Table 1. Arm lengths of the chromosomes numbered 1, 2, 3, 4, and 5 in Fig. 2.

Chromosome number	Long arm (cm)	Short arm (cm)	Arm ratio*
1	0.85	0.48	1.77
1	.83	.54	1.54
1	.77	.46	1.67
2	.61	.35	1.75
2	.60	.32	1.88
2	.61	.32	1.91
3	.55		
3	.55		
3	.55		
4	.40	.15	2.66
4	.36	.11	3.28
4	.39	.12	3.26
5Z	.24	.24	1.00
5Z	.28	.26	1.08
5Z	.25	.24	1.04

* Arm ratio = long ÷ short.

In all, 30 well-spread metaphase and prometaphase figures were counted. Eleven, or 36.7 percent, of the metaphases were diploid, with a ZZ constitution. Nineteen, or 63.3 percent, were triploid, with a ZZZ constitution. A metaphase showing the 3A-ZZZ chromosome constitution is shown in Fig. 1, and a reproduction of the metaphase, in which the largest chromosomes are identified by number, is shown in Fig. 2. Measurements of arm lengths for the chromosomes numbered 1, 2, 3, 4, and 5 in Fig. 2 are given in Table 1. The measurements were made from Fig. 1.

Ohno (*3*) demonstrated that triploidy can be compatible with viable postnatal life in the chicken. In the present study, the triploid (3A-ZZZ)–diploid (2A-ZZ) condition was found in an embryo exhibiting retarded development and little mitotic activity. It may be suggested that chromosome number deviations of the type reported here for the chicken may be one of several possible factors causing embryonic mortality. However, before such a relationship may be accepted, additional cases should be found.

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Porotic Hyperostosis, Anemias, Malarias, and Marshes in the Prehistoric Eastern Mediterranean

Abstract. *Porotic hyperostosis, formerly called osteoporosis symmetrica, is an overgrowth of the spongy marrow space of the skull. In children, other bones may also be affected. The disease is a consequence of one of the thalassemias or sicklemia. These anemias are balanced polymorphisms which are apparently maintained by falciparum malaria. Falciparum malaria spread over the anopheline belts of the Old World in coincidence with porotic hyperostosis, but did not penetrate the New World. Here some other parasitism or deficiency anemia must have been the cause of porotic hyperostosis in ancient times. In Anatolia, Greece, and Cyprus from the seventh to second millennia B.C., porotic hyperostosis occurred frequently in early farmers who lived in marshy areas, but rarely in inhabitants of dry or rocky areas or in latest Paleolithic hunters. As shown by skeletal samples from Greece, the frequency of the disease decreased as farming methods improved. However, from Hellenistic to Romantic times it again increased together with increases in the incidence of malaria and in poorer farming. There are correlations between porotic hyperostosis and adult stature and fertility. The mutations producing falciparum malaria therefore must antedate seventh millenium B.C. and I think may have an Eastern Mediterranean origin.*

Skulls of the earliest farmers from Western Anatolia [6500 B.C. at Çatal Hüyük (*1*)] and from Macedonia [6000 B.C. at Nea Nikomedeia (*2*)] often have extraordinary thickening of the diploë together with thinning of the outer layer of compact bone. In ten skulls which have medium to pronounced porotic hyperostosis, the parietal boss area averages 12 mm in thickness; this is about 4 to 5 mm thicker than the normal bone. Since the inner and outer layers of compact bone make up only about one-quarter of the thickness of the skull vault, the diploic thickening must reflect a red cell production by the hyperostotic bone almost double that of normal diploë. Skulls with a slight degree of porotic hyperostosis have less thickening of the diploë, but the outer table is always irregular and somewhat porous. Apparently the disease in these bones is vascular in origin and affects either the outer table ["symmetrical osteoporosis" (*3*)] or the orbit roofs ["cribra orbitalia" (*4*)], or usually both the vault and the orbits. Porotic hyperostosis of the skulls of young children and older infants often extends to sphenoid and zygomatic bones and sometimes affects the long bones. Some of the long bones from a child from Bamboula in southern Cyprus, an infant from Lerna in southeastern Greece [both Bronze Age (*5*)], and an infant from Nea Nikomedeia (Early Neolithic) have an "inner shell" of bone attached to the cortex by a few trabeculae. This finding indicates that hypertrophy of the red marrow may have prevented normal remodelling of the bone as it grew (Fig. 1).

