picture of the electrolyte status would have been of great interest. This is certainly attributable to the time consuming procedures required to separate the commonly used isotopes. The use of the cyclotron-produced ^{43}K and ^{77}Br with ^{24}Na greatly facilitates the simultaneous measurements of K_E , E.C.F.V., and Na_E but beta counting of $^3\text{H}_2\text{O}$ must be carried out after decay of the gamma emitters to measure T.B.W. Further work on the development of a radiochemically stable iodoantipyrine and on the distribution space and metabolism in man of this substance may be worth while. In-vitro instability has been shown in some presently available products,²⁵ and in-vivo instability has been shown in some species,²⁶ although this effect was found to be small in man.²⁷ Should iodoantipyrine be suitable for T.B.W. measurement, a stable product labelled with ^{125}I or ^{123}I would be desirable for use with ^{24}Na , ^{43}K , and ^{77}Br , since the gamma energy of either isotope would permit the estimation of T.B.W. with Na_E , K_E , and E.C.F.V. by counting untreated plasma and urine samples on a multichannel gamma spectrometer.

Although the use of the above equations and the measurement technique developed by us²² should enable a more widespread application of the isotope dilution method, the confidence limits of the prediction are wide, and thus limit the immediate value for diagnosis. Perhaps the additional use of simple indices of body fat content such as multiple skinfold measurements would help in narrowing the confidence limits of the predicted normal range.

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Contemporary Themes

Thalassaemia in Cyprus

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Summary

Frequencies of the thalassaemias in Cyprus were examined by a survey of hospital inpatients and haematological investi-

gations of adult and newborn population samples. The data indicate that 15% of the Greek and Turkish Cypriots are carriers of beta-thalassaemia genes, while 10% of the population carry alpha-thalassaemia genes. These are the highest frequencies of thalassaemia genes found today in any Caucasian population.

Introduction

Population genetic studies in areas where thalassaemias are common in addition to their theoretical interest are of much practical importance. In planning management facilities a consideration of the types and frequencies of the various thalassaemia genes in the population is necessary because the public health problems created by the various forms of thalassaemia differ. Information about the frequency of thalassaemia types in a population is also instrumental to the design of preventive

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programmes. While prevention of homozygous beta-thalassaemia through population screening and counselling of the subjects at risk is theoretically possible, a similar approach for alpha-thalassaemia is not justified because of the intra-uterine death of the alpha-thalassaemia homozygotes and the inability to identify the matings at risk for offspring with Hb H disease.

The purpose of this study was to appraise the problem of thalassaemia in Cyprus, where preliminary evidence had indicated that both forms of thalassaemia, alpha and beta, were common. Cyprus is the third largest island in the Mediterranean, having 633,000 inhabitants; 77.0% of Greek origin, 18.3% Turkish, and 4.7% of various other national backgrounds.¹ There have been two studies on thalassaemia in Cyprus. One was conducted before biochemical tests for thalassaemia were available, and it indicated that the frequency of thalassaemia trait among Greek Cypriots was 28%.² Plato *et al.*,³ using a combination of haematological and biochemical tests, found that the frequency of beta-thalassaemia in Greek Cypriots was 6%. In this study we estimated the frequencies of thalassaemia in Cyprus by surveying hospital inpatients and unselected adult and newborn population samples. The data indicated that among Caucasian populations Cyprus is characterized by the highest frequencies of the alpha and beta-thalassaemia genes.

Subjects and Methods

To study the frequencies of thalassaemia traits in persons of Greek origin 326 recruits of the National Guard and 176 newborn infants were examined. Venous blood samples from the adults and umbilical cord samples from the infants were collected in tubes containing edetic acid or citrate dextrose anticoagulant and shipped under refrigeration to Athens. Thalassaemia subtyping was done with procedures already described.⁴ Haemoglobin was measured as cyanmethaemoglobin and red blood cells were counted using an electronic counter. Haemoglobin electrophoresis was done on paper at pH 8.2 using a barbitone buffer and on starch gels using a TEB buffer at pH 8.6 and a phosphate buffer at pH 7.0. Hb A₂ was quantitated with starch block electrophoresis using a barbitone buffer at pH 8.6, while Hb Bart's was quantitated with starch block electrophoresis using a phosphate buffer at pH 7.0. Fetal haemoglobin was measured as described by Betke *et al.*⁵

To estimate frequencies of beta-thalassaemia on the basis of hospital admissions the records of all inpatients for the period 1960-71 in five State hospitals (Nicosia, Limassol, Famagusta, Larnaka, and Paphos) were examined and patients with a diagnosis of Cooley's anaemia ascertained. Frequencies of births of infants with Cooley's anaemia each year were calculated using the estimated yearly number of births in the Greek and Turkish communities.

