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RESEARCHES ON THALASSEMIA AND MALARIA IN ITALY AND THE ORIGINS OF THE "HALDANE HYPOTHESIS"

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SUMMARY

This paper aims to shed light on Italian contribution to the origins of malaria hypothesis, also known as Haldane hypothesis. The first studies on the association between hemoglobinopathies and malaria, in fact, were done in Italy since the end of the 1920s. These studies tried to explain the correlation between malaria and thalassemia observed by clinicians in various Italian regions. Later, since the beginning of the 1940s, this singular correlation was documented by thorough and wide-ranging epidemiological researches that revealed a strong geographic correspondence between the frequency of the thalassemic features and endemic malaria in Italy. These researches raised clearly the question of maintaining the frequency of a gene that, at the time, doomed homozygotes to death within the first two years of life. In 1948, Silvestroni, Bianco and Montalenti started investigating the causes of the persistence of the thalassemic foci in Italy. In 1949 J.B.S. Haldane finally hypothised for the first time an evolutionary advantage of thalassemic condition due to the concomitant presence of malarial infection. Since 1948, Montalenti and Haldane had various occasions to discuss on this topic. I try to demonstrate the role of Silvestroni, Bianco and Montalenti's research and data on the formation of Haldane hyphothesis.

Introduction

In 1954, Anthony Allison's research on sickle cell anemia in East Africa demonstrated for the first time that a specific human disease

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can have a selective role in evolution and, therefore, can modify the genetic composition of a population by favouring certain genotypes rather than others[1].

However, the first studies on the association between hemoglobinopathies and malaria were done in Italy long before Allison's research. These studies tried to determine both the possible origin of the high frequency of thalassemia in certain malarial areas of the country, and whether its cause could be, as hypothesized by J.B.S. Haldane in 1949[2], an advantage of thalassemic condition due to the concomitant presence of malarial infection.

The story of the origin of "malaria hypothesis," also known as the "Haldane hypothesis," has been pieced together by several authors[3]. However, in these reconstructions there is no trace of the fundamental premises of the hypothesis of a causal relationship between malaria and thalassemia, from the standpoint of the epidemiological observations and researches carried out in Italy since the end of the 1920s. This historiographic void is mostly due to the fact that this research was published only in Italian, in Italian journals poorly read abroad. Incidentally, this is also the reason why the important Italian contribution to the discovery of the genetics of thalassemia is so widely ignored[4].

1. *Early observations on the association between malaria and thalassemia*

The problem of the links between Cooley's anemia and malaria was discussed in Italian medical literature since the end of the 1920s. Different studies considered the possibility that malaria was an etiological factor for thalassemia, as it was recognized that a particularly high frequency of cases of Mediterranean anemia existed in a number of malarial zones in families affected by Cooley's anemia[5]. These coincidences were confirmed by subsequent observations pointing to the presence or the frequency of the disease in malarial

zones[6], or the existence of thalassemic foci in Italy (Sicily, Sardinia, Ferrara area, Puglia) corresponding to endemic malaria zones[7].
An even more significant fact was the exact localization of Cooley's anemia in intensely malarial zones such as Sardinia, in which the frequency of Cooley's disease was very high in the malarial coastal areas and practically absent in the non malarial internal mountainous zones[8]. Working at the pediatric clinic of Cagliari University, Cadeddu collected a large number of case histories from all over Sardinia and systematically analysed all the relevant environmental variables:

> *in the entire mountain area of the island* – he wrote – *in which malaria occurs sporadically there is an absence of observations except for one observation from Macomer but due to the family community moving there from a malarial zone. [...] The towns from which our cases have been drawn almost all have an altitude of less than 100 m. above sea level*[9].

The hypotheses about the relationship between malaria and thalassemia put forward by clinical practitioners who had observed the geographic links between them remained restricted to the medical domain, that is, at the level of pathogenetic explanation. Physicians had too little knowledge of genetics and evolutionary biology to allow those working in a clinical environment to elaborate interpretations capable of focussing on the links between a population's gene pool and selective pressures.
Furthermore, the hypothesis of a direct etiology regarding the explanation of the links between malaria and thalassemia was quite weak, though the most widespread at that time. It had to come to terms with a series of other anomalies pointed out by Gino Frontali and F. Rasi working at the pediatrics clinic of Padua University[10]: 1) the absence of thalassemia in populations with a high incidence of malaria; 2) conversely, the existence of cases of Mediterranean anemia unrelated to any malarial influence; 3) the inefficacy of antimalarial

therapy observed by many clinicians[11]. Marino Ortolani, director of the Ferrara Provincial Institute for Childhood pointed out that the hypothesis of direct malarial etiology was based on a body of case histories collected through a systematic diagnostic error that led to a series of obvious cases of malaria being listed as Cooley's anemia and for this reason apparently sensitive to quinine therapy[12].

