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Biology of the Gene: The Ergon/Chronon System

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Summary

The concordance of physiological and pathological times in human identical twin pairs induced the authors to postulate the existence of a hereditary biological time.

Having formulated the hypothesis that the information of each gene has a given period of existence and that, therefore, every gene has its own inherited temporal dimension, the authors report on five different experimental studies intended to verify their hypothesis.

In the first study (cf II.1*) a twin research on bone age and dental age is performed. The chronological study of the appearance of ossification nuclei in carpal bones and of mineralization of the gems of permanent dentition, in 20 MZ and 20 DZ human twin pairs, indicates that these well-known " biological timetables " exhibit about 70 % of genotypical control.*

In order to verify whether biological time is a function of the genotype as a whole, or a property of each individual gene, the authors carried out an experimental study on the mean lifespan in different strains of Drosophila melanogaster *whose genotypes were fully known (cf* II.2*). Their results indicate that the specific information of certain genes controls the insect's lifespan; it may also be inferred that the differential persistence of its specific information is an attribute of each individual gene. This* chronological dimension *of the gene is called* chronon, *which the authors also define as " the period during which the original information of the gene remains unchanged " — whether it is used for transcription or duplication, or it remains at the potential stage.*

The determination of alkaline phosphatase activity in the same strains of D. melanogaster *(cf* II.3*) affords an estimate of the amount of genic information (intensity of the individual trait) and the variation thereof during the gene's* chronon. *The authors observe that the amount of information decreases gradually during the gene's* chronon, *suggesting that this be due to the gradual exhaustion of a given specific energy. The decrease in the amount of information in the longitudinal study of* chronon *leads the authors to identify a further fundamental parametric unit of the gene which they call* ergon.

Ergon *is defined as " the degree of stability of a gene ".*

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In the fourth study (*cf* II.4) *the twin test is applied to the chromosome association index in subcultures of lymphocytes from MZ and DZ twins at age 6 and age 60. This study affords a parallel estimate of* chronon (*i.e., duration of information*) *and* ergon (*i.e., stability of information*). Chronon *and* ergon *are found to be interrelated; they may be considered as variables in a dimensional equation of the gene. Thus, the existence of the* Ergon/Chronon (E/C) system *is postulated.*

Nine parameters of development and of senescence (first smile, first word, first steps, first pubic hair, menarche, first white hair, first loss of a permanent tooth, first use of reading glasses, onset of menopause) are studied in an experimental population of 666 twin pairs of either zygosity, leading the authors to formulate several conclusions concerning the characteristics of the E/C system (*cf* II.5).

The interpretation of their experimental findings leads the authors to consider the ergon (*energy of stability*) *of a gene as the total result of the stabilities of all the nucleotides making up the DNA sequence of that gene. Since it is well known that the stability of adenine-thymine (AT) bonds exceeds the stability of guanine-cytosine (GC) bonds, and that different combinations of codons (differing in at least one nucleotide) may provide the same information, it is clear that identical polypeptide chains may be produced under the control of genetically different* ergons *resulting in genetically different* chronons.

The authors summarize these concepts in the following two aphorisms: "one gene, one stability" (ergon) *and "one gene, one time"* (chronon).

Biological time, development, senescence, homeostasis and disease are interpreted by the authors in the light of the E/C system.

I. Introduction

Time is a fundamental parameter in every process of life.

Biological time is a peculiar attribute of every species, affecting its growth rate as well as its lifespan. Further variability exists within the species: thus different populations may have different biological times. In man, this variability is reflected down to the family level, whereby several time constants may be shared by close relatives.

Those who are devoted, as we are, to the study of twins can hardly avoid noticing that biological time is determined at the individual level, since it is impressively coincident in MZ twins.

The latter finding (i.e., identical biological times in isogenic individuals such as MZ twins) leads us to believe that biological time may be under genetic control.

On the other hand, the chronological modalities of a living organism are many; in some cases the individual time is one (as in growth and development), while in other cases there are repetitive times (as in heartbeat or breathing) that may be considered as physiological clocks. Other individual biological times are related to environmental influences or to habit, as is the case in the circadian sleep/wake rhythm.

Physical and biological time obviously interact, complicating the chronological mechanism in the phenotype and making any interpretation difficult.

Having decided to study the genetics of biological time, we formulated a working hypothesis and attempted to verify it experimentally. Only subsequently, the resulting model is used as a foundation in the interpretation of more complex phenomena.

According to our basic hypothesis, the information of each gene has a given period of existence; therefore, every gene has its own inherited temporal dimension.

For semantic convenience, we adopted the word *chronon* for this temporal dimension of the gene.

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II. Experimental Verification

The experimental verification was developed along the following patterns:

1) Studies on bone and teeth development rates in MZ and DZ twins;

2) Studies on genetic conditioning of lifespan in *D. melanogaster;*

3) Studies on alkaline phosphatase in pure and hybrid strains of *D. melanogaster* at various stages of development;

4) Studies on the chromosome association index in subcultures of human lymphocytes obtained from MZ and DZ twins of different ages;

5) Studies on nine parameters of development and senescence in MZ and DZ twins.

II.1. Studies on bone and teeth development rates in MZ and DZ twins

II.1.1. *Introduction*

The timetables that medical science has drawn up to assess an individual's age on the basis of several average phenotypical traits of the species are proof of the existence of biological time. Such timetables are especially concerned with growth and development, and we refer to the two timetables that are more widely used: the timetable of *bone age*, based on the occurrence and shape of ossification nuclei in the carp and in the distal end of the forearm, and the timetable of *dental age*, based on the degree of mineralization of tooth gems in permanent dentition.

The tables of bone and dental age afford a fairly correct appraisal of an individual's age; conversely, when the latter is known, they can verify whether body growth and development are within the normal range.

We have adopted these two timetables not only because they are generally used, but also because they refer to the same age period, thus permitting simultaneous study of the same subjects.

The present study was intended to ascertain the causes of the succession of phenomena underlying the calculation of bone and dental age, in order to verify whether such ontogenetic times undergo individual genetic conditioning.

II.1.2. *Material and Methods*

In order to verify our hypothesis, we had to adopt a test that would afford a direct estimate of heredity vs. environment in any human trait; the interzygotic comparison of a twin test appeared to fit the above requirement.

Our sample consisted of 40 healthy twin pairs, 20 MZ and 20 DZ, distributed as follows:

Age (years)	MZ		DZ	
	♂♂	♀♀	♂♂	♀♀
5	2	2	2	2
5½	2	2	2	2
6	2	2	2	2
6½	2	2	2	2
7	2	2	2	2

Bone and dental ages of each subject were estimated as follows.

Bone age. The presence (p) and shape (s) of the ossification nuclei of different bones (corresponding to the various ages, as shown below) were recorded on the basis of the X-ray pictures of both hands.

Bone age (years)	Ossification nuclei
3	Pyramidal (p) Metacarpal epiphysis (p)
4	Semilunar (p)
5	Trapezium (p) Scaphoid (p)
6	Trapezoid (p) Ulnar epiphysis (p)
7	Trapezoid (s)
8	Ulnar epiphysis (s)

Whenever only one of each pair of parameters was found in one hand, the corresponding age was reduced by ½ year.

Individual bone age was recorded as the mean of the two hand values.

Dental age. Completed mineralization of the gems of different permanent teeth (corresponding to the various ages, as shown hereafter) was recorded on the basis of oblique cranial X-ray picture. Only right side teeth were taken into account, mirror-image factors appearing to be irrelevant in this case.

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Dental age (years)	Completed mineralization of the gems
3	Maxillary (6th) Mandibular (6th)
4	Maxillary (1st and 2nd) Mandibular (1st and 2nd)
5	Maxillary (4th) Mandibular (4th)
6	Maxillary (3rd) Mandibular (3rd)
7	Maxillary (5th) Mandibular (5th)
8	Maxillary (7th) Mandibular (7th)

As already mentioned for bone age, intermediate values were assigned whenever the number of mineralized gems was incomplete. In the 4-year class, a reduction of 1/4 year was made for every non-mineralized gem; in all other classes, the reduction was obviously of 1/2 year per non-mineralized gem.

II.1.3. *Results and Discussion*

The bone and dental ages of all 80 individual twins in our sample were estimated as shown in Tables I and II. Intrapair correlations (r) were assessed for both phenomena; Holzinger's formula was then used to calculate the degree of genetic conditioning ($\hat{H}$), as shown in Tab. III. A visual representation of the same data is found in Fig. 1.

Hereditary components were calculated as 66.7% and 68.5% for bone and dental ages, respectively: both values thus lie between 65% and 70%. These values of genetic conditioning are already quite high, and might have been even higher but for the sensitivity of the test, which is based on the one-year intervals of international tables.

The values of $\hat{H}$ represent the influence of heredity (i.e., the degree of control by individual genotypes) on the time of ossification and the time of dentition, respectively.

Our results are highly significant as to the nature of the phenomena involved; they relate individual variability to the genotype.

Tab. I. Bone age in 40 twin pairs (20 MZ and 20 DZ)

MZ ♂♂			MZ ♀♀			DZ ♂♂			DZ ♀♀		
Twin register serial N.	Age	Bone age	Twin register serial N.	Age	Bone age	Twin register serial N.	Age	Bone age	Twin register serial N.	Age	Bone age
9132	5.0	4.50 4.50	6972	5.0	5.25 5.00	6756	5.0	5.25 5.25	10942	5.0	5.00 5.25
6845	5.0	5.00 5.25	9025	5.0	4.50 4.25	6747	5.0	4.75 5.00	11248	5.0	4.50 5.00
9041	5.5	5.50 5.50	10802	5.5	5.25 5.50	8514	5.5	5.75 5.25	6807	5.5	5.25 5.75
9083	5.5	5.75 5.50	6720	5.5	5.50 5.00	6857	5.5	5.75 5.50	10903	5.5	5.25 6.00
9081	6.0	6.00 6.00	4523	6.0	5.75 5.25	9436	6.0	6.00 5.50	11543	6.0	5.75 6.25
6735	6.0	5.75 6.00	9483	6.0	5.25 5.50	10880	6.0	5.75 6.00	9112	6.0	6.25 6.00
6628	6.5	6.50 7.00	8629	6.5	5.75 6.25	9155	6.5	6.00 6.50	4652	6.5	6.50 6.00
6602	6.5	6.50 6.25	6639	6.5	5.75 5.50	6430	6.5	7.00 6.00	6764	6.5	6.25 6.50
6832	7.0	7.25 7.00	6418	7.0	6.50 6.25	4541	7.0	7.25 7.00	10123	7.0	6.50 7.25
6558	7.0	7.50 7.50	10950	7.0	6.75 6.75	6801	7.0	6.75 6.75	6817	7.0	7.50 7.25

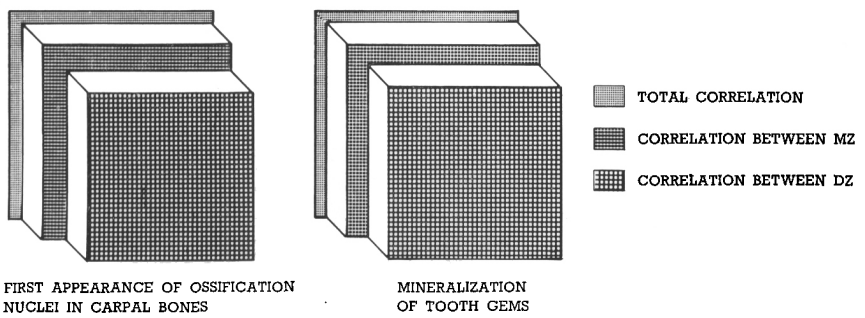

Fig. 1. Within-pair correlation of bone age and dental age in MZ and DZ twin pairs.

