THE THALASSAEMIA TRAIT IN MALTA AND GOZO

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Introduction

An increased osmotic resistance of the erythrocytes can be tested for, and detected, by mixing a measured amount of fresh blood with a hypotonic solution of inorganic salts of an osmolarity such that the majority of erythrocytes with normal resistance will rupture in it and produce a clear solution; if the erythrocytes are more resistant than normal under the same conditions, the mixture remains an opaque suspension. A solution of sodium chloride in water which contains 0.35 grams per 100 ml has this critical osmolarity (120 milliosmoles per litre). By making a suspension of erythrocytes from fresh blood in this solution, in a haemocytometer pipette (blood 1 part, hypotonic solution 199 parts) and allowing time for lysis to occur (2 to 5 minutes), individuals with a raised osmotic resistance can clearly be distinguished from those in whom the resistance is normal.

In 1943, Silvestroni and Bianco described this simple test, making use of a

diluted Tyrode solution (osmotically equivalent to a 0.35 per cent. solution of sodium chloride) as the hypotonic medium. These workers were the first to describe, by this method of testing, a familial incidence of increased erythrocyte resistance in otherwise healthy persons ("constitutional microcythaemia" or "thalassaemia minor", this abnormality being associated with certain morphological characteristics of the erythrocytes in the majority of subjects. They also established a relationship between this anomaly and the clinical condition which had been described by other authors as "haemolytic icterus with increased osmotic resistance" (Rietti - Greppi - Micheli Syndrome), such that the anomaly was often found in healthy siblings or parents of such icteric patients. They also describe the genetic relationship between "constitutional microcythaemia" and Cooley's anaemia, such that the former represented the heterozygous state while the latter represented the homozygous state for the thalassaemia gene or genes. By this method of testing, Silvestroni and Bianco have, in a series of population surveys carried out over the last two decades, mapped the geographical pattern of incidence of this anomaly in Italy and Sicily, while numerous investigators have used the same, or a similar, test for the same purpose in Sardinia, Cyprus, Greece, India, Portugal, Portuguese East Indies, Algeria and other parts of the world.

The Thalassaemia Trait

Great interest has developed since 1948 (when Vecchio described the presence of foetal haemoglobin in the blood of patients with Cooley's anaemia) in the study of the structure and synthesis of the haemoglobin molecule in thalassaemia. These studies have led to a realization that what had been considered to be a simple genetic and clinical entity, in reality encompasses many different genetic conditions, all of which exhibit the abnormal erythrocyte morphology which had come to be considered as characteristic of thalassaemia. According to Fessas (1965): "Recognition of thalassaemia trait or thalassaemic conditions in general is still a haematological problem, at least at the initial stages of the diagnostic procedure. It may be necessary to emphasize this point, as it is sometimes overlooked. Accepting a deficiency in the production of a major chain (of the haemoglobin molecule), we can expect the effects of this deficiency to become manifest in the aspect of the red cells: microcytosis, hypochromia, a low M.C.H., poikilocytosis. It is realized that the alterations of erythrocyte morphology may vary in extent and, indeed, have been reported as entirely absent in a minority of cases; conversely the presence of an abnormal haemoglobin in the heterozygous form may also be responsible for mild alterations of erythrocyte morphology. Such findings do not alter the basic facts concerning the alteration of erythrocytes in all forms of thalassaemia and I am rather hesitant to accept that the morphological alterations are separable from a true "thalassaemia effect."

The criteria that have been used by different investigators for the detection of thalassaemia are many. Those which have been most frequently used are summarized in Table I. It is worth noting that the microscopical appearance of blood smears was the only criterion used in assessing the frequency of thalassaemia in a recent study in Yugoslavia (Fraser, Grunwald and Stamatoyannopoulos, 1966). Silvestroni and Bianco (1966) have this to say on the criterion of increased osmotic resistance: "This method, if properly carried out, has proved itself useful for the recognition of nearly all microcythaemics and is, even today, the most valuable and rapid single criterion for the identification of microcythaemia": translation from the original in Italian).

Since the erythrocyte morphology is the same in the various presently recognized forms of thalassaemia (alpha thalassaemias, beta thalassaemias, delta thalassaemias, etc.), a test based on this morphological abnormality should detect, without distinguishing between, these different forms. The techniques necessary for identifying the different possible haemo-

globin patterns are still too complex, time-consuming and expensive to be used for extensive population studies. It is not surprising then that a simple, rapid and inexpensive test such as that of Silvestroni and Bianco has been used by many workers for extensive population studies.