In 1929 Giovanni Careddu suggested an interesting indirect action by malaria on the germ cells of the parents that was capable of leading to an alteration of the hemopoietic and osteogenetic mesenchyma[13]. Federico Vecchio, at the pediatric clinic of Naples University, in 1947 made explicit reference to germ plasma mutation, referring to Hermann Muller's experiments with radiations and the recent (at the time) demonstration that thermal shock seemed capable of producing genetic mutations[14]. It was postulated that gene variation indeed emerged as a consequence of "a direct action of malarial parasitism carried out by the repetition, especially in the pre-quinine era, of comparatively violent thermal shocks[15]". In the light of the growing evidence that thalassemia was not exclusively a Mediterranean disease, but one of the most widespread hereditary diseases in the world, Vecchio claimed that the cause should be sought in the exposure "of several ethnic groups to given environmental factors"[16], rather than in a given genotypic constitution of the populations affected.

Some explanations relied on a strong lamarckian point of view. In 1939, Michele Bufano actually considered the link between malaria and thalassemia a case of inheritance of acquired characters. He postulated that malaria produced a series of pathological transformations of the bone marrow capable of being transmitted to the offspring and giving rise to the clinical symptoms of Cooley's anemia. On the other hand, in a long discussion in *Clinica Pediatrica*, Renato Pachioli, a lecturer at Bologna University, suggested viewing Cooley's anemia

also as a hereditary transmissible malarial hemodystrophy originating from a mutation somehow induced by malaria[17].
Pachioli claimed that this hypothesis would allow a single interpretative model to be used to explain the apparently hereditary nature of Mediterranean anemia and the singular similarity between several fundamental parts of the pathogenetic processes in malaria and in Cooley's anemia, in particular the alterations of erythropoiesis. The explanatory hypothesis was thus constructed by linking together several early speculations on the genetic determinisms of Cooley's anemia with a series of pathological data. On the one hand, there was the idea put forward by Heinrich Lehndorff in 1936 that Cooley's anemia is the effect of a genetic mutation following which the erythroblastic system becomes incapable of producing mature red corpuscles[18]. On the other, the (incorrect) observations made above all by Virgilio Chini, seemed to show that malarial infection electively damaged the hemopoietic system and that this was somehow transmitted to the offspring, thus determining bone lesions that could be likened to the pathognomonic lesions of Cooley's anemia. This idea however allowed Pachioli to claim that the eradication of malaria would lead to a gradual reduction in the frequency of the thalassemic gene:

> *the causal relations, although indirect, between malaria and Cooley's anemia [...] allow it to be envisaged that the rehabilitation of malaria-infested zones can, in the course of generations, lead to the gradual exhaustion of this morbid hereditary defect*[19].

Although in a completely nebulous and speculative explanatory framework, Cesare Cocchi put forward the hypothesis that Cooley's anemia, just like favism, represented a defensive process typical of subjects in populations that had been exposed at length to malaria[20]. The idea was that

> *Cooley's anemia, with its clinical and pathological features, can manifest itself only in subjects prepared by a broad malarial inheritance in the sense that it represents a particular reaction of enhanced defence (instead of increased vulnerability). What I mean is that it is possible to imagine that the same morbid cause (toxic, infectious or due to deficiency) determines this disease, in that particular way, only in subjects that have malarial forebears, and are thus better protected (we know they are better protected against malaria itself than a subject who is not a descendant of malarial patients), better prepared to defend themselves against all hemolizing action*[21].

On the basis of Cocchi's hypothesis, in 1943 Marino Ortolani carried out research

> *on the immune state regarding malarial infection in subjects, some of whom present the classic Cooley type anemia symptoms and others affected by erythroleukemic myelosis with or without hyperhemolysis*[22].

Ortolani repeatedly tried to graft the "plasmodium vivax" into some patients by inoculating them with blood taken from soldiers from the Greek-Albanian front suffering from malaria. Without stating the number of cases precisely, he reported having failed to infect the subjects involved in the research. Malarial patients lacked the clinical signs of malaria, while the search for parasites in the blood was always negative, even after spleen contraction as well as the search via medullary and spleen puncture. The only exception was a 10 year old girl who, after being subjected to a cycle of 4 inoculations over four months of blood that was particularly rich in parasites drawn from four malaria patients, developed malaria two years later, displaying clinical symptoms and showing the presence of the benign tertiary plasmodium. The author also reported the case of a baby with Cooley's anemia who died about two months after being inoculated with infected blood and who tested negative for the plasmodium even on autopsy via bone marrow examination[23].