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Tab. II. Dental age in 40 twin pairs (20 MZ and 20 DZ)

MZ ♂♂			MZ ♀♀			DZ ♂♂			DZ ♀♀		
Twin register serial N.	Age	Dental age	Twin register serial N.	Age	Dental age	Twin register serial N.	Age	Dental age	Twin register serial N.	Age	Dental age
9132	5.0	4.75 4.50	6972	5.0	4.75 4.50	6756	5.0	4.75 4.75	10942	5.0	4.25 5.00
6845	5.0	4.50 4.25	9025	5.0	4.50 4.25	6747	5.0	4.25 5.00	11248	5.0	3.50 4.25
9041	5.5	5.25 5.50	10802	5.5	5.25 5.50	8514	5.5	4.75 5.25	6807	5.5	5.00 5.50
9083	5.5	5.50 5.00	6720	5.5	5.50 5.00	6857	5.5	4.75 4.50	10903	5.5	4.75 5.50
9081	6.0	5.75 5.25	4523	6.0	5.75 5.25	9436	6.0	5.00 6.00	11543	6.0	5.25 5.50
6735	6.0	5.25 5.50	9483	6.0	5.25 5.50	10880	6.0	5.50 5.75	9112	6.0	6.25 5.50
6628	6.5	5.75 6.25	8629	6.5	5.75 6.25	9155	6.5	5.75 6.50	4652	6.5	6.00 5.50
6602	6.5	5.75 5.50	6639	6.5	5.75 5.50	6430	6.5	6.75 6.25	6764	6.5	5.75 6.25
6832	7.0	6.50 6.25	6418	7.0	6.50 6.25	4541	7.0	7.00 6.50	10123	7.0	6.25 6.50
6558	7.0	6.75 6.75	10950	7.0	6.75 6.75	6801	7.0	6.50 6.75	6817	7.0	7.25 6.50

Tab. III. Analysis of bone and dental age in 40 twin pairs (20 MZ and 20 DZ)

		Bone age	Dental age
Within-pair correlation coefficient:	r_{MZ}	0.94	0.95
	r_{DZ}	0.81	0.84
Holzinger's index of heredity:	$\hat{H}$	0.67	0.68
Probable error of $\hat{H}$:	$Pe(\hat{H})$	0.13	0.14

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The present study is an approach to the solution of the genetic problem of biological time; from it, we may draw the following conclusions:

1) X-ray studies of ossification of the hand in 20 MZ and 20 DZ twin pairs, aged 4 to 6 years, indicate that 67% of the control of the ossification process is due to the genotype;

2) X-ray studies of permanent dentition on the same twin sample indicate that 68% of the control of the mineralization process in second-dentition gems is due to the genotype;

3) The above ossification and mineralization traits are used to estimate bone and dental ages; it may be inferred that human biological time, as related to such traits, exhibits 2/3 of genotypic conditioning.

II.2. Studies on genetic conditioning of lifespan in *Drosophila melanogaster*

II.2.1. *Introduction*

In our previous study (II.1) we showed that biological time is largely conditioned by the individual genotype. At this point, a logical question arose: is such conditioning due to the entire genotype or to individual genes?

In order to find an answer we performed experimental studies on *D. melanogaster*, one of the few living species whose genotypic structure is very well known. The temporal parameter we chose for genetic analysis in this vinegar fly was its lifespan.

Several authors, and especially Gonzales (1923), already carried out genetic analyses of lifespan in *D. melanogaster*, but the experimental conditions they had adopted did not seem to fit our requirements. In our working hypothesis, the individual gene controls one specific time in the phenotype as a function of its own structure, and not as the result of any outside influence.

In fact, Gonzales and others carried out genetic analyses of lifespan in *D. melanogaster* without taking into account some sub-experimental factors that may be relevant, such as the age and generative experience of the parents. Therefore, we decided to test the hypothesis of correlation between lifespan and genotype through an original and more complete structure of the experiment.

II.2.2. *Material and Methods*

For this purpose our test was based on 11 experimental strains, as follows:

1 and 2: parental strains, represented by one wild strain (*Oregon R*) and one strain with three mutant genes: *brown* (*bw; II, 104.5*), *cinnabar* (*cn; II, 57.5*) and *vestigial* (*vg; II, 67.0*);

3: F_1 hybrids resulting from the cross 1 × 2;

4-11: strains obtained by back-crossing F_1 hybrids and then selectively back-crossing individual strains.

The eight strains thus obtained correspond to the parental genotypes and to those homozygous for one, two or three mutant genes.

As already indicated, there were 11 experimental strains, of which two are homozygous P (*Oregon R*; *bw-cn-vg*), one heterozygous F_1 (*Oregon R* × *bw-cn-vg*) and eight homozygous F_2 (*Oregon R*; *bw*; *cn*; *vg*; *bw-cn*; *bw-vg*; *cn-vg*; *bw-cn-vg*).

Each strain was cultured separately in order to verify the mean lifespan of the respective individuals. Reproducibility was granted by the constancy of the following sub-experimental factors:

1) *Genotypical environment.* All the strains were derived from the same wild strain (*Oregon R*, from the California Institute of Technology, Pasadena), stabilized through 466 inbreeding generations and 150 mass-culture generations. The other P strain (the triple mutant *bw-cn-vg*) was derived, in the same Institute, by mutation from *Oregon R.* It was similarly stabilized. Mass cultures were carried out at the Institute of Genetics, University of Rome. Thus, all our strains have the same genetic material (apart from the specific mutations), i.e., the same genotypic environment;

2) *Progenetic environment.* Parental age was standardized, since all matings took place five days after the insects reached the *imago* stage. The possible differential maturation according to parental sex was verified by using one container for males of one strain and females of the other, and a second container with the opposite disposition;

3) *Generative experience.* We took the generative experience into account by providing two strata for each sample, composed respectively of virgin (V) and previously mated (M) individuals. This was obtained by separating all individuals by sex and by strain on the day of their final metamorphosis, and mating 50% of them five days after separation;

4) *Experimental conditions.* Temperature, lighting, culture medium, etc were standardized.

Possible chance variations were taken into account: all experiments were repeated five times.

II.2.3. *Results and Discussion*

Tables IV, V and VI list the results obtained by estimating the respective mean lifespan in the parental strains (*Oregon R*; *bw-cn-vg*) and in their F_1 (*Oregon R* × *bw-cn-vg*); each table includes the various classes of sex (♂; ♀) and generative experience (V;M).

The results listed in these tables enabled us to exclude any interaction of sub-experimental factors "sex" and "generative experience" with the experimental factors. In fact, the "♀" and "V" conditions may be related to the mean lifespan, but this influence is uniformly distributed among the three strains, *Oregon R*, *bw-cn-vg* and *Oregon R* × *bw-cn-vg*. There follows that, as long as the numerical relationship between strata is held constant, the mean lifespan is a characteristic of each strain. It was considered that the same would apply to all strains and all subsequent analyses were based on one value (♂, V) per strain. The resulting

Tab. IV. Lifespan of the *Oregon R* strain

		N.	$\bar{x}$	S
♂	V	117	40.6	5.9
	M	117	39.8	3.9
	Total	234	40.2	5.5
♀	V	111	46.3	4.8
	M	111	39.6	5.8
	Total	222	43.4	8.4
Total	—	456	41.7	10.6

Tab. V. Lifespan of the *bw-cn-vg* strain

		N.	$\bar{x}$	S
♂	V	19	22.1	5.8
	M	19	21.6	2.9
	Total	38	21.7	6.5
♀	V	15	25.6	4.5
	M	15	23.2	4.2
	Total	30	23.9	6.1
Total	—	68	22.4	11.6

Tab. VI. Lifespan of the F_1 *(Oregon R × bw-cn-vg)* strain

		N.	$\bar{x}$	S
♂	V	73	25.7	4.5
	M	72	26.3	4.5
	Total	145	25.9	6.1
♀	V	64	36.8	6.8
	M	64	30.2	7.4
	Total	128	34.0	11.0
Total	—	273	29.6	13.6

data are shown in Tab. VII. The total experimental pattern and the respective mean lifespan values are illustrated in Fig. 2.

We believe that the structure of the test guarantees that the variation in

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Tab. VII. Lifespan of the homozygous F_8 strains (obtained by backcrossing F_1 individuals) *

Strain	N.	$\bar{x}$	S
Oregon R	634	43.4	10.8
bw	561	38.1	10.6
cn	504	35.6	13.2
vg	255	24.2	11.3
bw-cn	166	32.9	15.2
bw-vg	294	23.5	13.5
cn-vg	168	23.2	18.1
bw-cn-vg	185	21.6	9.0

* Analysis based on one value (♂, V) per strain (cf text: II.2.3).

mean lifespan is in fact to be ascribed to the different genotype of the various strains. Thus, the analysis of our results seems to justify the following statements:

a) A relevant difference in mean lifespan exists between parental strains, the value for *Oregon R* being twice that for *bw-cn-vg;*

b) The mean lifespan in the F_1 strain is intermediate between those characteristic of the parental strain, apparently as a result of the presence of one single mutant at the three loci involved;

c) The variation in mean lifespan between F_8 strains, homozygous for mutant genes, is related to the number and type of mutants involved. This is consistent with the hypothesis that the mean lifespan be decreased with the increase of homozygous mutants. The decrease per mutant varies between mutants.

From a more general point of view, these studies seem to support the following conclusions:

1) Mutant genes responsible for the *brown*, *cinnabar* and *vestigial* phenotypes in *D. melanogaster* are shown to be directly and specifically related to lifespan;

2) The three mutants share a qualitative relationship (decrease) to lifespan; the quantitative relationship is specific for each mutant, since the decrease in lifespan is a specific characteristic of each single mutant;

3) The influence of a gene on lifespan represents a specific factor *ad tempus:* such influence may be assumed to be either direct (primary effect of the gene) or secondary (interaction with other structural and/or functional genes);

4) The effect of mutation on the gene, as studied through the lifespan of *D. melanogaster*, results in a specific decrease of the temporal dimension of the gene;

5) Each mutant gene and each corresponding wild gene in our system exhibits a specific temporal dimension that differentiates it from the others. In other words, each gene has its own differential persistence of information, identified through the exhaustion of the same information;

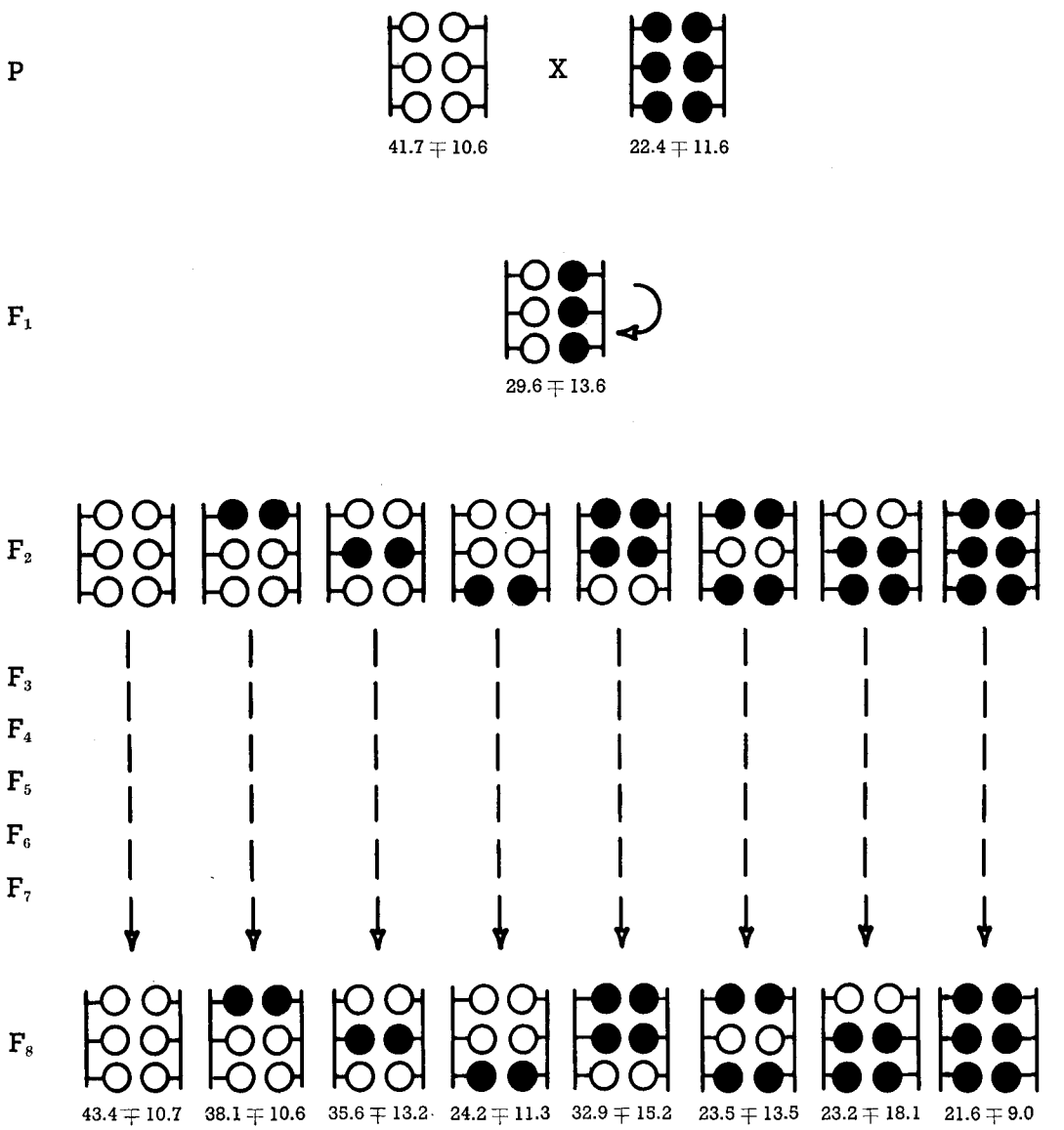

Fig. 2. Mating models and mean lifespan (in days) in different strains of *D. melanogaster*.