Thalassaemia trait in Malta and Gozo

During March 1960, the present writer had an opportunity of visiting the "Centro per lo Studio della Microcitemia e delle Anemie Microcitemiche", at the Istituto d'Igiene of the University of Rome (directed by Professor E. Silvestroni) and of studying, at first hand, the method used by Silvestroni and Bianco in surveying large populations for the incidence of thalassaemia. He also took part in a population study in two localities near Ferrara where the frequency of thalassaemia is amongst the highest yet described. This method was then used (together with microscopical study of peripheral blood smears from those who gave positive tests) in a study carried out in Malta and Gozo during the spring months of 1960, 1961, 1962 and 1963. The results have already been described in detail (Vella, 1961; Vella and Sant Cassia, 1961; Vella, 1962; Vella, 1964).

The occurrence of thalassaemia is well known in patients of Maltese origin. It was first described in an indigenous Maltese family by Mooney (1951). The second report of its occurrence in Maltese patients came from Tunisia (Roche, Derrien, Diacono and Roques, 1953). Since then twelve instances of thalassaemia major (i.e. Cooley's anaemia) have been seen in Malta by Dr. E. Cachia (personal communication) and a set of identical twins with the disease have been seen by Dr. T. J. Agius Ferrante and their haemoglobin pattern studied by the present writer. No indication was available, however, as to the general frequency of the thalassaemia trait and its distribution in Malta and Gozo until the work that will be summarized here.

A preliminary survey was made during April 1960, amongst 2,700 healthy school children of both sexes attending two large government primary and three private schools in Malta. This sample was considered to be representative of the general population as it was drawn from various parts of the Island. The method of Silvestroni and Bianco was used, the Tyrode solution having been made available by Professor Silvestroni. An increased osmotic resistance was found in 4.4 per cent. of this sample. Noticeable differences in the frequency of positive tests were revealed when the subjects were grouped by village or town of origin. The following percentage frequencies were found: Msida 0.0, Zabbar 1.8, Gzira 1.9, Hamrun 3.2, Qormi 3.4, Valletta 3.4, "Three Cities" 3.7, Birkirkara 4.4 and Sliema 4.6, though the sample size from each of these localities was by no means uniform. The smallest group, that from the "Three Cities", numbered only 54 while the largest, that from Birkirkara, numbered 608. Of 1000 subjects not classed as originating from these above localities, 5.6 per cent. gave positive tests.

A systematic survey was started in 1961 and continued in 1962 and 1963. Healthy pupils of both sexes attending government elementary schools in various towns and villages in Malta and in some parts of Gozo were tested. A hypotonic solution was used which contained 3.5 grams sodium chloride (Analar grade, desiccated) per litre (made up in double-distilled water at the temperature of calibration of the volumetric flask, stored well stoppered at 4°C when not in use and equilibrated to room temperature before use). In the larger schools, an attempt was made to test some 200 pupils, but when the results indicated the need, more pupils were tested. In several places, the majority of the pupils in the village school were tested. The results confirmed and extended those of the preliminary survey.

The complete investigation covered 10,400 children in 39 towns and villages in Malta and revealed an over-all frequency of positive tests of 5.6 per cent. In Gozo, 1,150 children were tested in 5 towns and villages and the over-all frequency for this island was 7.8 per cent. In 14 localities in Malta, the frequency

was above the average and the villages of Mellieha and Marsaxlokk had, by far, the highest frequencies. Only the village of Zebbug in Gozo had a frequency above the mean for the island and this was the highest found in the whole survey.

The following localities gave frequencies of positive tests of about 10 per cent and above and constituted peaks of high frequency surrounded by areas of much lower frequency: Zebbug (Malta) 9.4, Mgarr (Malta) 10, Pawla 10.5, Qrendi 11, Cospicua, 11, Marsaxlokk 15.5, Mellieha and Zebbug (Gozo) 24.5. In Table II are given the percentage frequencies for towns and villages nearest to those with a frequency of about 10 per cent. and above.

The agricultural villages of Mellieha, Mgarr and St. Paul's Bay are of recent origin. The present parish of Mellieha was established in 1844, while St. Paul's Bay and Mgarr became centres of population around 1880. All three localities were first settled by villagers who came mostly from Rabat and Mosta. It is worth noting that the frequencies in the villages nearest to Rabat and Mosta are surprisingly low (Dingli 1.2, Gargur 2.0, Naxxar 2.1, Birkirkara 4.4). It would be interesting to know if the first inhabitants of Mgarr and Mellieha were of the same family stock. None of the localities situated in the north-western half of Malta are as isolated geographically as Mellieha. This village was, until the opening of the bus service to it some thirty years ago, the most inaccessible part of Malta. Even today, most marriages solemnized there are between local residents, while some 17 per cent. of all the adults aged 21 years and over in 1959 and registered in the Electoral List, bore the same surname. From the information made available during the survey, it was possible to group the 741 pupils tested in that village, into 180 sibships each numbering between 2 and 6 children. In 87 sibships only negative results were found, in 60, one child only gave a positive test, and in 33, two or more children gave positive tests.