These observations point to anemias as causes of the hyperostosis. The lesions produced by thalassemia and sicklemia (*6*) match those found in these skulls and long bones. However, the extreme ballooning of the bone marrow cavities seen in modern children hospitalized with thalassemia (*6*) is very rare in these samples because under prehistoric conditions ill children died at an earlier age. Moseley (*7*) shows that hereditary spherocytosis or iron deficiency anemia coupled with prolonged suckling, as in Bahima disease, can cause changes in the skull very similar to those found in patients with abnormal hemoglobin. Anemia caused by chronic amebiasis, severe bacillary dysentery, hookworm, any one of the malarias, or even altitude anoxia might cause identifiable bone changes.

I believe the infants and children from which the severely affected skeletons came were homozygous for one of the thalassemias or sicklemia and that the adults represent a spectrum of heterozygotes which were severely to very mildly affected by the disease. Such heterozygotes are apparently more resistant to falciparum malaria than normal people (*8*). This advantage allows a reproductive increase in the genes for abnormal hemoglobin which is balanced by the deaths of homozygotes from anemia. I know of no studies yet which show that some other chronic parasitism may also maintain these polymorphisms.

Williams (*9*) and Vogt (*10*) first suggested that "osteoporosis symmetrica" in American Indians might be Cooley's anemia (*11*). Then Chini and Valeri (*12*) and Neel (*13*) showed it probable that the "osteoporosis" in

Table 1. Porotic hyperostosis related to ecology, deaths, and stature in Mediterranean populations. The percentages of adults and juveniles who had slight (Sl.) or moderate to severe (+) hyperostosis are given, N is the total number in a sample. The environment from which the samples were taken have been classified as not marshy (−), variably marshy (±), moderately marshy (+), and severely marshy (++). The proportion of deaths in the populations are given in relation to an arbitrary figure of 10 for the adults and are given as the deaths of infants (I), children (C), and adults (A).

Place	Date (B.C.)	Percentage porotic hyperostosis						Marsh	Proportion of deaths			Total No.	Male stature estimate (cm)
		Adults			Juveniles								
		Sl.	+	N	Sl.	+	N		I	C	A		
Classic Greece	450	1	0	108	14	0	7	±	5	3+	10*	144	169+
Bamboula, S.W. Cyprus	1400	6−	0	26	0	31	13	+	(1)	3−	10	81	166?
Lerna, Argolis	1800	12	4	68	17	9	81	++	8	5	10	230	167
Corinth	2400	36	0	14	29	29	7	+	?	5	10	21	162
Karatas, Lycia	2400	9	1	82	0	0	17	±	?	4	10	160	166
Kephala, Kea	3000	7	0	28	0	11	9	−	(1)	(2)	10	53	167?
Khirokitia, S.C. Cyprus	6000	9	0	33	(33)	0	3	−	5−	2?	10	120	163?
Nea Nikomedeia, Macedonia	6000	36	32	22	40	16	23	++	5?	8	10	105	168
Çatal Hüyük, Turkey	6500	31	23	13	?	?	1	++	?	(2)	10	34	171
Taforalt, E. Morocco (*25*)	11000	2	0	41				−	6	5	10	186	174

* For example the proportion of deaths in infants : children : adults is 5 : 3+ : 10.

people from the Mediterranean and Africa is a symptom of thalassemia and sicklemia. In Greece, I showed that the frequency of "osteoporosis symmetrica" decreased during the Bronze Age to under 2 percent in the Classical period and then increased from Hellenistic to modern times (*13, 14*). Using the data of Letterer (*16*) and others, Hamperl and Weiss (*15*) redefined this condition as "spongy hyperostosis," clearly separating it from other osteoporoses, avitaminoses, effects of hemorrhage, and so forth. The name porotic hyperostosis seems to me slightly more descriptive than theirs.

I have found porotic hyperostosis in some family groups but not in other family groups from the Middle Bronze Age cemetery at Marshy Lerna (*17*) and from Late Bronze Age Bamboula (*5*). This suggests there was segregation of recessive genes for thalassemia (or sicklemia) during population mixture after intrusion of the first Greek-speaking people. I would expect this observation since these abnormal hemoglobins are balanced polymorphisms which are selectively maintained in heterozygotes by the effects of *Plasmodium falciparum* [here carried by *Anopheles saccarovi* or *A. superpictus* (*18*)] on the one hand and by juvenile deaths of both unprotected and anemic individuals on the other. If Greek-speaking people or other intruders came from areas which were relatively free from malaria, they would tend to lack the high gene frequencies for abnormal hemoglobins which selection by malaria would develop in native populations of the marshy areas of Anatolia, Greece, and Cyprus.