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6) Since the experiment was set up as if, *coeteris paribus*, each gene involved had its own temporal dimension, we assume that the existence of the temporal dimension of the gene has thus been proved.

We call the temporal dimension of the gene "*chronon*".

II.3. Studies on alkaline phosphatase in pure and hybrid strains of *Drosophila melanogaster* at various stages of development

II.3.1. *Introduction*

Once ascertained in *D. melanogaster* that individual genes are related to lifespan (i.e., they have a temporal dimension or *chronon*), our next step was to study the time factor in the primary effect of a single gene. In fact, this group of studies aims to investigate the manifestation of *chronon* in *D. melanogaster* using an enzyme that is already well known to geneticists: alkaline phosphatase.

Alkaline phosphatase represents a normal trait in the haemolymph of *D. melanogaster*; it is controlled by a system of three alleles: Aph^F, Aph^S, Aph^O, located on the third chromosome (*III; 46.3*).

Genetic control of alkaline phosphatase in peripheral leukocytes of human twins has already been studied at the Mendel Institute (Cardinali et al, 1964; Gedda et al, 1969). Individual phosphatasic indexes were obtained in 40 MZ and 40 DZ twin pairs, distributed in four age classes covering the age interval 0-15 years. The twin test indicated that the genetic component in the phenomenon (index of heredity, $\hat{H}$) amounts to 88.1% with a probable error, $Pe(\hat{H}) = 1.9\%$.

It has been found that alkaline phosphatase in human blood leukocytes decreases by about 0.75 milliunits (mu) per ml per year, with occasional increases in special physiological or pathological cases (such as pregnancy and sepsis). By analogy, care was taken to protect *D. melanogaster* cultures in order to guarantee the constancy of environmental factors.

II.3.2. *Material and Methods*

The design of the experiment was based on the study of alkaline phosphatase in the parental strains *Oregon R* and *bw-cn-vg* (cf II.2.2), and in the resulting F_1.

Every strain was sampled on the first, second, third, fifth, seventh and ninth day after fertilization, i.e., at the following stages of development: (1) egg; (2) first moult; (3) second moult; (4) puparium formation; (5) pigmentation of eye; (6) pupa ready to emerge.

Alkaline phosphatase activity was estimated by a colorimetric technique, based on the principle that phosphatases catalyze hydrolysis of orthophosphoric esters (Andersch and Szczpynsky, 1947). Activity is estimated by incubating alkaline phosphatase with p-nitrophenilphosphate and measuring the amount of separated p-nitrophenol. P-nitrophenol has an absorption peak at 405 mμ; quantitation is obtained by comparison against a standard. Taking into account the dilution, phosphatasic activity is given by the following

formula: $\Delta E = E_2 - E_1 \times 200$; E_2 and E_1 being the extinction coefficients for the sample and the standard, respectively.

The small size of the samples (0.2 ml per test, obtained from 10 individuals) increased the probability of variation due to the method.

In order to minimize such variability, a Beckman DK_2 spectrophotometer was used instead of the normal colorimeter, thus providing simultaneous reading of both sample and standard. All readings were taken at 37.0°C ± 0.1.

Alkaline phosphatase values in the parental and hybrid strains are listed in Tab. VIII. Each figure represents the mean value for 18 readings. In fact, three operators each repeated every dosage six times. Since no causal variability was found between the results of the three operators, it became possible to consider the respective results as chance repetitions.[1]

Tab. VIII. Alkaline phospatase values during development in different strains of *D. melanogaster* (mu/ml)

Stage	Days after fertilization	Strain		
		Oregon R	*bw-cn-vg*	*Oregon R* × *bw-cn-vg*
Egg	1	7.5	9.4	10.6
First moult	2	8.0	7.8	7.6
Second moult	3	6.8	6.1	8.2
Puparium formation	5	5.5	3.3	1.7
Pigmentation of eye	7	3.8	4.7	2.6
Pupa ready to emerge	9	2.8	1.6	2.0

[1] The sensitivity and variability of the biochemical dosage were checked histochemically.

Six squash slides per sample were stained with sodium-naphtil-phosphate and brentamine fast garnet. The slides were mounted after treatment with methyl-green. This staining method reveals the granules with phosphatasic activity within the cytoplasm.

The following classification was adopted:

Class 0 = cells without phosphatasic granules;
Class 1 = cells with 1-5 phosphatasic granules;
Class 2 = cells with granules occupying less than half of the cytoplasm;
Class 3 = cells with granules occupying more than half of the cytoplasm;
Class 4 = cells with granules occupying the whole cytoplasm.

An index of phosphatasic activity was obtained as follows: 100 cells are distributed in the above classes; each class index number is multiplied by the number of cells in the class; the sum of the five numbers thus obtained is divided by 100 (the number of cells).

The results of the histochemical test based on the phosphatasic index (obtained at the fifth day of age for each of the three strains), when compared to those obtained by spectrophotometry, appear to be practically superimposable. The correlation index ($r = 0.96$) demonstrates the high degree of concordance between the two tests.

II.3.3. *Results and Discussion*

On the basis of the results listed in Tab. VIII we have drawn up a graph (Fig. 3). Phosphatasic activity is clearly inversely proportional to age. The decrease is shared by all three strains.

Two points are worth making here: (1) as already seen in the previous lifespan studies, each strain has a definite survival value, genetically determined, which is

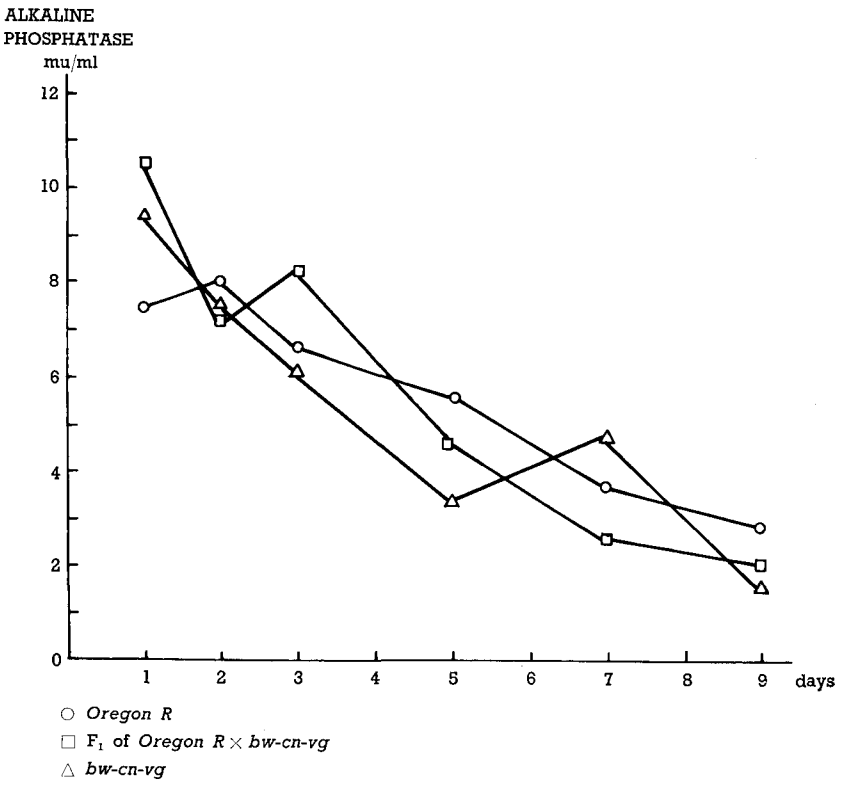

Fig. 3. Chronological regression of alkaline phosphatase values (mu/ml) in different strains of *D. melanogaster*.

ascribed to specific genes that become extinguished in a predetermined time; and (2) alkaline phosphatase levels are known to be under genetic control.

The results of this group of studies may be interpreted as follows:

a) Alkaline phosphatase index values, in two strains of *D. melanogaster* (*Oregon*

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R and *bw-cn-vg*) and in their F_1 hybrids, decrease in direct proportion to the age of the individual insect;

b) Phosphatasic activity at any given stage of development shows no relevant variation between the three strains, in spite of the differing respective lifespan;

c) Since the different lifespan of the three *D. melanogaster* strains does not affect phosphatasic activity, it may be inferred that the time trend of the information of the genes controlling alkaline phosphatase is separate and independent from the *chronon* of the genes controlling lifespan in the same insect;

d) The gradual decrease in alkaline phosphatase activity in different strains of *D. melanogaster* (directly related to individual stages of development but independent from the lifespan) seems to reflect a gradual decrease in the primary effect of the respective gene. In other words, the decreasing activity of alkaline phosphatase reveals a gradual extinction of specific information.

On the basis of these findings the following conclusions seem justified:

1) The decreasing manifestation of genic information reveals the gradual degradation of the stability of such information;

2) The gradual extinction of genic information during the gene's *chronon* proves the existence of some kind of energy providing stability of genic information; this energy becomes degraded with time.

We call the degree of stability of a gene "*ergon*".

II.4. Studies on the chromosome association index in subcultures of human lymphocytes obtained from MZ and DZ twins of different ages

II.4.1. *Introduction*

In the foregoing studies on *D. melanogaster* we have demonstrated the existence of *chronon* (the temporal dimension of the unit of inheritance) and *ergon* (the stability dimension of the unit of inheritance). We now revert to human twin studies for a survey of the behavior of these two gene dimensions (*chronon* and *ergon*) in a process controlled by a complex genotype: mitosis.

We believe that a study of the time factor in the behavior of chromosomes in induced mitosis in isolated human cells affords an intimate knowledge of the relationship linking *chronon* and *ergon* to the fundamental unit of biology: the cell. Experimental studies of *chronon* in isolated cells also help to eliminate any variability due to organismic influences which might mask the fundamental phenomenon and confuse the meaning of the inheritance of time.

II.4.2. *Material and Methods*

In order to guarantee standardization of experimental and sub-experimental conditions, we adopted the standard techniques of karyotype studies. Peripheral lymphocytes were obtained from human MZ and DZ twins of widely differing ages, according to the following distribution:

2 ♂♂ MZ pairs aged 6 years
2 ♂♂ DZ pairs aged 6 years
2 ♂♂ MZ pairs aged 60 years
2 ♂♂ DZ pairs aged 60 years

In order to standardize sub-experimental conditions, the following precautions were taken:

a) Clinically healthy pairs were selected from the large Twin Register of the Mendel Institute, Rome;

b) Blood was drawn at the same time, by the same technique, using the same material and equipment.

Each blood sample was used for two cultures, stimulated by phytohaemoagglutinin and arrested by cholchicine after 72 hours. The resulting slides were used to assess the association index. Association occurs whenever D and G group acrocentric chromosomes are joined through the satellites or (if not joined) their centromeres are separated by less than one chromosomal diameter. Four classes were formed according to the above criteria: *Class 0*: plates without associations (Fig. 4); *Class 1*: plates with two associated chromosomes (Fig. 5); *Class 2*: plates with three associated chromosomes (Fig. 6); *Class 3*: plates with 4 or more associated chromosomes (Fig. 7). Thirty metaphasic plates from a sample slide were photographed for each culture and assigned to the corresponding classes of chromosomal association (Tab. IX). In the following discussion only *Class 0* (no association) is considered; there follows that our "association index" is really a non-association index, reflecting the number of *Class 0* cells in the total cell population of a slide.

II.4.3. *Results and Discussion*

Our experimental data indicate that the association index varies with age. The variability of our index has been studied taking the following sources of variability into account: (1) individuals; (2) age groups; (3) probable error (Tab. X). The analysis shows that the age factor accounts for a large part of the variability in the phenomenon; the significance of this finding exceeds the 0.01 level. The twin test indicates that the number of associations is largely controlled by heredity (index of heredity: $\hat{H} = 92.0\%$; $Pe(\hat{H}) = 5.0\%$), as shown in Tab. XI.

Tab. IX. Chromosome association in lymphocyte subcultures from human MZ and DZ twin pairs (cf II.4.2)

Zygosity	Age	Pairs	Individ.	Classes				
				Association			Non-association	
				3	2	1	0	Δ_x
MZ	6	I	1	4	19	41	36	3
			2	6	23	32	39	
		II	1	7	14	39	40	2
			2	3	22	37	38	
	60	I	1	7	25	42	26	4
			2	5	29	36	30	
		II	1	14	28	40	18	6
			2	11	28	41	24	
DZ	6	I	1	4	16	48	32	12
			2	7	12	37	44	
		II	1	2	23	30	45	5
			2	3	21	36	40	
	60	I	1	8	26	51	15	6
			2	13	28	38	21	
		II	1	17	22	33	28	9
			2	5	35	41	19	

Mean non-association index (na): age 6 $\simeq$ 0.4; age 60 $\simeq$ 0.2.
Mean association index (a = 1 − na): age 6 $\simeq$ 0.6; age 60 $\simeq$ 0.8.

