. It may well be that investigators who have at their disposal long experience and extensive statistical data, together with the possibility of continuing their research, would be able to contribute on a formal basis to some aspect of interest to the Army, or to furnish to Army laboratories samples representative of particular conditions. There are shortcomings: the lack of intimate connections with a large clinical center may be difficult to overcome. Others are technical, such as the failure, up to now, to identify

the G6PD variants; these could be rectified. Suggestions or expressions of interest from Army Laboratories would be welcomed.

#### VII. SUMMARY

Drs. A. Pellicer and A. Casado of the Institute of Genetics and Anthropology, Center for Biological Research, Madrid, have accumulated extensive data on the incidence of thalassemia trait and G6PD deficiency in Spain, with particular reference to the former incidence of malaria. They find no correlation, in the case of thalassemia trait. The G6PD deficiency gene they believe to be uniformly distributed in Spain. They have failed to find any cases of sickle cell trait despite the presence of an African negroid component in segments of the population. The studies are being extended to acatalasemia: three cases of presumably heterozygous hypocatalasemia have been found.

The paper also contains outlines of the research programs of the other sections of the Institute of Genetics and Anthropology, and the Departments of Genetics and of Anthropology in the "original" University of Barcelona.

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