In his comment on these results Ortolani explicitly cited the passage from Cocchi's work mentioned above, then claiming that his study seemed to support the hypothesis that Cooley's anemia ought to be considered as a hereditary form of defence against malaria that developed through long exposure of certain populations to the infection[24]. Although unsystematic and ethically dubious, Ortolani's experiment takes on an exceptional historical value as it preceded by ten years Allison's work demonstrating the greater resistance of sickle cell anemia patients to malarial infection[25]. For reasons that we shall try to understand in the following, Ortolani's experiment was not replicated even in Italy.

The absence of any consistent classification of the pathogenetic mechanisms of thalassemia as a function of the etiology and pathogenesis of malarial infection materially stood in the way of any emerging hypothesis that malaria could represent a selective factor capable of favouring a mutation of the erythropoietic system. Moreover, there was no exact overlapping of the malarial zones with the zones with a high frequency of Cooley's anemia or the microcythemic trait. Reported exceptions were the Rome rural area and the Tuscan Maremma, which for centuries had been intensely malarial but characterized by a low incidence of Mediterranean anemia and microcythemia. In his introduction to the course of medical pathology and clinical methodology at Bari University, Virgilio Chini[26], one of the greatest Italian experts in Mediterranean anemia of the time, postulated that the absence of any link between the distribution of malaria and that of Cooley's anemia could be accounted for by biological difference among the types of malaria distributed through the various different geographic regions. The same year, Chini reported certain similarities in the radiological examinations of 40 of his malaria patients with those observed in Cooley's anemia[27].

In 1941, Franco Toscano reported a geographic correspondence between malaria and Rietti-Greppi-Micheli disease, or thalassemia

intermedia, postulating a link with a single etiopathogenesis deriving from an ancestral malaria[28].
Again with reference to the malarial hypothesis, two obvious theoretical anomalies were pointed out: the relatively small number of Cooley's patients also in the more intensely malarial zones and the fact that not all the offspring of chronic malarial patients were affected by Mediterranean anemia. Still, some researchers, such as Paradiso[29], believed that the fact that Cooley's anemia was found in non malarial zones did not rule out the possibility that malaria might be part of the more or less remote ancestry of these patients.

2. *The Italian contribution to the knowledge of thalassemia genetics and epidemiology*

Halfway through the 1940s, Italian and US physicians independently demonstrated the mechanism of inheritance of thalassemia. In 1943, Ezio Silvestroni and Ida Bianco, at the time working at the Istituto di Clinica Medica of the University of Rome, described an inborn and hereditary hematological anomaly in healthy people that they subsequently called microcythemia[30]. At the same time, Silvestroni and Bianco showed the genetic relationship between thalassemia and microcythemia, studying several people with Cooley's anemia[31]. At the end of these investigations, Silvestroni and Bianco documented the Mendelian inheritance of microcythemia as the heterozygous condition, and the homozygous condition in Cooley's disease[32]. These results confirmed the evidence obtained in similar studies conducted in USA by Dameshek[33], and especially by William Valentine and James Neel at the University of Rochester[34]. Literature on the history of thalassemia, with the works of David Weatherall[35] and Maxwell Wintrobe[36] in the forefront, has extensively documented the contribution made by US research, but displays only a partial knowledge of the research and debate on the genetics of Cooley's disease that developed in Italy since the 1920s[37].

Silvestroni and Bianco carried out a series of epidemiological studies all over Italy[38], using a specific method to detect the microcythemic trait through the reduction of globular fragility[39]. The results of the research, which by 1950 had involved some 50,000 persons, enabled the two to map, for the first time ever, the disease's distribution for a whole country. This distribution revealed a peculiar epidemiological profile, with numerous microcythemic foci scattered all over the country, particularly in the areas of the Po Delta, Sardinia and Sicily, where the incidence of carriers exceeded 20% of the population. The map revealed a strong geographic correspondence between the frequency of the thalassemic features and endemic malaria. This singular correlation, which had already been observed by clinicians, was now documented by thorough and wide-ranging epidemiological research, thus raising even more clearly the question of maintaining the frequency of a gene that, at the time, doomed homozygotes to death within the first two years of life.