Tab. X. Analysis of variance for the association index

Sources of variability	Deviance	df	Variance	F	P
Between classes of age	1106	1	1106	50.27	<0.001
Error	313	14	22		
Total	1419	15	9560		

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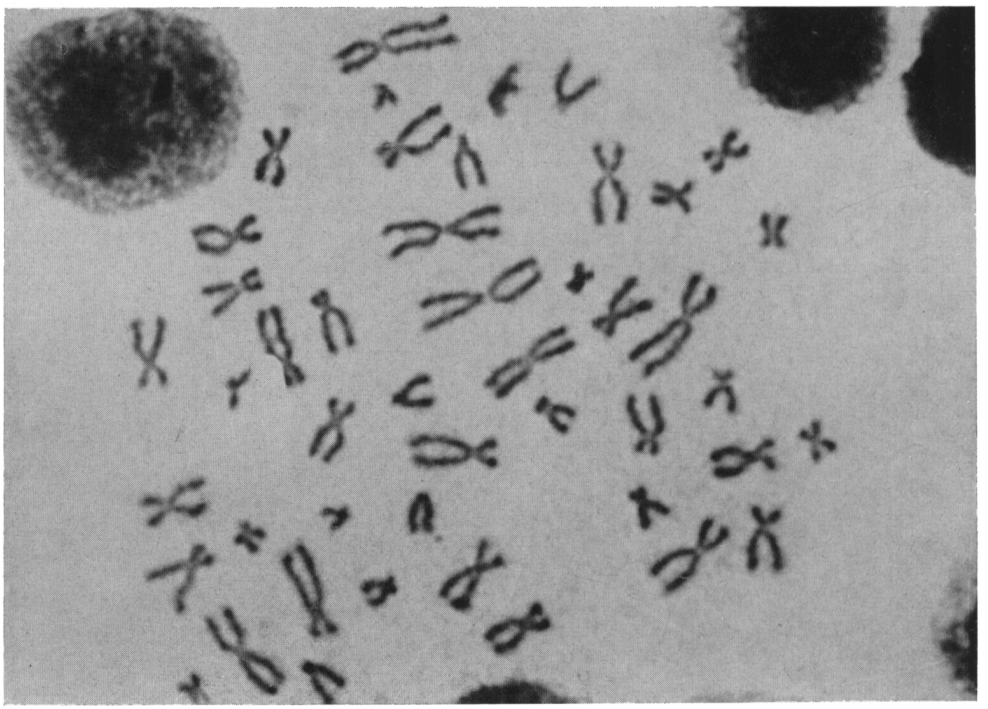

Fig. 4. Metaphasic plate without association (*Class 0*).

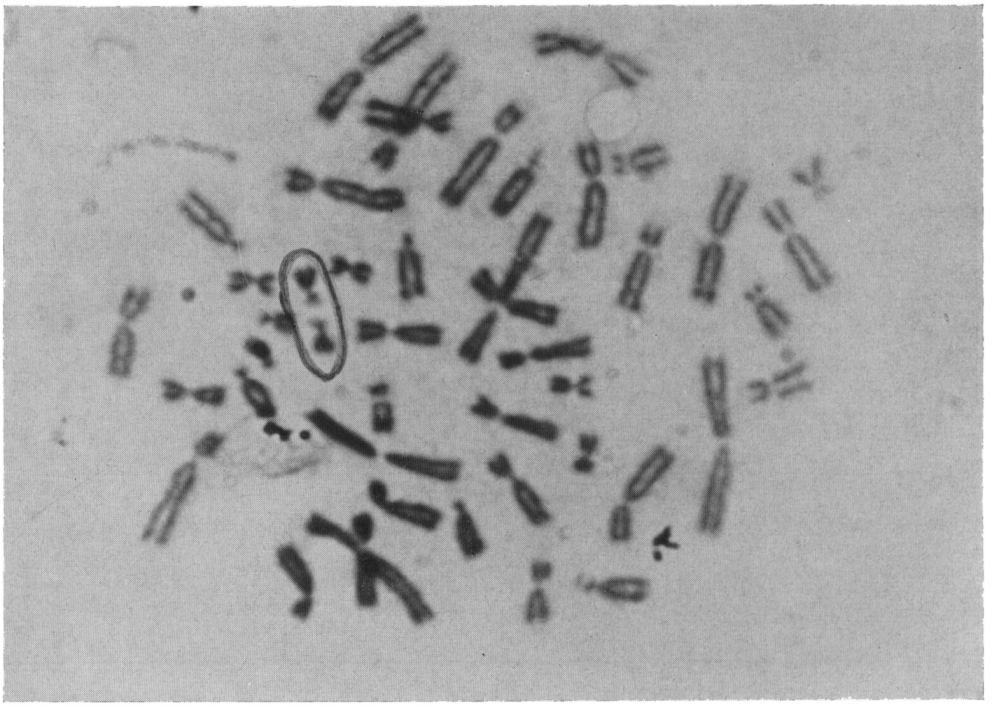

Fig. 5. Metaphasic plate with two associated chromosomes (*Class 1*).

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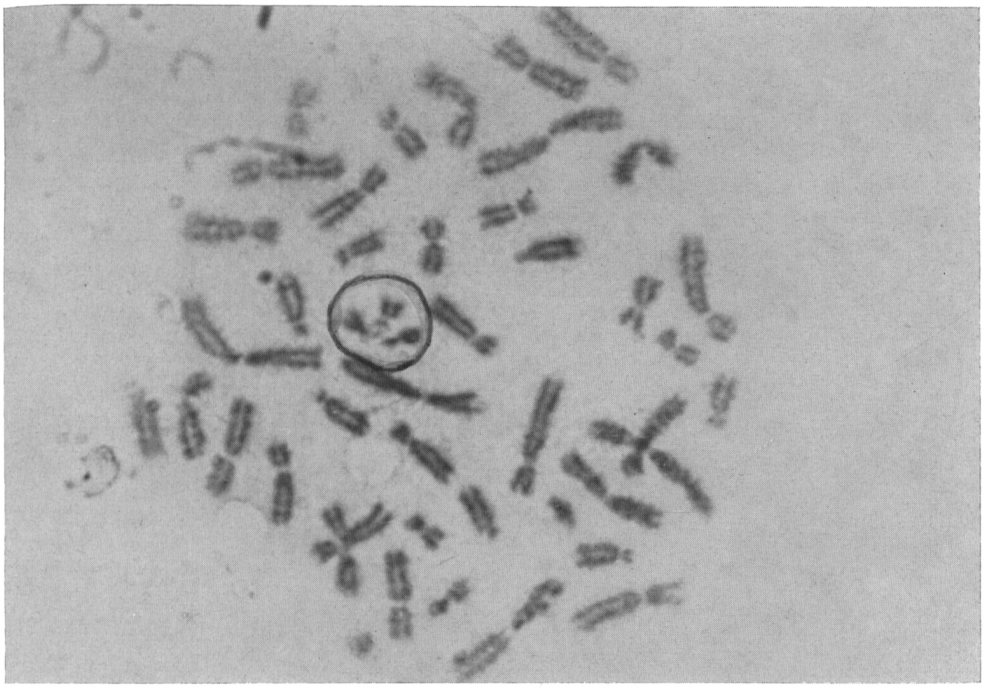

Fig. 6. Metaphasic plate with three associated chromosomes (*Class 2*).

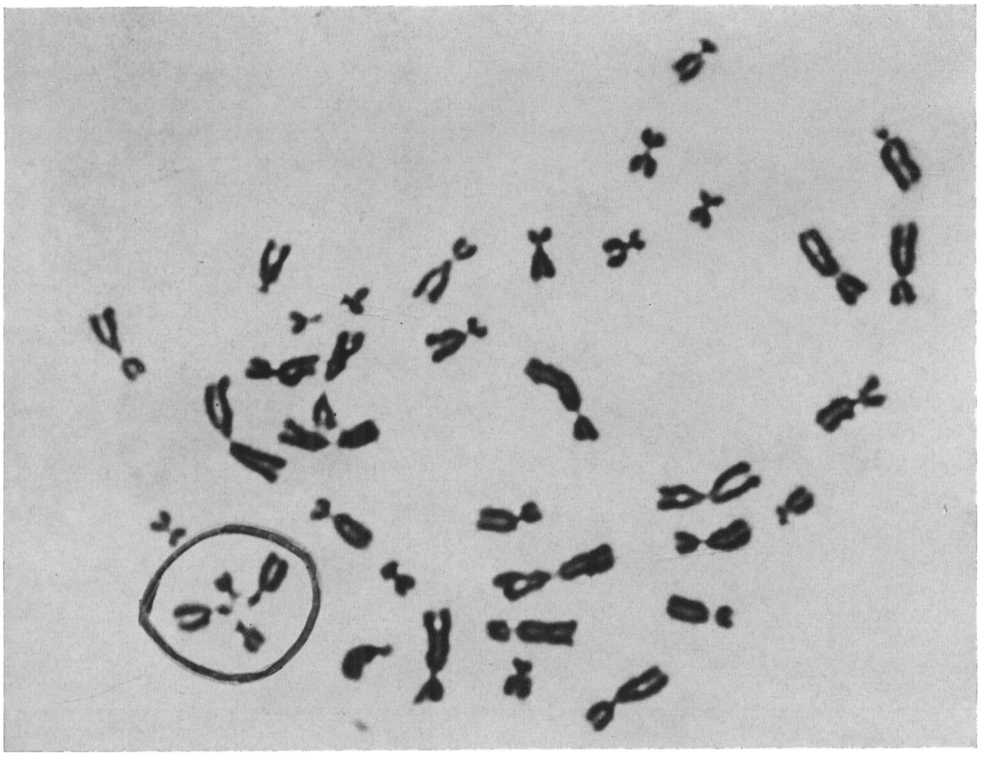

Fig. 7. Metaphasic plate with four associated chromosomes (*Class 3*).

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Before drawing any specific conclusions from these findings we believe that the following general considerations may be made:

a) In the first place, the use of the twin test in the study of lymphocyte subcultures from human MZ and DZ twins indicates that the genetic differences characterizing the two types of twins can be identified and used at the level of the basic biological unit: the cell. In this case they were applied to the study of a fundamental function of the cell, i.e., reproduction;

b) Secondly, our results confirm in man that mitosis is under genotypic control, as already indicated by several studies in fundamental genetics, since Morgan to the present time. The phenomenon of association between acrocentric chromosomes may be interpreted by admitting that the genotype responsible for mitosis has a certain degree of stability, resulting from the stability of each component information, one of which would condition the normal separation of acrocentric chromosomes;

Tab. XI. Analysis of correlation (r), Holzinger's index of heredity ($\hat{H}$) and probable error thereof [Pe($\hat{H}$)] for the association index

Zygosity	r	$\hat{H}$	Pe($\hat{H}$)
MZ	0.98	0.92	0.05
DZ	0.70		

c) Thirdly, the phenomenon of association appears to be a function of the age of cell donors.[1]

As to the specific subject of our study, the following conclusions may be drawn:

1) The application of Holzinger's formula to the interzygotic comparison of the mean value of the association index proves that the index of heredity in this phenomenon approaches unity. Since the association index represents a parameter of the phenomenon of mitosis, we believe we can state that mitosis, in our clones of lymphocytes, reflects an information which is chronologically controlled by the genotype. In other words, the genotype responsible for mitosis has its own temporal diameter, i.e., its own *chronon*;

[1] The study of the type of function involved cannot be based on our data alone, since the two points represented by our sample may be placed on any number of curves representing as many different analytical functions. Yet, if we combine the association indexes we may derive from our data (association index at 6 years = 0.6; association index at 60 years = 0.8) with those obtained by Prokofieva (1966, 1967) for the association index in the lymphocytes of the newborn (association index = 0.5), which appear to be homogeneous with ours as to methodology and sampling, we obtain by interpolation a single analytical function of a logistic type.

In order to underline the analogies between analytical function and phenomenon observed, we should mention the rapid increase of the association index during embryofetal life and the slow increase during independent life.

It should be pointed out that Prokofieva extended her study of the association index to embryonic fibroblasts, finding a value of 0.3. We were unable to include this figure in our analytical curve (cf *graph*) be-

2) When the same data are compared by age (comparing the mean values of the index for all 6-year-old twins to the corresponding values for all 60-year-old twins) experimental values are found to decrease with age. Thus, genic information is found to decline with time, as if it were the product of some kind of energy that becomes progressively exhausted. This represents further confirmation of the concept of *chronon* as a function of the stability of the gene, i.e., of the *ergon* of the gene. In other words, *chronon* and *ergon* are so closely related as to constitute a system.