Results

Adult Greeks.—Of the 326 recruits 49 were considered to be carriers of beta-thalassaemia on the basis of a mean corpuscular haemoglobin concentration, red cell morphological abnormalities, and a rise in Hb A₂ and occasionally also of Hb F. Thus in this population sample the frequency of the high A₂ type of beta-thalassaemia was 15.0%. Two recruits with mild anaemia had Hb H inclusions in properly incubated red cells⁶ and small amounts (3 to 5%) of Hb H on electrophoresis and were considered to have Hb H disease. Samples from 14 recruits showed morphological abnormalities in red blood cells and depression of the mean corpuscular haemoglobin concentration but normal levels of fetal and A₂ haemoglobins. Since, these subjects were not investigated further it is not known whether the observed abnormalities were due to heterozygosity for

alpha-thalassaemia or beta-delta-thalassaemia of the low A₂, low F variety⁷ or due to iron deficiency. The findings in newborn infants, however, (see below) make the first possibility more likely. Finally, in this population sample there was one subject to sickle-cell trait and a second who had small quantities of a haemoglobin with slow electrophoretic migration.

Greek Newborn Infants.—Of the 176 newborn infants 21 had raised levels of Hb Bart's. From inspection of the electrophoretic preparations these infants fell into two groups, one (10 infants) with about 1% Hb Bart's, and the second (11 infants) with 4 to 6% Hb Bart's. Hb Bart's was measured in six of the first group of infants and ranged from 1.0 to 1.5% (mean 1.2%). In the 11 infants in the second group the range was 3.2 to 5.7% (mean 4.9%). If the infants with Hb Bart's near the level of 1% were carriers of alpha-thalassaemia-2 and those with Hb Bart's between 3 and 6% were carriers of alpha-thalassaemia-1⁸ the probable frequencies of these two forms of thalassaemia in Greek Cypriots are: alpha-thalassaemia-1 trait 6.3%; alpha-thalassaemia-2 trait 5.7%. These are the highest frequencies of alpha-thalassaemia found to date in any Caucasian population.

HOSPITAL SURVEY

Calculations of the frequencies of Cooley's anaemia on the basis of hospital admissions are subject to biases which lead to considerable underestimates. Patients whose parents decide against treatment of the fatal disease in their child will most likely not be found in such records; cases of thalassaemia intermedia are not likely to be ascertained by a survey of hospital inpatients; management of a large proportion of patients in private hospitals or by physicians in private practice will cause significant error. In spite of these shortcomings the survey of hospital admissions provided information substantiating the frequencies derived from the study of the unselected population sample.

Cooley's Anaemia in Greeks

Of the patients with Cooley's anaemia born between 1960 and 1970 394 were admitted to the five State hospitals at least once. During the same years 121,278 births occurred in the Greek population. Thus on the basis of these hospital admissions the *minimum* frequency of Cooley's anaemia in the Greek population of Cyprus is 3.3 per thousand births. Detailed findings for the period 1960 to 1970 are presented in table I. The decline in the frequency of Cooley's anaemia after 1966 most probably reflects the lower ascertainment of the youngest

TABLE I—Frequency of Cooley's Anaemia among Greek Cypriot Livebirths*

Year	Estimated No. of Births	No. of Cases of Cooley's Anaemia
1960	11,179	42
1961	11,514	30
1962	11,179	40
1963	11,179	52
1964	10,948	45
Total	55,999	209† (0.373%)
1965	11,156	37
1966	11,102	33
1967	10,948	26
1968	10,871	34
1969	10,794	29
1970	10,408	26
Total 1960-70	121,278	394‡ (0.325%)

*Number of patients with Cooley's anaemia born each year was derived from in-patient records of five State hospitals.

†Frequency of heterozygotes for beta-thalassaemia 11.4%.

‡Frequency of heterozygotes for beta-thalassaemia 10.8%.

affected patients, several of whom are admitted to hospital only after the second or third year of life. Thus ascertainment of patients with Cooley's anaemia through hospital admissions appears more complete in the earliest years of the survey; for the years 1960 to 1964 the incidence was 3.7 patients per thousand births. By comparing the hospital admission data with the records of the Central Nicosia Laboratory and those of blood banks we concluded that 30 to 35% of cases do not appear in the State hospital records. If a correction for this is applied to the hospital admission data, the probable frequencies of homozygotes for Cooley's anaemia becomes 4.9 per thousand births and the frequency of beta-thalassaemia trait 13.0%.