Two checks on the double hypothesis that porotic hyperostosis is a result of thalassemia or sicklemia and that it is related to malaria are (i) a comparison of the world distribution of the three and (ii) a correlation of their local occurrences in space and time.

The range of porotic hyperostosis extends over the malaria belt across Africa and Asia (*5, 19*). The occurrence of porotic hyperostosis in many American Indian groups implies that the first immigrants across the Bering platform brought *Plasmodium falciparum* and one of the hemoglobin mutations with them into the New World sometime between 30,000 and 20,000 B.C. Even under warmer modern conditions they would have had to march 6000 to 7000 miles in perhaps half a generation through territory too cold for the survival of *Anopheles sundaicus* to *Anopheles freeborni*. It is unlikely, but just barely conceivable, that a pioneer band of food-gatherers could do this. Gabaldon (*20*; see also *12*), speculates and Zaino (*21*) assumes that falciparum malaria was endemic in parts of the Americas in pre-Columbian times. Zaino necessarily argues that the first migrants brought thalassemia to the New World even though Basketmaker and Pueblo Indians and Coastal Peruvians who had porotic hyperostosis lived in places ecologically atypical for malaria. Arends, Lisker, and others (*22, 23*) find no traces of abnormal hemoglobins in unmixed Indian populations. Dunn (*23*) shows that human malarial parasites evolved in Old World Primates; *Plasmodium malariae* and *P. vivax* became adapted to Primates perhaps before Miocene times in contrast to the much more recently evolved *P. falciparum*. Hence in the New World, porotic hyperostosis appears to be a direct effect of iron deficiency with prolonged lactation, or of a severely restricted childhood diet (*7*), or of severe dysentery, or even of production of abnormal hemoglobin in response to some parasite other than *P. falciparum*, rather than of thalassemia or sicklemia.

In Greece and the Eastern Mediterranean, on the other hand, *Plasmodium falciparum* and the other plasmodia and at least three groups of abnormal hemoglobins plus the deficiency of glucose-6-phosphate dehydrogenase all have overlapping distributions (*18, 19, 8*). The malarias have been a very serious health problem at least from Roman times to the present (*24*). If falciparum malaria were the key selective factor in the maintenance of relatively high frequencies of genes for abnormal hemoglobins in prehistoric times, there ought to be correlations of its incidence with ecology, with chronologic changes in farming methods and land use, with juvenile mortality, with human fertility and fecundity, and with human growth.

Table 1 sets forth some of my data gathered in Greece, Turkey, and Cyprus (*25*). I use as the point of departure a large sample of Mesolithic snail-eaters at the Mouillian site of Taforalt in a dry valley in eastern Morocco (*25*). This relatively "inbred," but fairly typical, latest Paleolithic population had a high juvenile death rate. There is only one example (in illustrations of adult skulls) of slight "osteoporosis" in the total sample. I studied samples from Çatal Hüyük, an enormous earli-