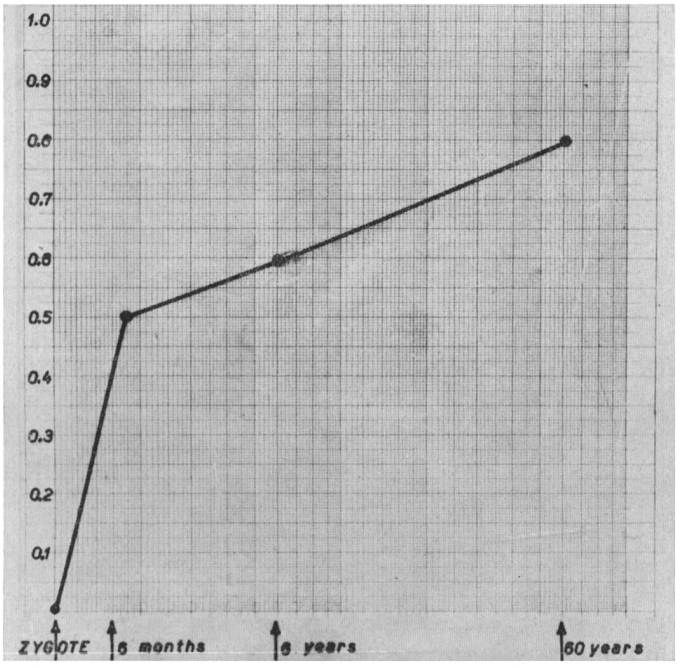
We call this the "*Ergon/Chronon system*" (*E/C system*).

II.5. Studies on nine parameters of development and senescence in MZ and DZ twins

II.5.1. *Introduction*

The present group of studies is intended to take us back to those phenomena of phenotypical synchronism which, by their frequent occurrence in MZ twins, originally led us to investigate the problem of biological time.

cause the exact embryonic age is unknown; yet we believe that this value fits other data well enough, providing further indication of the steep rise in the incidence of chromosomal association during intrauterine life, followed by a slower rate of increase during independent life.



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When we proceed from the study of isolated human cells (cf II.4) to the study of phenomena involving the entire organism, any analysis of biological time obviously becomes complicated. The genotype involved in our experiments on *D. melanogaster* (cf II.2 and II.3) was a simple one, whereas the genotype involved in our experiments on human lymphocytes (cf II.4) was probably polymeric. Now we revert once more to the study of the behavior of the *E/C system*, at the complex level of several converging genotypes (cf II.1). We do realize that we are relinquishing the sound field of clear-cut experimental design for the quicksands of complex phenomena. As a consequence, the concepts of *ergon*, *chronon* and *E/C system*, applying *sensu strictiori* to the individual gene, will now be applied only *sensu latiori* to the final phenotypical result of a large number of converging genotypes. The results of such convergences in our case will be a number of macroscopic somatic phenomena; we will apply the usual methodology of Medical Genetics to this study.

While the definitions of *ergon* and *chronon*, as applied to the gene in the previous context, remain unchanged, we now extend the concept of *chronon* to apply to a series of phenomena of human development that are controlled by a number of individual *E/C systems*. In other words, we extend the concept of *genic chronon* to include an *organismic chronon*.

The present group of studies concerns the action of the *E/C system* at the level of several landmarks in the course of human life.

II.5.2. *Material and Methods*

Chronological details of ossification and of permanent dentition have already been considered (cf II.1). The same methodology has now been applied to a twin study of the age at which the following parameters are first observed: (1) first smile; (2) first word; (3) first steps; (4) first pubic hair; (5) menarche; (6) first white hair; (7) first loss of a permanent tooth; (8) first use of reading glasses; (9) onset of menopause.

The twin sample was drawn from the Twin Register of the Mendel Institute (Rome), which listed data on 13104 twin pairs as of 1.V.1969.

Two different questionnaires were mailed to each of 2000 same-sexed twin pairs of either zygosity, of which 1500 were aged between 6 and 15 years while 500 were over 45.

The first questionnaire, identical for both age groups, was conceived as an aid in completing previously recorded information. (Upon registration, the case history of each pair is taken and supplemented by zygosity-oriented data such as photographs, dermatoglyphics, blood groups). The questionnaire contains Gedda's equivocity test for zygosity determination (Gedda, 1960) on the basis of the ease with which the twins were mistaken for each other (by their parents? by other relatives? by others? up to what age?). The first questionnaire also contained questions about the twins' current health.

A second questionnaire mailed to each pair differed according to the respective age group. Twins aged 6 to 15 years were asked to specify the ages at which first smile, first word, first steps and first pubic hair were observed; females were also asked to specify the age of menarche.

Twins over 45 years of age received a different questionnaire, concerning first white

hair, first loss of a permanent tooth and first use of reading glasses; females were also asked to specify the age of onset of menopause.

A total of 666 pairs answered, resulting in 1332 questionnaires. The following steps were then taken:

1) All acceptable answers were recorded;

2) Zygosity was verified, assigning each pair to one of the following classes: (1) monozygotic (MZ); (2) dizygotic (DZ); (3) zygosity unknown (ZU);

3) The analysis was performed of the distribution of individual data for one twin per pair, in order to calculate mean and variance for each parameter (thus excluding the influence of " cotwin " data on variability);

4) The intrapair correlation index for each parameter in the classes of known zygosity (MZ and DZ) was calculated;

5) For each parameter, Holzinger's index of heredity, ĥ, was calculated.

II.5.3. *Results and Discussion*

Relevant findings concerning each parameter of development and of senescence are listed in Tab. XII.

The implications of these findings in the fields of growth and development, of senescence and of sex studies will be discussed at a later date.

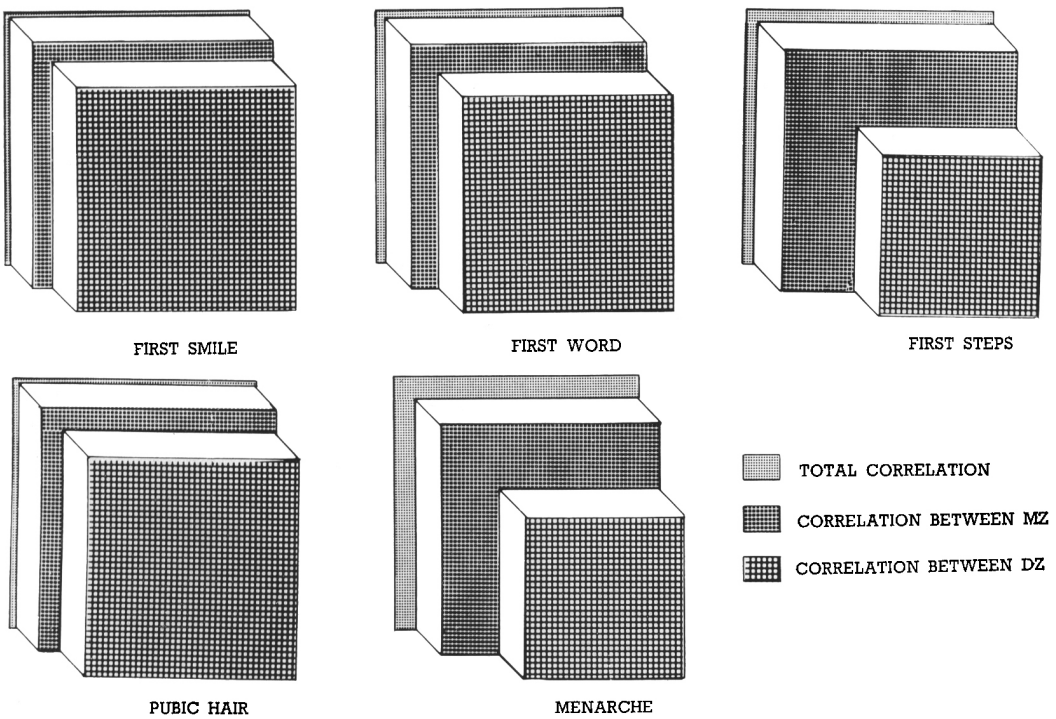

Fig. 8. Phenomena of development in human twins: within-pair correlations.

At the present time, we are concerned with the contribution these findings may provide towards a better understanding of the *E/C system*. The discussion on the subject will be divided in the following four points.

1) The first point concerns the differences in standard deviation between phenomena of development and phenomena of senescence (cf S values in Tab. XII).

Taking into account only data that are homogeneous as to the unit adopted (year), we find a striking difference in the standard deviation between phenomena of development (first pubic hair and menarche) and phenomena of senescence (first white

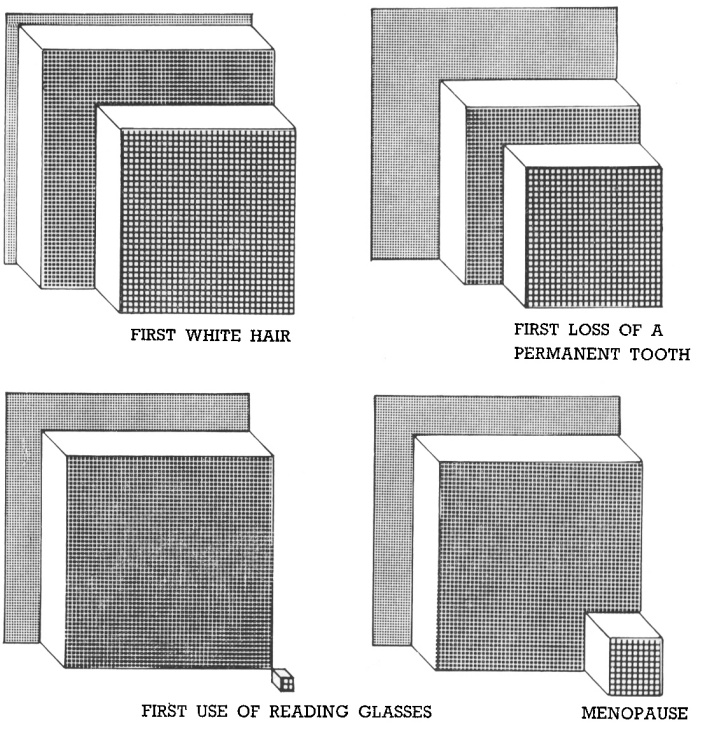

Fig. 9. Phenomena of senescence in human twins: within-pair correlations.

hair, first loss of a permanent tooth, first use of reading glasses and menopause): deviations from the mean in the latter group of phenomena clearly exceed those found in the period of development.

We believe that this difference reflects a difference of the genetic mechanisms responsible for escalation in development and descalation in senescence, respectively.

Tab. XII. Phenomena of development and senescence in human twins: mean values ($\bar{x}$) and standard deviations (S), correlation index (r), Holzinger's index of heredity ($\hat{H}$) and probable error thereof [Pe($\hat{H}$)]

	Phenomenon	Zygosity	N.	r	$\bar{x} \pm S$	$\hat{H}$	Pe($\hat{H}$)
Development	First smile	MZ	153	0.99	3.4 ± 2.2	0.95	0.07
		DZ	108	0.90			
	First word	MZ	155	0.99	9.5 ± 3.8	0.91	0.16
		DZ	230	0.86			
	First steps	MZ	187	0.96	12.4 ± 2.7	0.90	0.09
		DZ	128	0.66			
	First pubic hair	MZ	63	0.99	11.0 ± 2.1	0.94	0.10
		DZ	71	0.88			
	Menarche	MZ	167	0.92	12.8 ± 1.5	0.75	0.02
		DZ	119	0.66			
Senescence	First white hair	MZ	38	0.96	36.9 + 9.5	0.85	0.03
		DZ	35	0.73			
	First loss of a permanent tooth	MZ	17	0.72	30.0 ± 14.4	0.36	0.18
		DZ	16	0.56			
	First use of reading glasses	MZ	35	0.86	48.8 ± 5.9	0.86	0.03
		DZ	24	0.04			
	Menopause	MZ	14	0.85		0.79	0.07
		DZ	6	0.23			

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The experimental differences between ontogenetic and involutive chronological phenomenon once more confirm our hypothesis, suggesting a *particulation* of both energy and time at the level of the individual gene.

2) The second point refers to the twin quality of our material.

The values of the mean within-pair correlation index in MZ and DZ twins indicate that, in the absence of causal factors of environmental diversification, cotwin similarity as to the age of onset of each phenomenon is significantly different in the two classes of zygosity, with much higher values in the MZ class.

The finding of a lesser *chronological* variability within MZ twin pairs may be ascribed to the constancy of genetic factors both in terms of specific information and in terms of the respective *E/C systems.*

3) The third point concerns the analysis of the index of heredity ($\hat{H}$).

The minimal decrease of $\hat{H}$ in the order of increasing mean age of the experimental phenomena shows that most of the chronological variability is due to hereditary (i.e., genotypical) variability.