3. *Collaboration of Silvestroni and Bianco with Montalenti and the formulation of Haldane hypothesis*

In 1947 Silvestroni and Bianco made the first arrangements in order to collaborate with the Institute of Genetics of Naples University, directed by Giuseppe Montalenti[40]. It had been the lack of appreciation and the criticisms displayed by the medical community towards the genetic aspects of their work that led the two clinical pathologists to seek the support of the most authoritative Italian specialist in the field.

Montalenti advised the two researchers to primarily investigate the causes of the persistence of the microcythemic foci, in spite of the negative selection of the thalassemic genes due to the fact that the homozygotes could not reproduce. Montalenti also suggested studying several well-known Mendelian traits in the populations investigated by the two clinicians, such as sensitivity to phenylthiocarbamide, and also to check any possible link with the microcythemic trait[41].

At the VIII International Genetics Congress held on 7 - 14 July 1948 in Stockholm, Montalenti presented a talk on distribution of thalassemia in Italy based on the epidemiological data collected by Silvestroni and Bianco[42]. In the report he reasoned about the existence of still unknown environmental factors capable to counteract the strong selective disadvantage of this hemoglobinopathy.
At the same congress, as reported by the Proceedings, John Haldane for the first time put forward the malaria hypothesis.
While discussing the problem of the mutation rate in man with reference to Neel and Valentine's studies on thalassemia – namely, the hypothesis advanced in 1947 by the two US geneticists that in order to maintain such a high frequency the mutation rate should have been 1/2,500, and that this rate might have an ethnic basis - Haldane considered the rate to be too high, and briefly stated that "the possibility that the heterozygote is fitter than the normal must be seriously considered" and that

> *it is at least conceivable that they are also more resistant to attacks by the sporozoa which cause malaria, a disease prevalent in Italy, Sicily and Greece, where the gene is frequent*[43].

The Haldane hypothesis stood out as an obvious alternative once it could be ruled out that polymorphism was due to a 'special' mutation rate.
Yet, some evidences cast some doubts as to whether Haldane got the idea before the Stockholm congress or while attending it.
Just a couple of weeks after Stockholm congress, on July 31st - august 2nd, Haldane met Montalenti at Pallanza, near Milan, on the occasion of a Symposium on the ecological and genetic factors of animal speciation, organized by Adriano Buzzati Traverso in the biophysics section he directed at the Physiopathology Study Centre of the National Research Council. In this forum Haldane presented a report on the links between disease and evolution, which mostly

hinged on the role of diseases and infectious agents as factors of selection and thus of evolutionary change[44].
In the discussion that followed, Montalenti pointed to Haldane the case of thalassemia, studied by Silvestroni and Bianco. In this case, Montalenti said: "a lethal gene in homozygotic condition is so diffuse in heterozygous state in several Italian regions that we have to admit it confers an advantage for the carriers[45]".
Montalenti then added an important thing for the reconstruction of the origin of malaria hypothesis:

> *Since some researches seem to demonstrate that this gene is more diffuse in malarial zones, Haldane suggested in a verbal communication that microcythemics could be more resistant to malaria infection*[46].

Haldane agreed with Montalenti, adding that the advantage of the microcythemic heterozygote could also derive from an enhanced capacity for iron absorption in populations with dietary deficit of this element.
This Haldane's report was published by the Italian review *La Ricerca Scientifica*, and titled "Disease and Evolution". It is the most frequently cited as the one where the malaria hypothesis was first formulated, even though in that context it was actually Montalenti and not Haldane to first suggest the link between thalassemia and malaria.
Joshua Lederberg later noted that in this paper Haldane did not refer directly to the relationship between thalassemia and malaria and that a generic role of evolution in modifying genetic resistance to infectious disease in crops, such as wheat, was long known before Haldane[47].
On the basis of the reading of *Disease and evolution*, Allison gave credit to Montalenti for suggesting the malaria hypothesis to Haldane[48], but Krishna Dronamraju have recently ruled out this possibility using two arguments[49]: first, "it was Montalenti himself