Cooley's Anaemia in Turks

Owing to the urban concentration of the Turkish population, as well as other socioeconomic factors, estimates of the frequency of thalassaemia using State hospital admissions are considerably more accurate for the Turkish than for the Greek population. For political reasons, however, admissions of Turks to these hospitals have declined in recent years. Data for the years 1960 to 1963 are given in table II, and they show that among the Turkish population the *minimum* frequency of Cooley's anaemia is 5.5 per thousand neonates and the frequency of beta-thalassaemia trait 13.8%.

TABLE II—Frequency of Cooley's Anaemia in Turkish Cypriots*

Year of Birth	Estimated No. of Births	No. of Cases of Cooley's Anaemia
1960	2,639	17
1961	2,718	19
1962	2,639	11
1963	2,639	12
Total	10,635	59† (0.55%)

*Data based on inpatient records of five State hospitals.

†Frequency of heterozygotes for beta-thalassaemia 13.7%.

Load on the Public Health System

As in most other chronic genetic diseases the impact of thalassaemia on the health resources of a nation is quite disproportional to the number of patients. Under conditions of optimal medical care a patient with Cooley's anaemia will require a minimum of 12 outpatient visits a year; an equal number of transfusion sessions, 12 to 24 units of blood, antibiotic and other pharmaceutical support, and, whenever chelating agents are administered in spite of the absence of convincing evidence for their efficacy, up to 200 g of highly expensive drugs. In addition the patient will require periods of hospitalization for aplastic crises, other complications of the disease, infections, fractures, splenectomy, etc. Calculation of the actual cost of thalassaemia to a nation requires that these factors in addition to the loss of earning power of the patients and effects on the productivity of their parents should be considered. Such calculations could not be done with the limited data collected in our survey. Nevertheless, analysis of the hospital admissions illustrates how sharply the public health load from a chronic genetic disease is increased when an improved but not "optimal" form of management of these patients is introduced. Admissions for Cooley's anaemia in the paediatric wards of the five State hospitals showed a sharp increase after 1968 (fig. 1); admissions during 1971 were almost 400% higher than 1966. As is shown in fig. 2, this increase was not due to more efficient diagnosis of Cooley's anaemia, for in the recent years admissions increased drastically while the number of new patients diagnosed each year remained constant. The reasons for this "epidemic" of admissions are

FIG. 1—Number of cases of Cooley's anaemia admitted to five State hospitals in Cyprus. Note sharp increase after 1968.

FIG. 2—Number of admission to Famagusta General Hospital compared with number of new cases diagnosed each year. Between 1960 and 1970 number of new cases yearly remained constant.

related to the changing approaches in management of patients. In the past, transfusions were administered only when a patient was extremely anaemic; recently, however, multitransfusion schemes have been used in the management of Cooley's anaemia in an effort to satisfy the tissue's need of oxygen and suppress endogenous erythropoiesis.⁸ The result of the introduction of this management scheme is illustrated in table III; much of the increase in admissions reflects an increased number of transfusion sessions. Thus over the years the number of patients treated with multiple transfusions increased and the number of patients treated with occasional transfusions decreased in an almost linear fashion. Currently, however, only

TABLE III—Frequency of Hospital Admissions of Patients with Cooley's Anaemia in Decade 1960-70

Year	Percentage of Patients		
	1 to 3 Admissions	4 to 7 Admissions	More than 8 Admissions
1960	100	0	0
1961	100	0	0
1962	98.3	1.7	0
1963	84.0	16.0	0
1964	89.0	11.0	0
1965	87.0	13.0	0
1966	81.3	17.0	1.7
1967	78.3	16.9	4.8
1968	64.9	29.7	5.4
1969	62.4	24.8	12.8
1970	57.0	24.8	18.2

18% of the patients attending the State hospitals are undergoing multiple transfusions. If the present trend of change in management continues, by 1975 the number of admissions for Cooley's anaemia will be at least three times that of 1970—that is, approximately 2,100. This will produce a severe strain on the delivery of paediatric care and the blood resources of the island. At present, with 18% of the patients in a multi-transfusion programme, almost 20% of blood donated to the Cyprus blood banks is used for the treatment of Cooley's anaemia.