On the other hand, the peculiar behavior of chronological variability indicates that the latter, in turn, is a function of the genic time, as postulated by the degradation of *ergon* and, more generally, by the theory of the *E/C system.*

4) The final point, prompted by our study on the onset of new functions and the disappearance of exhausted *E/C systems* in MZ twins, concerns the accuracy of the genetic mechanisms in preserving over the years the timing originally established at amphimixis.

III. Discussion and Conclusions

The problem of the genetics of biological time, from which we started and which we investigated in five groups of studies, led us to observe two phenomena:

a) The existence of a basic gene-related time, which we called *chronon*;

b) The existence, within the gene, of a variable amount of energy, intended to stabilize its information, which we called *ergon.*

The two properties of the gene, mentioned above, the role of which we believe to be fundamental in gene biology, are interrelated. Therefore, in our conclusions, *ergon* and *chronon* will be considered together under the heading of *E/C system.*

Our conclusions will also concern the problem of the genetics of biological time and its implications in several phases and conditions of life.

Therefore, the structure of our conclusions will be as follows:

1) The *E/C system*;

2) Biological time;

3) Development;

4) Senescence;

5) Homeostasis;

6) Disease.

III.1. The E/C system

The concept of *ergon* may be foreshadowed by the following aphorism: " One gene, one stability ".

We call *ergon* of a gene the energy that ensures the degree of stability of its information. The concept of *ergon* allows a distinction between energy of information and energy of stability.

Energy of information concerns the *specificity* of the information, while energy of stability concerns the *preservation* of such specificity in time. Energy of stability is the total result of the stability of each component nucleotide. Stability of information is also a function of the physico-chemical field in which the gene operates, and especially of bond energy.

The example of the template, so widely used in molecular genetics, may be usefully adopted for *ergon*. The symbol represented in the template (a letter, a number, etc) is one thing, while the material of which the template is made is another. The template of a given symbol may be made of wood, lead, bronze, steel or many other materials. The resistance of these materials varies, resulting in different wear and duration.

It is conceivable that the same may apply to *ergon*: two different genes, although coding for the same information, may have different *ergons*, thus differing as to stability of information.

We do know that (1) the degree of stability of a nucleotide differs according to whether it includes the adenine-thymine (AT) combination (higher stability) or the guanine-cytosine (GC) combination (lower stability); and (2) the same information may be provided by different combinations of synonymous codons.

There follows that the AT/AT + GC ratio of a gene determines its energy of stability; also, two genes coding for the same information may differ for such ratio.

Considering that the average human gene includes 500 codons, i.e., 1500 nucleotides, and that synonymous codons normally differ only in one base, it follows that genes coding for the same polypeptide may differ from 1 to 500 nucleotides. Assuming equiprobability of AT and GC, the possible *ergons* of the genic information are distributed as shown in Fig. 10.

The value of *ergon* also depends on the number of times the information may

be repeated in the same gene (redundancy), and on the possibility of " repair " of the gene when damaged.[1]

On account of its variability, the stability of a gene (*ergon*) may be normal, or it may extend into the pathological. Therefore, different individuals from the same species may differ as to the *ergon* of similar genes, according to the aphorism " one gene, one stability ".[2]

In a diploid organism, the *ergon* of each genotype is the total result of the *ergons* of its two component alleles. Such a behavior duplicates the Mendelian behavior of the so-called " primary gene action ".

Following amphimixis, all genes undergo gradual degradation of the respective *ergon*, closely paralleling the concept of entropy at the level of the unit of inheritance. Upon activation of the gene (whether it be for transcription or for duplication) the process of degradation becomes quicker. It appears that duplication entails an even greater degradation than transcription.

A peculiar form of degradation of *ergon* is found in mutation, which represents a sudden, violent, generally irreversible, sometimes total loss of *ergon*; its effect is also different according to whether it occurs in the gametic or the somatic line of cells.

The concept of *chronon* may be foreshadowed in the following aphorism: " One gene, one time ".

We define *chronon* as the period during which the original information of the gene remains unchanged (whether it is used for transcription or duplication, or it remains

[1] One possibility of " repair " concerns the pre-duplication stage, eliminating the undesired nucleotides by transferring them from the insoluble to the soluble fractions of DNA: in the following stage of DNA synthesis the resulting free positions are filled by the right bases, reconstituting the original structure. In this case, " repair " represents restoration of gene structure and thus restoration of *ergon*. " Repair " may also result from the action of operator genes which increase the error ratio during transcription and translation. Since the " errors " include correct readings of the original genic sequence, the damaged gene is not restored but its function is partly restored. In this case " repair " represents a degree of regeneration of information which affects the corresponding organismic *chronon* but does not affect *ergon*.

[2] Thus, the variability of individual *ergons* is a consequence of the degeneration of the biological code. Watson (1965), dealing with the value of the AT/GC ratio in different species, writes: " Higher plants and animals all have an excess of A + T over G + C in their DNA, whereas among the viruses, bacteria, and lower plants, there is much more variation, and both A + T-rich and G + C-rich species occur. These variations, however, are not purely random, and the base ratios of taxonomically related organisms are quite similar. No one yet knows the reason for the wide base-ratio spread. It may be a consequence of but certainly not a prerequisite for extensive evolution. Witness extreme differences between higher plants and animals despite roughly similar percentages of the four main bases ". We believe that the meaning of this phylogenetic variability is to be found in the resulting chronological variability: the excess of AT bonds provides an increased *ergon*, i.e., more stability of information. Thus, the persistence of genic information may be proportional to lifespan.

On the other hand, the concept of stability of information is not in opposition to the current hypotheses concerning degeneration. Nirenberg's (1963) mechanistic model is not an alternative to Sonneborn's (1965) and Woese's (1965) selective models when related to the concept of *ergon:* stability of information is an objective fact, regardless of its cause. The originality of the concept of *ergon* lies in the fact that it goes beyond the degeneration of the biological code to consider the resulting degree of stability of information.

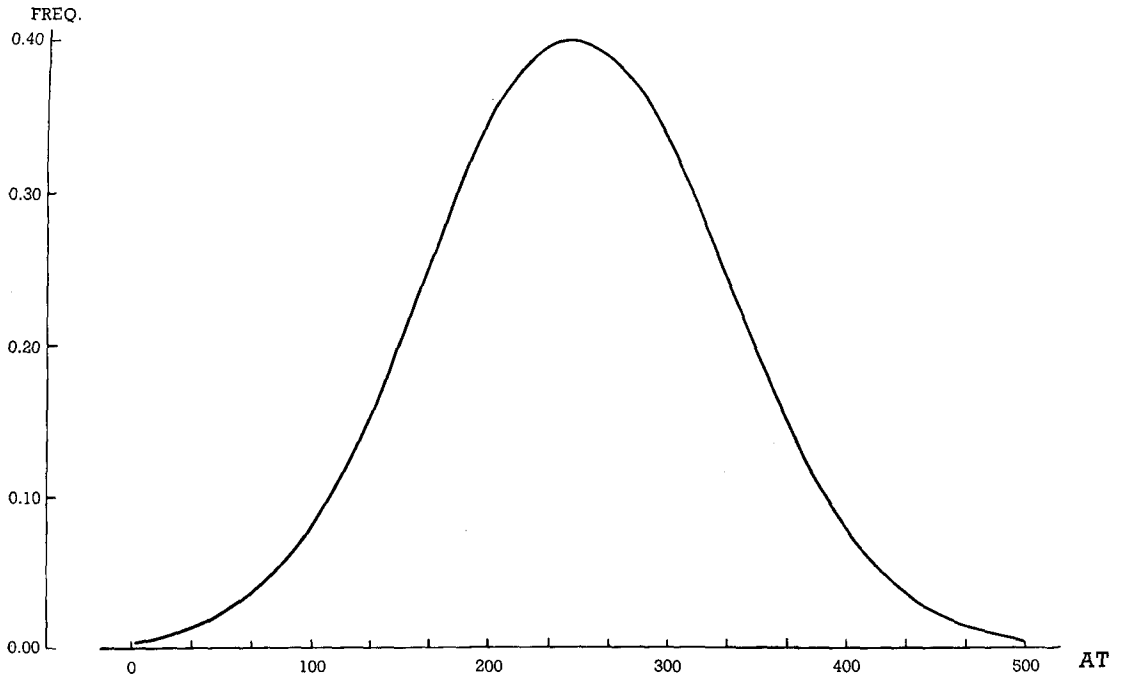


Fig. 10. Distribution of genes in man by number of AT nucleotides.

at the potential stage). There follows that *chronon* is the temporal diameter of the gene as well as the period of availability of its information.

Sensu strictiori, *chronon* is a genic trait. By extension, " *organismic chronon* " is related to the extinction of original information in such a majority of somatic cells as to become insufficient for the requirements of the organism.

It must be remembered that *chronon*, *sensu strictiori*, is a function of the *ergon* of the gene, i.e., a function of the energy which ensures stability of information (cf II.4.3).

We have already seen that *ergon* is a function of five variables: (1) the physico-chemical structure of the gene; (2) the physico-chemical field in which the gene operates; (3) the number of synonymous codons corresponding to a given aminoacid; (4) the possible repetition of information within a gene (redundancy); and (5) the possibility of " repair ". Since *chronon* is a function of *ergon*, it is also a function of the above-mentioned variables.

Organismic chronon is related to several additional variables. The genotypical variables involved are: (1) the type of cells carrying the information (perennial or not); and (2) the type of development of the cell clone and especially the number of mitoses that follow the zygote.

Environmental variables are: (1) the type of environmental requirement: cyclic (e.g., circadian), sporadic (e.g., immunological) or other; (2) the intensity of environmental requirement; and (3) phenomena of interaction between the requirements of the various environments: external, organismic, cellular and genotypical.

Organismic *chronon* is also a function of the threshold number of cells carrying the information required to perform the genotypical program of the organism.[1]

The energy of stability of genic information (*ergon*) and the time during which genic information is available to the phenotype (*chronon*) are fundamental and mutually related parameters of the unit of inheritance. They may be considered together, in terms of factors of a dimensional equation which goes under the name of *E/C system.*

Ergon is the efficient cause of *chronon*, since the energy of stability of information results in the availability of information and the duration thereof.

The two parameters are directly proportional in the sense that higher *ergon* corresponds to longer *chronon* and viceversa.

The particulation of energy ensuring chronological stability of genic information radically changes the problem of gene quantification.

Some authors attempted such quantification by measuring the primary gene action; yet, this seems to be a defective approximation, since it only appraises *ergon* at a given time. The problem should be considered in terms of quantification of the cause, not of measure of the effect. In this sense the best approximation may be provided by a simultaneous estimate of both parameters of the *E/C system.*

Fig. 11 shows a theoretical graph illustrating the system. In a cartesian space two parallel planes represent the genotype and the phenotype. On both planes the ordinate represents energy and the abscissa represents time.

In spite of their correlation, the meaning of the two variables is different on each of the two planes. On the genotypical plane, energy represents stability of information (*ergon*), while time represents the duration of the gene (*chronon*). On the phenotypical plane, instead, the values are related to the manifestation (m) of the corresponding trait; therefore they are conditioned by both the *E/C system* and operator-environment influences. The theoretical gene, whose *E/C system* is represented in Fig. 11, has the following characteristics on the genotypical plane:

1) An initial *ergon* (E_i);

2) A curve of degradation;

[1] Paigen and Ganschow (1965) have proposed the following classification: structural genes, regulator genes, architectural genes and temporal genes. Rieger et al (1968) observe that experimental proof of the existence of architectural and temporal genes is still lacking.

As far as temporal genes are concerned, we doubt their existence, whereas we believe all genes possess a temporal diameter, i.e., the capacity to produce a given phenomenon for a given time.

Paigen and Ganschow's temporal genes are probably regulator genes, whose temporal incidence is probably easier to show, since their activity conditions the activation of structural genes (which also have their respective *chronon*).