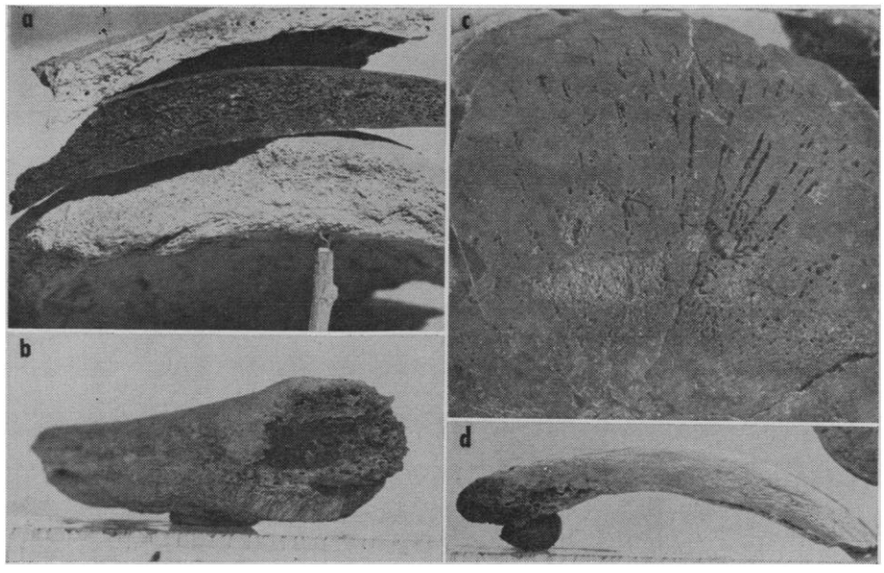

Fig. 1. Examples of porotic hyperostosis: (*a*) normal and thickened sections of parietal bone, one blackened by fire, from Catal Hüyük; (*b*) 'inner shell"; and trabeculae at the distal end of the femur of a newborn infant from Nea Nikomedeia; (*c*) porotic outer table of parietal bone of 6- to 8-month-old infant from Lerna; (*d*) thickened diploë in the frontal bone of the newborn infant from Nea Nikomedeia.

est Neolithic town with buildings having wall paintings and rooms for ritual healing (*1*), in an inland drainage marshy plateau region southeast of Konya; from Nea Nikomedeia, a related but smaller village in the marshy Macedonian plain close to the present Haliakmon estuary (*2*); from Kephala, a latest Neolithic site (*26*) on a rocky headland of Kea island; and from Karatas (*27*), an early Bronze Age village a few miles from a lake in a stream-watered fertile valley in the Lycian mountains (southwestern Anatolia). In 1949 I studied the Early Neolithic people from Khirokitia (*28*), a dry valley in the hills of south central Cyprus, and the Late Bronze Age people from Bamboula near Episkopi which is close to large salt marshes on the south coast west of Limassol. The other groups form part of my samples of ancient populations in Greece (*14*).

The five prehistoric populations living close to "permanent" marshes have a substantial frequency of medium to marked porotic hyperostosis especially among children; the five groups from dry environments have a lower frequency of hyperostosis. Different incidences of malaria, especially falciparum malaria, could explain this contrast.

There are interesting chronological changes correlated with changes in farming and nutrition as well as with malaria. As the frequency of malaria increased, that of hyperostosis increased from a low level in latest Paleolithic times up to 50 percent among the first successful farmers, which is unusually high for thalassemia. At the same time the average body size decreased and the proportion of juvenile deaths increased. From this time onward, in Greece at least, as farming methods improved (*29*) and the population density increased, the frequency of hyperostosis decreased to 8 percent in Late Bronze Age, to 4 percent in Early Iron Age, to a very small frequency in the Classical period. At the same time the number of child deaths in relation to infant deaths decreased. This finding indicates that the incidence of falciparum malaria also decreased. Increases in stature began again in the Middle Bronze Age during the period of extreme population mixture. After Classical times the frequency of adult hyperostosis increased as follows: Hellenistic, 10 percent; Roman, 24 percent; Medieval, 12 percent; Turkish, 45 percent; and Romantic, 37 percent. This increase paralleled the historically recorded increase in the incidence of malaria. The average stature fluctuated during the post-Classical period; I have no more data on juvenile deaths in periods up to the Romantic period. My total sample for judging hyperostosis in Greece is 1500; many of these individuals could be used for demographic analysis but fewer could be used for the determination of stature.

There may have been some direct effect of malaria on fecundity and fertility. As estimated from changes in the female pelves due to stress there were about five pregnancies per woman at Nea Nikomedeia and at Lerna. At Nea Nikomedeia 2.4 children reached adulthood, and the rate of population increase was about 16 percent per generation; at Lerna 2.2 children reached adulthood and the rate of population increase was 7 percent per generation. The population increase during the Classical period may have been greater. However, according to inscriptional evidence (*30*) fertility decreased during Hellenistic times. Women died at a younger age than men throughout this whole period and, in Greece, even up to and through the end of World War II when malaria was eradicated.

Perhaps, during this period of transition from Paleolithic hunting and gathering to successful Neolithic village hoe farming and hunting, there was, in many areas, an immense increase in infestation by all malarial parasites because early farmers in preferring soft and marshy soils (*31*) mishandled standing water so that anopheline mosquitos increased greatly (*24*), just as did farmers in the recent West African expansion described by Livingstone (*32*), and also because the density of settled human populations increased.

I know of no solid data which show the provenience of the mutations which produced *Plasmodium falciparum*. The mutations appear to be recent and seem to have originated in the Eastern Mediterranean. Likewise there are as yet no data which locate the first mutations for hemoglobin S, the thalassemias, or glucose-6-phosphate dehydrogenase deficiency. These variants may be very old, even antedating the human species, as do the adjustments of primates to *Plasmodium malariae* and *vivax*.