who stated that Haldane communicated the idea to him"; second: "Haldane had already discussed his hypothesis at the International Genetics Congress in Stockholm in 1948, *long before* the Milan symposium[50]" (emphasis added).
Weatherall had already used this second argument in 2004, in a book edited by Dronamraju himself[51].
On the contrary, the lapse of time between Stockholm and Pallanza is very short (just two weeks), and this could be very important to understand the origins of malaria hypothesis. In fact, Dronamraju and Weatherall's thesis is not documented, in the sense that it must in any case be demonstrated that the address published in the proceedings of the Stockholm Congress corresponds to the one publicly delivered at the Congress.
Some doubts as to whether Haldane got the idea before Pallanza seem to emerge from the fact that in the article *Disease and Evolution* he does not propose the example. Why? If he had already presented it in Stockholm, where Montalenti was also present, why did he not talk about it again in Italy? And why Montalenti had to remind him of it? However, Montalenti himself recognized that Haldane communicated malaria hypothesis to him. In their correspondence found in the Montalenti Archive there is no clear trace of this idea[52]. Montalenti wrote to Haldane in 1948 about a paper that Montalenti, Silvestroni and Bianco were writing in that period and that appeared in *Nature* in 1950[53] with acknowledgements to Haldane for a scheme calculation. The paper suggested that mating between thalassemia heterozygotes produced more children than did other mating. Lederberg also revealed that, in a letter found by Dronamraju, Haldane added some extensive (and almost illegible) algebra, showing that the fertility of this mating would have to be at least twice that of the others to yield a stable equilibrium, contrary to the data[54]. But malaria is not mentioned in any of these and the positive selective value of the heterozygous condition was found inconsistent with data about the

average age of death of microcythemics in comparison with non-microcythemic individuals.
Consequently, Haldane should have suggested this only by word of mouth, presumably at Stockholm or at Pallanza during the summer of 1948.
Could it not be that, after a discussion with Montalenti, the Italian having the most extensive data about the malaria hypothesis at the time, Haldane modified the written text of his address to the Stockholm Congress, including the hypothesis of heterozygous advantage vis-à-vis malarial infection? The question may be solved only by documentary evidence concerning what Haldane actually said in his report to the Stockholm Congress.
Anyway, it seems certain that Haldane, and not Montalenti, was the first to recognize the right perspective to frame the correlation between thalassemia and malaria diffusion in Italy. Years after the first formulation of malaria hypothesis, Montalenti continued to express doubts regarding the idea of a possible heterozygous advantage, even on the basis of the strong evidence from the more and more extensive Silvestroni and Bianco's researches[55].
In 1953, at Bellagio, on the occasion of the IX International Genetics Congress, in presenting a long report on microcythemia genetics based on Silvestroni and Bianco's research, Montalenti continued to express doubts regarding the idea of a possible heterozygote advantage[56]. The first reason was the partial overlap in Italy of the epidemiological map of microcythemia and malaria distribution: a point, as we have already seen, considered central by clinicians in order to refute the hypothesis of possible links between malaria and Cooley's anemia. Another point against the hypothesis was the total absence of any causal explanation linking the microcythemic gene to the increased resistance to malarial infection. Insights of the possible heterozygote advantage in this sense could be provided at the level of macroscopic functions. Yet, it was practically impossible to identify any peculiar traits of the

heterozygote that may yield a selective advantage. Furthermore, this opinion was shared at the time by Neel who wrote that:

> *in our own experience (Valentine and Neel, 1948) individuals with thalassemia minor have averaged two grams of hemoglobin less than normal persons. While there is undoubtedly a large margin of safety in normal hematological physiology, it is difficult to see how such a departure from the norm can per se be of adaptive value to the organism. The possibility remains that the hematological trait is linked to some yet unrecognised characteristic of distinct value*[57].

Conclusions

As soon as J.B.S. Haldane put forward the hypothesis that the distribution of thalassemia in Italy was the result of a thalassemic heterozygous advantage, Montalenti started and coordinated a wide, long lasting and complex research programme, even thanks to funding from the Rockefeller Foundation. The directions he chose and the inferences he drew from the development of that programme from Fifties to the Sixties demonstrated that he was looking for the definitive proof to believe in malaria hypothesis, quantifying the many variables involved, so that the advantage of the thalassemic traits carriers and the exact dimensions of this balanced polymorphism could be determined. Several theoretical and methodological factors made this objective impossible to achieve at the time[58].

The results of that programme can not be considered as definitive proof that the thalassemic trait provides protection from malaria, above all because of the epidemiologically and genetically complex signs displayed by thalassemia. Nevertheless, by means of this research a huge quantity of data was obtained, collected and analysed, and the cognitive and politico-cultural presuppositions were created for the anti-thalassemia campaign launched in Italy in the second half of the 1950s: the first national anti-thalassemia campaign ever realized in the world[59].

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Acknowledgements

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