Discussion

These data indicate that the thalassaemias constitute a major health problem for Cyprus. Among Greeks the most probable frequency of beta-thalassaemia trait is 15% and among Turks 14%. One in every 44 couples risks having a child with homozygous thalassaemia; one out of 166 newborn infants has Cooley's disease. With the exception of some restricted areas in Greece and Italy, Cyprus is characterized by one of the highest frequencies of beta-thalassaemia among Caucasian populations. Similar frequencies were obtained by Model *et al.*⁹ on the basis of a hospital survey of Cooley's homozygotes for Cooley's anaemia in London and by Kattamis *et al.*¹⁰ in a survey of Cypriot army recruits.

This island population is also exceptional for its high frequencies of alpha-thalassaemia. The presence of alpha-thalassaemia in Cyprus was suspected from the finding of the only known Caucasian case of Hb Bart's hydrops fetalis in the offspring of a Cypriot couple¹¹ and the detection of several patients with Hb H disease in our laboratory in Nicosia. The findings of the survey on neonates showed that up to 10% of the island population may carry alpha-thalassaemia genes. It is, however, difficult to use the data on newborn infants to calculate expected frequencies of the homozygous or doubly heterozygous alpha-thalassaemia forms for the reasons discussed below.

Recently evidence has been presented for¹²⁻¹⁴ as well as against^{15,16} the duplication of alpha-haemoglobin loci in man. The possibility that redundant alpha-haemoglobin loci may exist only in a proportion of populations has also been raised.^{15,17} Various genetic hypotheses have been proposed to accommodate the phenotypes of alpha-thalassaemias¹⁷⁻²⁰ using models involving single or duplicated alpha loci. If in a population the alpha-haemoglobin loci are duplicated, the frequency of the symptomatic forms (Hb H disease, Hb Bart's hydrops fetalis) will depend on the relative frequency of thalassaemia at each locus. As Lehmann¹⁹ indicated, it is possible, with a duplicate loci hypothesis, for thalassaemia trait to be quite frequent in a population in the absence of symptomatic forms if only one of the two alpha-chain loci is affected.

In addition to this possibility limitations on calculation of the frequencies of Hb H disease and Hb Bart's hydrops fetalis on the basis of the frequencies of alpha-thalassaemia heterozygotes are imposed for the following reason. It is quite possible that what is regarded as alpha-thalassaemia-1 or alpha-thalassaemia-2 trait is not the expression of one genetic entity each but the phenotypic outcome of the action of various thalassaemia alleles, each characterized by a different degree of alpha-chain suppression.¹⁷ If this is so, certain "homozygotes" for alpha-thalassaemia-1 will, in fact, be double heterozygotes and may not manifest with Hb Bart's hydrops fetalis but with Hb H disease. Such a possibility has already been considered in order to explain the absence of Hb Bart's hydrops fetalis in Negroes.²¹

In view of the gaps in our knowledge on alpha-thalassaemias accurate calculations of frequencies of Hb Bart's hydrops fetalis and of Hb H disease in Cyprus are impracticable; however, some rough estimates can be made with the data at hand. If one supposes that the hypothesis of one locus with multiple

thalassaemia alleles applies in the Cypriot population, the frequencies of normal alpha chain (T), severe alpha-thalassaemia (t^0), and mild alpha-thalassaemia (t^+) alleles can be calculated from the data on newborn infants as $T=0.937$, $t^0=0.033$, and $t^+=0.030$. The expected frequency of homozygotes t^0/t^0 is 0.00109, while the frequency of double heterozygotes t^+/t^0 is 0.00198. If homozygosity for alpha-thalassaemia-1 were to express as Hb Bart's hydrops fetalis, 10 to 15 infants with this condition would be born in Cyprus each year. There is no indication that this is correct, but a study of blood samples from stillborn infants on the island may provide an answer to that inquiry. If double heterozygosity t^0/t^+ were to express as Hb H disease, this condition would present in one in 500 births. It seems that Hb H disease is at least as common as that: two cases were found among the 326 unselected recruits (table I). Since Hb H disease most probably does not affect life expectancy, 1,500 to 2,500 patients with Hb H disease may exist in the Cypriot population.

This small Mediterranean nation is thus burdened with one of the highest frequencies of thalassaemia genes in man. The local public health service has to face the problems of a severe disease whose management is completely inadequate, whose cure is not in sight, and whose prenatal diagnosis is unavailable. What could the solution of the problem of thalassaemia in a nation like Cyprus be? Present trends suggest establishment of preventive measures aimed at reduction of the frequency of homozygotes for Cooley's anaemia through discouragement of matings between heterozygous carriers. The question, however, is whether prevention of genetic disease through testing, education, and counselling is effective. While it is assumed that in the setting of established genetic clinics genetic counselling is effective,²² it is completely unknown whether prospective genetic counselling applied at the level of populations has beneficial effects. Data showing the effectiveness of population screening and genetic counselling are required before preventive genetic programmes are established on a "service basis," particularly in nations with restricted financial and health resources.