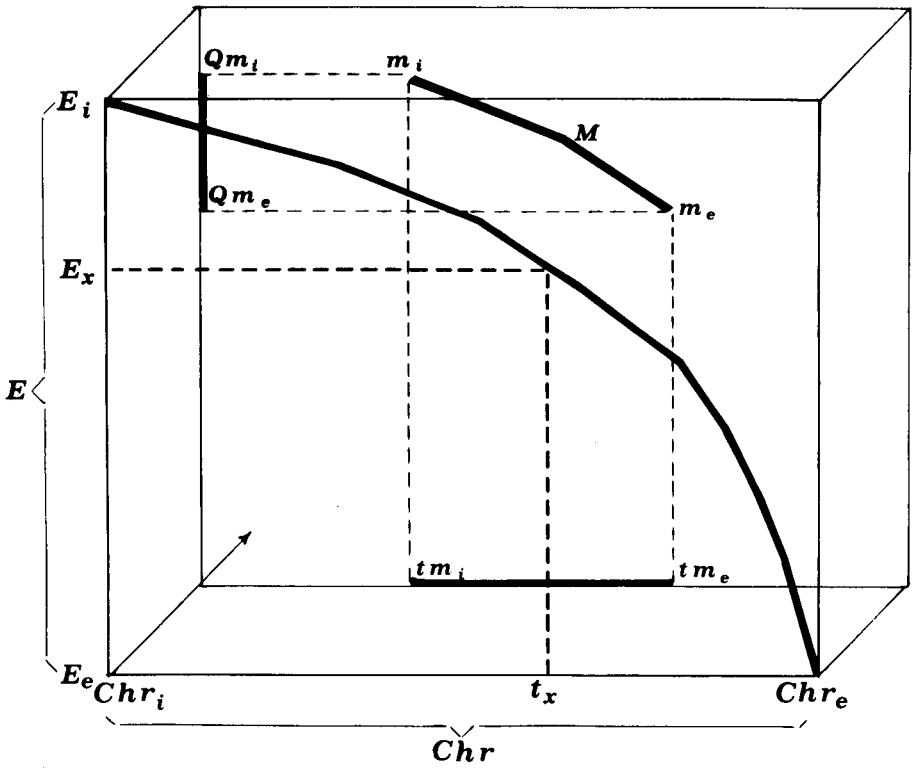


Fig. 11. Genotypical model and phenotypical projection of the *E/C system*.

3) A time of extinction of information, i.e., of exhaustion of *ergon* (E_e), which corresponds to:

4) The limit of the duration of information, i.e., the exhaustion of *chronon* (Chr_e).

Parallel to the persistence of information on the genotypical plane, one or more periods of manifestation may be found on the phenotypical one.

For the sake of simplicity, our graph shows only one period of manifestation (M) extending from the initial manifestation (m_i) to the end of manifestation (m_e). The quantity (Q) of information in the corresponding interval decreases according to the formula: $Q = Qm_i - Qm_e$. Q is obviously related to, and conditioned by, the degradation of *ergon* on the genotypical level.

The graph may be applied to the estimate of the *E/C system* of a gene. If we as-

sume that the degradation of *ergon* follows the law of simple chance accumulation of errors, we obtain:

$$E_x = 1 - \frac{\log\ t_x}{\log\ Chr}$$

which gives the value of E_x (*ergon* at a given time t_x) if the total *chronon* (Chr) is known.

III.2. Biological time

Biological time is a function of *chronon.* The particulation of time at the level of the gene affords a new outlook concerning biological time.

As all biological phenomena have a chronological component, it therefore ensues that such a component is under close genetic control: it is a fact that there are many biological times, varying among species, among populations, among families, among individuals.

The variability of *chronon* is the result of selective forces, resulting in different times of development and of response in different species and even different populations, in order to cope with the requirements of different environments (Haldane, 1932).

The concept of *chronon* provides a typically Mendelian interpretation of chronological variability. The normal methodology of fundamental genetics may be employed in the study of the genotypical variability of *chronon*, since the latter represents, by definition, the temporal diameter of the unit of inheritance. Genetic time may be analyzed through the complex interplay of its components (i.e., of individual genic *chronons*), just as inheritance in general may be analyzed on the basis of the mosaic of its genic component.

The final phenotypical result of genetic time is often more complex than appeared to be the case in our five groups. Yet, the key to the interpretation of phenotypical temporal phenomena is always to be found in the temporal diameter inherent to the *E/C system* of each gene.

Biological time is always related to external time. While basic (astronomic) time is the same for all living beings, physical time may change from one earthly environment to another. The living organism responds to environmental stresses through the *E/C system* of primarily or secondarily involved genes, originating biological rhythms which are studied in chronobiology. Biological rhythms may be annual, monthly, daily, or even more frequent, as in the case of heartbeat or breathing. Such phenotypical rhythms or "physiological clocks" are related to physical time through the immediate or chain reactions of the *E/C systems* of the various genes involved.

There follows that the temporal diameter of the gene and biological rhythms are separated but interrelated concepts. *Chronon* is a universal genotypical phenomenon, since every unit of inheritance has its own temporal parameter. A biolog-

ical rhythm, instead, is a phenotypical phenomenon, resulting from the interaction between *chronon* and exogenous stimuli; such stimuli activate the corresponding genes in the cells that are responsible for cyclic responses. Since the phenomenon of biological rhythm involves the *E/C systems* of a certain number of genes, inheritance also affects biological rhythms, as shown by Halberg's studies (1967).

The nature of genetic time, as we consider it, is different from that of biological rhythms: it concerns the period of availability of information, irrespective of the type of manifestation (whether it be sustained, sporadic, or cyclic).

The existence of a genetic time based on the characteristics of the *E/C system* explains the nature and the genetic mechanism of the physiological and pathological synchronism so characteristically found in MZ cotwins. The chronological discordances induced in such twins by different ecological influences provide an approach towards the appraisal of the characteristics of the *E/C system*, making it possible to discriminate the two individual parameters: energy and time.

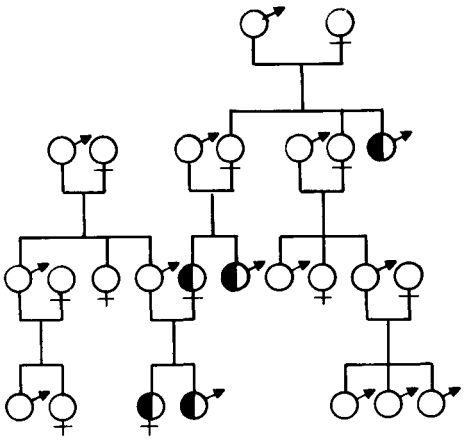
III.3. Development

Development, i.e., the process whereby the organism leads itself from the one-cell condition of the zygote to the (often extremely complex) adult condition, is governed by the orderly sequence of the action of many genes. Development is programmed by regulator genes in the ontogenetic sequence, and thus by the *E/C system* of each such gene. The chronological dimension of these genes (i.e., their respective *chronon*), within the succession of biological times that identifies the species, regulates the duration of each such time, resulting in the normal variability. As a consequence of environment-induced selection, the intensity of *ergon* and the length of *chronon* of ontogenetic genes are responsible for the chronological difference in the development periods of the various races, populations and families.

Regulator genes may exhaust their entire *E/C system* in the course of their developmental duties, or else they may then revert to a silent condition, keeping their information at the potential stage, only to be reactivated in case of need (homeostasis).

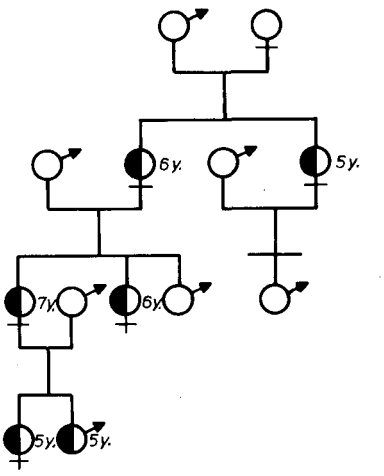
Structural genes normally remain at the potential state for any such need. Genetic variability of developmental *chronon* is demonstrated by those transitional paramorbid conditions which we call developmental anomalies (not to be confused with developmental disorders, i.e., irreversible diseases induced by errors of ontogenetic information). In developmental anomalies the ontogenetic drive is bound to balance at a later stage.

Let us take two examples from a paper by Gedda et al (1970). In the first family tree (Fig. 12), we find an inherited delay in speech: first words at about six years, instead of the normal 12 months, in several family members. In the second family tree (Fig. 13), the delay concerns hair growth, which normally starts before birth, but began around the fifth year of age in six members of this family.



FIRST WORD AT THE AGE OF 4 yrs.

Fig. 12. Familial repetition of retarded speech.



DELAY IN HAIR GROWTH

Fig. 13. Familial repetition of delay in hair growth.

In another study, Bigozzi et al (1961) examined the families of 14 propositi of either sex with delayed puberty, indicating that puberal delay may be transmitted to descendants of either sex by a simple dominant mechanism.

Such chronological anomalies of development may be explained by the concept of *threshold* which the intensity of information must reach to produce the primary gene action. Below the threshold, the gene remains unexpressed. An inherited deficiency of *ergon* may keep the information below the threshold during the normal chronological variability; only at a later date will the normal developmental sequence become expressed.

Such a deficiency of *ergon* may affect either a regulator or a structural gene. In such a way, chronological anomalies of development seem to reveal a deficiency in a mechanism which otherwise functions normally.

As we shall explain more extensively when dealing with disease (cf III.6), anomalies of development represent a case of pathological inheritance affecting the " onset " of the *E/C system.*

III.4. Senescence

The genetics of senescence is especially significant in the perspective of the *E/C system.* Development, as we have seen, brings the organism to adulthood through an " escalation " resembling the *crescendo* of a concert. After a variable period of

balance in the " ideal " condition of development, the organism starts on the descent of senescence, which is symmetrical to development both because it leads to the opposite extreme of life and because it involves a discontinuous exit of units of inheritance from their active, informative period.

Upon closer scrutiny, the process of senescence may be referred to two different genetic mechanisms, both related to the *E/C system*. The first mechanism differs from the mechanism of development since it involves the exhaustion of *ergon* (and the extinction of *chronon*) of structural genes, which hardly occurs during development but is a hard fact of life during senescence. The second mechanism is related to a process similar to that seen in development, involving the extinction of a regulator gene resulting in the reactivation of a no longer useful (and possibly harmful) operon.

An example of the first mechanism may be found in human hair growth. Hair growth represents the balanced phenogenesis of a process of development resulting from the synchronous activity of the genotypes responsible for structure, color and all other characteristics.

During senescence the genotype responsible for color often exhausts its *E/C system* earlier than the genotype responsible for structure, resulting in the occurrence of white hair. In other cases the *E/C system* of the genotype responsible for the function of hair bulbs becomes exhausted earlier than that responsible for color, resulting in the occurrence of baldness before hair turns white.

The likelihood of the involvement of the second mechanism of senescence is based on the mean ratio of active to silent genes in the average cell of the body (Strehler, 1964; Medvedev, 1966). According to the most reliable estimates, the ratio would be about 500 active to 60 000 silent genes; the latter would include 10-12 000 regulator genes.

Since the probability of degradation of the *E/C system* over a given time (and thus the probability of extinction) may be assumed to be similar for all genes, most of the extinctions are likely to occur among silent genes. Yet, it must be remembered that regulator genes are only apparently silent, and in fact whenever they exhaust their *E/C system* the organism is likely to suffer from the resulting derepression of the corresponding developmental genes that have become unnecessary or even harmful at later ages. This mechanism will be discussed again under III.6.

The degradation of the *E/C system* of a structural gene is less likely to cause serious damage. Both the activation of the genotypes responsible for development and the exhaustion (in senescence) of those responsible for normal structure and function follow the respective inherited temporal sequences. Yet, while the former sequence follows an architectural pattern, involving the participation of the entire genotype, the latter depends solely on the *E/C system* of individual genes.

Generally speaking, the termination of exhaustion of *E/C systems* in senescence does not duplicate the harmony of the ontogenetic sequence: it reflects a sort of biological anarchy which only homeostatic mechanisms try to neutralize.

The operative termination of genes during senescence is ascribed to the inherited characteristics of individual *E/C systems* and exhibits typical repetitions within populations and within families. Family and population models in the " emerging disorders " of senescence are thus consistent (and foreseeable) within the genetic variability of the *E/C system.* In different families the onset of senescence may tend to affect preferentially the skin, the bones, the cardiovascular system, the sense organs or any other structure or function, in any characteristic chance combination of discontinuous terminations of the respective *E/C system.*

A general consequence of the progressive exhaustion of *ergon* is the longer time required for the phenogenesis of any trait during senescence. In this sense, we share Strehler's view (1964) that metabolic processes in the aged tend to be slower. This slow-down would result from the scattered exhaustion of *ergon* in the various cells carrying a given information. The gradual increase in the number of cells in which the specific information has become exhausted leads to a proportional decrease in manifestation. Viewing this phenomenon from the opposite side, we agree with Carrel (1935) in underlining the importance of Lecomte du Noüy's equations concerning the " scar index " of wounds (which is in direct proportion to the youth of the individual).

Yet, our concept of senescence (seen as a finite degradation of *E/C systems* in the economy of the organism) does not seem to agree with Carrel's theory of potentially infinite life (based on the survival of his *in vitro* cultures started in 1912). Some authors (cf Swim and Parker, 1957) suggested that such apparent continuity of life might be explained in terms of new cellular DNA supplied to the culture by nutritional embryo extracts; others (cf Puck et al, 1958; Hayflick and Moorhead, 1961) showed that the number of possible mitoses in a cell clone is limited. In our view, the unavoidable degradation of *ergon* in the genotypes responsible for mitosis would not allow unlimited life in a cell clone.