In the south-eastern (the most densely populated) half of Malta, the highest frequency was found in Marsaxlokk. This secluded fishing village in the extreme south-east end of the island has a population of nearly 1000 and dates back almost one century. It is known that its population nearly doubled between 1943-1948 and that half of the children who gave positive tests had come from five sibships. This suggests that the abnormality is not as widespread as at first appears, and that it may in fact be restricted to only a few families, probably amongst the oldest in the village.

The only important localities in Malta which were not specifically covered by this survey were those of Mdina, Balzan, Lija, Attard and St. Julian's, though a number of individuals from these areas had been tested in the preliminary survey. Since the population of Mdina is similar to that of Rabat, and that of the other four villages is very similar to that of the large residential complex which comprises Birkirkara, Hamrun, Msida, Gzira, Sliema, Valletta and Floriana, in which the frequencies ranged between 1.9 and 5.1 per cent., there is no reason for suspecting that they may be regions of high frequency.

The village of Zebbug (Gozo) is similar to that of Mellieha and Marsaxlokk in its seclusion. It is one of the smallest villages in Gozo and was described by one prominent inhabitant as being "one large family". Unfortunately, it was not possible to investigate the family relationship of the children who were tested there.

The results of this survey are very similar to those found by Silvestroni and Bianco (1949, 1953) in Sicily, situated only 60 miles from Malta. There, the over-all frequency was 4.45 per cent. but ranged between 2.79 and 10.81 per cent. in the different regions studied. This survey only covered 8,000 persons and it is likely that regions may exist in Sicily with higher frequencies than these. There also, circumscribed foci were found in which the frequency of positive tests was much higher than that for the neighbouring countryside and adjacent to regions with a lower than average frequency not far away. Since the methods of testing and the criteria used for the detection of

thalassaemia in the Maltese Islands and in Sicily were to all intents identical, a comparison between the two studies is valid. The most common variety of thalassaemia found in the central Mediterranean region (Italy, Sicily, Sardinia, Algeria) is one form of beta thalassaemia. It is reasonable to infer that this is also the variety most frequently detected in Malta and Gozo in this study.

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TABLE I

Criteria used by different investigators for the detection of Thalassaemia

Criteria based on erythrocyte morphology

1. Microscopical appearance of the erythrocytes in peripheral blood smears.
2. Increased osmotic resistance.
3. Hypochromia of erythrocytes associated with normal serum iron levels and resistant to iron therapy.
4. Presence of erythrocytes containing foetal haemoglobin as detected by an acid-elution method.

Criteria based on haemoglobin pattern

1. Increased amounts of foetal haemoglobin.
2. Increased amounts of haemoglobin A_2.
3. Presence of an abnormal haemoglobin fraction demonstrable by electrophoretic methods (haemoglobin Barts, H, Lepcrte, Pylos, etc.).

TABLE II

Frequency of positive test in localities near to regions of high frequency (in per cent.)

Malta	Mellieha	22.0
	Mgarr	10.0
	Mosta	5.9
	St. Paul's Bay	5.5
	Marsaxlokk	15.5
	Gudja	8.0
	Birzebbugia	4.8
	Kirkop	4.7
	Safi	2.9
	Ghaxaq	0.6
	Zejtun	0.7
	Cospicua	11.0
	Senglea	3.9
	Vittoriosa	2.6
	Qrendi	11.0
	Zurrieq	8.2
	Mqabba	6.3
	Siggiewi	4.5
	Pawla	10.5
	Marsa	7.7
	Tarxien	6.7
	Fgura	4.8
	Zabbar	1.0
	Zebbug	9.4
	Siggiewi	4.5
	Qormi	3.4
	Luqa	2.0
Gozo	Zebbug	24.5
	Xewkija	7.0
	San Lawrenz	2.7
	Nadur	2.4
	Sannat	1.0

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THE FIRST CHAIR OF OPHTHALMOLOGY: a Biographical Sketch of Joseph Barth

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On the 7th April 1818, after a short illness, at the age of 73 years, Professor Joseph Barth, Imperial Councillor, Oculist to His Majesty the Emperor, Professor of sublime Anatomy and Physiology and first Professor of Diseases of the Eyes in the Imperial University, died in Vienna. Well known all over Europe for his ability as teacher and surgeon and for the numerous famous pupils, to whom he was a life long inspiration, his death passed unnoticed in Malta, his native country, and remained so for many years.

Joseph Barth was born in Valletta in 1745. His parents were Nicholas Barth and Maddalena Sceberras. He became a pupil of Professor Michelangelo Grima, Master in Surgery of the Order of St. John in