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Medical History

Some Discoveries on Myasthenia Gravis: The Background

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The experiments on two very severe cases of myasthenia gravis, showing that a curarizing substance is formed in the muscles themselves and enters the circulation, have not been published in full though they have been demonstrated before medical meetings many times and have been confirmed, though not by all observers. I think the difficulty is that the less severe the myasthenia the less the increase of weakness (sometimes only ptosis) and the longer it takes to come on—20 minutes or more. If the patients are fully under the influence of Prostigmin (neostigmine) or some such drug it does not come on at all.

The experiments show that increased destruction, defective production, or release of acetylcholine cannot be the immediate cause of myasthenia gravis but that something produced in the muscles themselves enters the circulation, causing the abnormal fatigability. Anything which delays the destruction or facilitates the release of acetylcholine would be antagonistic to this curarizing substance.

This experiment on two patients with approximately equally severe generalized myasthenia gravis was performed many times before it was shown at the clinical meeting of the Royal Society of Medicine, section of neurology, at the National Hospital, Queen Square, London, on 17 February 1938. Later in that year it was shown at the meetings of two other medical societies. It has been frequently repeated to show the effect to various doctors. The result was always the same (see Appendix).

Without Prostigmin neither patient could get out of bed or dress and neither could supinate the forearms except for a few feeble movements. Both had an injection of Prostigmin 2.5 or 3 mg with atropine $\frac{1}{2}$ grain (32 mg) and ephedrine $\frac{1}{2}$ grain at 8 a.m. each day. When the effect of Prostigmin was just beginning to wear off, though they were still strong, the circulation was cut off in both arms by sphygmomanometer armlets at a pressure of 200 mm Hg and the patients briskly pronated and supinated their forearms till they tired, which took about a minute.

While the circulation was cut off no weakness was noted in the rest of the body, not even in the eyelids, but a minute and a half after the pressure was released the eyelids began to droop, and by two minutes the weakness was very great, so that the patients

were about as weak as they had been before their morning injection of Prostigmin and had to have another injection of 2.5 or 3 mg, which restored their strength in the usual way. As a rule the pressure was released immediately after the pronation and supination had stopped, but it has been maintained for periods up to four minutes. The weakness always came on a minute and a half after the pressure was released and appeared always to be equally severe.

When one arm only was fatigued by pronation and supination with the circulation cut off, and when both arms were exercised in the same way but short of fatigue with the circulation cut off, the ensuing weakness was much less. Cutting off the circulation with the armlets in position for several minutes without exercise caused some weakness but very much less than with exercise, and it passed off with rest for a short time without Prostigmin.

All these experiments were done when the effect of Prostigmin was just beginning to wear off. When the patients were fully under its influence pronation and supination of both forearms for about a minute with the circulation cut off produced no obvious effect, though the patients thought that they felt a little weaker. In a normal subject no weakness was apparent after brisk pronation and supination for a minute with the circulation cut off. Performing the exercise with the venous but not the arterial circulation cut off was too distressing to continue till the muscles were fatigued but some weakness did come on.

Dr. F. Parkes Weber mentioned a man aged 31, an inpatient in the German Hospital in February 1938, with "myasthenia" symptoms of a few months' duration in neck muscles. "Prostigmin seems to act wonderfully but there is considerable wasting of some muscles. Diagnosis rests between myasthenia gravis, progressive spinal muscular atrophy with misleading symptoms, and atypical dystrophica myotonica (though no definite myotonia). We tried your arm constricting method this morning as you showed us but got no result whatsoever. Can this be regarded as a negative diagnostic test for myasthenia gravis? . . . It is noteworthy that the patient tells us that his sister died with similar symptoms to his, and we have heard from a hospital that the diagnosis on her case was progressive muscular atrophy with some wasting of one half of the tongue."

Comment

Dr. J. PURDON MARTIN, honorary consultant at the National Hospital for Nervous Diseases, London, writes: Dr. Mary Walker's article relating to some of her work 35 years ago perhaps requires a short introductory note.

In days when clinical physiology was still a new subject and almost unknown outside the medical schools and special hospitals Mary

Wigtown

MARY B. WALKER, M.D., M.R.C.P., Late Senior Hospital Medical Officer at St. Benedict's Hospital, London