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End-Organ Effects of Thyroid Hormones: Subcellular Interactions in Cultured Cells

Abstract. *Both actinomycin D and puromycin suppress the formation of colonies by cultured human kidney epithelial cells (T-1), but inactivation by puromycin is partially reversed with thyroid hormones. Uptake by the cells of* L*-thyroxine labeled with iodine-125, 60 to 80 percent of which is nuclear, is depressed by actinomycin and enhanced by puromycin. Genome and possibly nuclear membrane are implicated as initiating loci.*

While it is increasingly evident that many hormones act at the cellular level in producing peripheral effects, the subcellular sites that are involved require identification and the responsible mechanisms elucidation. In pursuing our earlier findings of the end-organ action of thyroid hormones on human leukocytes in vitro (*1*), we thought that cultured mammalian cells would be a particularly suitable test system. Although tissue-culture studies that deal with the mode of action of thyroid hormones were conducted from inception of the technique until three decades ago (*2*), no such applications appear to have been made of modern monolayer cell cultures. We found that several cultured mammalian cell lines simulate in vivo behavior in their response to pathophysiological concentrations ($1.78 \times 10^{-5}M$ to $1.78 \times 10^{-7}M$) of thyroid hormones, and we inferred that these effects involved modifications in protein and nucleic acid metabolism, with the nucleus participating as an initial locus (*3*). Studies were undertaken with the antibiotics puromycin and actinomycin D and with I^{125}-L-thyroxine to characterize these actions more precisely.

Human kidney epithelial cells (T-1) (*4*) were grown in a water-saturated mixture of CO_2 (5 percent) and air (95 percent) at 37°C in Eagle's minimum essential medium (*5*) supplemented with 10 percent fetal bovine serum. Plating efficiency (PE) was determined by seeding 300 cells, in the logarithmic phase of growth, that had been dispersed with trypsin into 100-mm plastic petri dishes; after 2 weeks of incubation the colonies that were made visible by staining with methylene blue were scored. As has been reported (*3*), the PE for this cell line, which ranged between 40 and 70 percent, could be enhanced substantially by addition of thyroid hormones L-thyroxine (T_4) or 3,5,3′-L-triiodothyronine (T_3). The modifications in PE produced by either puromycin or actinomycin in hormone-treated and untreated cells were compared. Exposure of the experimental cells to either antibiotic was started 1 week after seeding to permit unimpaired establishment of colonies.

Suppression of colony-formation by puromycin depends not only upon concentration of the antibiotic and the duration of exposure but also upon the presence and titer of thyroid hormones. Treatment for 2 to 4 hours with concentrations of puromycin below or equal to 2.5 μg/ml produced no inhibition of PE. When 5 μg/ml of puromycin was used PE was depressed almost linearly with duration of exposure from the 4th to the 15th hour, reaching zero by 24 hours (Fig. 1). For hormone-treated cells, however, there was a marked inactivation of the effect of puromycin on PE. Thus, the PE for cells incubated in $4.45 \times 10^{-6}M$ of T_4 and exposed to 5 μg/ml of the antibiotic for 9 hours was 37 percent greater than for cells grown without hormone; extending the exposure to 15 hours resulted in a relative rise of 67 percent in PE. Similar reversals of inhibition of colony-formation by puromycin occurred with other concentrations of hormone; moreover, these effects were independent of the sequence in which hormone and antibiotic were introduced.

Formation of colonies was obliterated by immersing the cells in concentrations of puromycin exceeding 0.5 μg/ml during the entire 2nd week. For this period of exposure, 0.25 μg/ml of puromycin caused a fall in PE to 16 percent from 75.5 percent for the controls, but the addition of $1.78 \times 10^{-7}M$ of T_3 produced twice as many colonies (PE, 37.6 percent). Figure 2 illustrates the variation of PE with the concentration of T_3 for cells grown in 0.15 μg/ml of puromycin.

Such reversal by thyroid hormones could not be demonstrated for cells treated with actinomycin. No colonies developed from cells incubated for the entire 2nd week in 0.005 to 0.5 μg/ml of this antibiotic. The formation of colonies began between 0.005 and 0.0005 μg/ml, but the suppression caused by actinomycin was uninfluenced by thyroid hormones. Similarly a shorter exposure to the antibiotic (3 hours) re-