While senescence is largely due to the generalized, gradual exhaustion of *ergon*, resulting in the extinction of *chronon* (according to the hereditary structure of each gene), phenotypical factors may also interfere. An example of such phenotypical influences is the deposit of catabolites (lipofuscins, chetonic bodies, free radicals, etc). In the latter perspective, even lifespan may be considered as a function of the chronological distribution of all the information that is essential for the organism: lifespan appears to be a function of the respective *chronons* of such information.

In the specific case of *D. melanogaster*, the longer lifespan of the wild *Oregon R* strain (cf II.2.3) is due to a later extinction of the *chronons* of genes which become

extinguished earlier in the mutant strains. This proves that lifespan is related to the extinction of the *chronon* of specific genes; their *chronon*, in turn, is related to the degradation of the *ergon* of the respective genes.[1]

III.5. Homeostasis

Any phenotype continuously undergoes a variety of factors tending to modify its *statu quo*. Such factors include the environment in which the organism lives, the accelerations of development and the extinction of genic *chronons* in senescence, physiological phenomena such as nutrition, fatigue, pregnancy, and harmful agents such as bacteria or viruses.

The organism reacts to such modifying agents by means of compensating mechanisms, resulting in a series of different equilibria, each one representing a balanced situation which we call " homeostasis ". The sequence and diversity of homeostatic equilibria is a function of the characteristics of the *E/C system* of every single gene which is activated to balance out the effect of modifying factors. If the *E/C systems* of the genes becoming derepressed in order to preserve homeostasis can cope with the emergency in terms of both specificity and amount of information, then either the same or a different homeostatic balance is reached. This is how the dynamics of homeostatic equilibria keeps most changes from becoming diseases. Homeostasis is a response by the organism as a whole (Mikal, 1967); it is conditioned by the efficiency of the *E/C systems* of the genes responsible for the homeostatic response.

Out of the many homeostatic patterns we may single out:

1) " Development homeostases ", occurring when developmental pressure produces what Locke (1968) calls " the emergence of order in developing systems ";

2) "*Statu quo* homeostases", intended to balance any factors from external or internal environment (the only homeostatic pattern occurring during adulthood);

3) " Senescence homeostases ", intended to neutralize the exhaustion of *E/C systems*.

The variability of homeostatic patterns may be compared to similar mechanisms operating during development: here too, regulator genes condition homeostatic responses. There follows that the efficiency of the *E/C system* of such genes conditions the attainment of a new dynamic equilibrium.

[1] Such a concept of lifespan may be related to parallel studies carried out by interpolation of mortality data obtained from vital statistics. Beard (1959), having discussed the various mathematical models proposed by previous authors, adopts the hypothesis of hit-induced faults, suggesting a model whose application provides data that are superimposable to those provided by vital statistics. On the basis of such an interpretation (apparently consistent with experimental data) death would occur at a given point in a process of gradual degeneration, which is, in our view, the process of degradation of *ergon*.

III.6. Disease

The experimental individuation of the *E/C system* through the five studies described (II.1-II.5) followed a study of the hereditary aspect of chronology that we carried out on different diseases, both in twins and singletons.

Our previous observations have been fully elucidated by our present theory of the *E/C system*; the latter has in turn been confirmed by its application, at the family level, to numerous cases of hereditary diseases.

It is in the area of disease that the limitations and alterations of the *E/C system* prevail; in fact, any damage to the system may be considered as the common denominator of disease. We have seen the fundamental role of the exhaustion of the *E/C systems* of structural genes in the understanding of senescence and of chronic diseases that go with it. Yet, the involvement of the *E/C system* is also essential in diseases that arise in other age periods, or in age-independent diseases.

The most obvious cases involve hereditary diseases in which mutation alters or destroys the stability of a gene. The affected *ergon* is directly responsible for such diseases, since its absence (or critical deficiency) results in the absence of specific information. Such absence may be partial (resulting in a defective or intermittent primary effect), or total (resulting in absence of information and of the primary effect thereof). Mutation-induced pathology may overlap and combine with normal degradation-induced pathology (like embroidery on cloth). An example of such combination is provided by the finding that the slowdown induced by degradation of *ergon* in elderly individuals involves a slowdown of neoplastic processes too.

Mutation-induced pathology bears the imprint of heredity, according to genetic laws, more clearly than does degradation-induced pathology.

Hereditary diseases are lethal (*quoad vitam*) whenever the deficiency of *ergon* results in serious, irreversible damage to an essential structure or function. In these cases, death follows, unless medical science succeeds in supplying from the outside the substance that the organism no longer produces. Diabetes is the obvious example of a hereditary disease that may be lethal unless insuline (which the organism no longer produces, following exhaustion of the *E/C system* of the corresponding genotype) is administered from outside.

Hereditary diseases are chronic (*quoad valetudinem*) whenever the deficiency of *ergon* results in a specific but non-lethal damage. Examples of hereditary diseases affecting health but not life, resulting in a chronic condition without final recovery, are especially frequent in dermatology.

The clinical, diagnostic and prognostic value of *chronon* in genetic diseases is great. Most hereditary diseases are especially variable as to time of onset. Within the limits of such variability, the manifestation of *chronon* is responsible for the disease's chronological imprint in the family. In other words, the fact that members of a family tend to share the same pathological *chronon* explains the fact that pathological times are often replicated in the family.

Diabetes, for example, may have early, adult or late onset. Shorter or longer

chronons may explain the differences in the time of onset; yet, in no such case should the *chronon* be considered as normal. The chronological variability of a disease may also be studied from the viewpoint of crossings, especially in the case of recessive, polyallelic diseases, as we have shown in a recent study of diabetes (Gedda et al, 1967).

The phenomena of " anticipation " deserve special mention at this point. They concern the functions of the *E/C system*, representing an aspect of hereditary chronological variability; they may originate in one of two ways. One origin would be the redistribution of units of inheritance in a consecutive series of matings within a family: the new genotypical combinations may bring about an abbreviation of *chronon*. This type of anticipation may best be called " anteposition ".

The second origin of anticipation concerns individuals sharing the same specific genotype but undergoing a faster consumption of *ergon* resulting from a more strenuous demand for the specific information. An example of this phenomenon is to be found in the chronological discordance as to hereditary diseases that MZ twins may exhibit when living in different environments. The term anticipation seems best suited for this latter phenomenon.

As we stated before, most hereditary diseases may arise from the abbreviation of *chronon*. Yet, some diseases may be caused by the late activation of some genes. In such cases, the defect may consist in the deficiency of the *ergon* of an operator gene. This could be the cause in the phenomenon of enuresis. A recent twin test, carried out on 55 MZ and 72 DZ twin pairs, confirmed the hereditary nature of enuresis (Holzinger's index of inheritance, $\hat{H} = 74.9\%$). The index of inheritance for the age of recovery from enuresis (i.e., for the operative activation of the corresponding genotype) has a value of $\hat{H} = 90.5\%$, confirming the reliability of *chronon* as a fundamental diameter in the quantification of the unit of inheritance (Gedda et al, 1969).

From the perspective of the *E/C system*, we may distinguish a " hereditary pathology of activation " from a " hereditary pathology of termination ", depending on how the *E/C system* affects the operative phase of any given gene.

A further example of hereditary pathology of activation is provided by protracted pregnancies. Recent studies on the " syndrome of protracted pregnancy " (Gedda et al, 1969) have confirmed its familial nature, referring it to damage of the *ergon* of the genotype responsible for the mechanism of parturition. The function of hypophysis (oxytocin, prolactin) is particularly impaired, as indicated by the fact that women with protracted pregnancy showed a significant eccess in the incidence of agalactia or hypogalactia.

As an example of hereditary pathology of termination we may mention gastric and duodenal ulcer: in a recent survey of 92 cases from 54 families, the characteristic " rendez-vous with disease " occurred in 19 cases from 8 families, with a mean within-family variability of less than three years (Gedda et al, 1970*c*). Another

example of the same type of pathology is found in psoriasis. In Fig. 14 we see a family tree (from Gedda et al, 1970*a*) in which the constancy of the age of onset, or the variability thereof according to genetic models, indicate, on the one hand, the existence of genes with different *chronons*, but sharing the responsibility for psoriasis; and, on the other hand, the Mendelian behavior of *chronon*.

A statistical study by Burch (1968) on patient populations suffering from hereditary diseases may be relevant at this point. Burch found that some such populations (including patients with psoriasis and patients with gastro-duodenal ulcer) included sub-populations differing by several modal classes as to age of onset. Burch interpreted his finding as due to the existence of " genetic heterogeneity " in such populations, much as they obviously shared the genetic predisposition towards a given morbid condition. We believe that Burch's " genetic heterogeneity " may best be explained in terms of genetic heterogeneity of the *E/C system*.

As far as most so-called exogenous diseases are concerned, the role of the *E/C system* is different but equally important. Within certain limits, in fact, an external *noxa* (whether viral, bacterial, chemical, or physical) may be pathogenic if the organism lacks specific defence mechanisms. The exhaustion or insufficiency of the *E/C systems* of the specific genotypes of these mechanisms may allow the *noxa* to invade the organism and produce various pathological effects.

In the case of an infectious disease, the destruction or limitation of the *ergon* of the corresponding genotype may either prevent the formation of antibodies, or reduce such formation below the required threshold. On the other hand, immunization may derepress the *E/C system* of the operator gene of an operon controlling specific antibodies, resulting in a critical anticipation. In this way, immunization produces, even before aggression, those substances that the organism would normally produce only after the noxious agent has been recognized. Since recognition would have taken a certain time (which the noxious agent might use for excessive invasion and damage) such anticipation is the critical factor in recovery and survival.

Allergic phenomena often exhibit a familial tendency, appearing in different members of a family at about the same age. Genetic control over this phenomenon has been shown by means of a twin study (Gedda et al, 1961). The onset of an allergic reaction may be explained in terms of *chronon*: during the maturation of the immune defenses, some genotype, responsible for a mechanism of immunological recognition, failed to provide the required information because its *chronon* was already exhausted. A different interpretation involves a chronologically limited functioning of the mechanism responsible for " non-self " recognition. In both cases, the exhaustion of the *chronon* involved, the normal mechanism of non-self recognition and response would fail, and " emergency " responses, of the allergic type, would be employed. It may also be that the exhaustion of the *E/C system* involves the genotype responsible for recognition in connection with specific degradation processes.

As for exogenous diseases caused by chemical or physical agents, we may mention that a study on the incidence of silicosis in Sardinian miners (Gedda et al, 1964) revealed the familial nature of the disease as to type of reaction and as to age of onset.

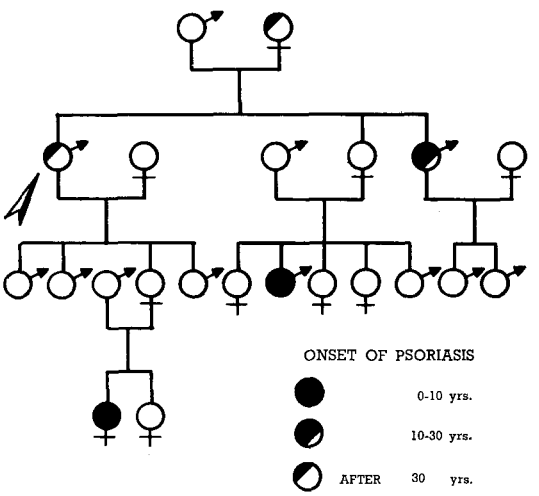


Fig. 14. Familial distribution of the age of manifestation of psoriasis.

Such a familial repetition of the age of onset may be explained by the extinction of the *chronon* of the genic information required for the defense against silicosis.

Even neoplastic phenomena become more understandable when considered in the light of the concept of the *E/C system*. The increased frequency of tumors in senescence may be explained in terms of exhaustion of *ergon*, undermining the stability and authenticity of information in any tissue, especially those in which the *E/C system* is genetically less efficient. Strong (1968) observed that " cancer is the resultant of a particular genetic state at a particular chronological period of time in the life of an individual. Extrinsic forces such as viruses and intrinsic forces such as hormones may play a very significant initiating role, but in the last analysis it is the cell, controlled to a great extent by the genes it contains, that must be involved either directly or indirectly to carry the neoplastic state through succeeding cell division ".

Strong's words are easily interpreted when considering that the failing *E/C system* of a regulator gene may derepress a developmental operator gene, resulting in the neoplastic activity of " silent " structural genes. Such a possibility has been considered in the section on senescence (cf III.4).

Bartalos (1967) referred directly to our thesis when he underlined the importance of the concept of *chronon* in neoplastic disease.

If only the *chronon* of some regulator genes is shortened in certain individuals, then the genes responsible for cell division become activated at the age of termination of those *chronons*. In the case of familial occurrence of cancer, the hypothesis of an inherited early degradation of the *E/C system* of regulator genes may supersede the hypothesis of the existence of specific neoplastic genes.

Our own finding (Gedda and Alfieri, 1966), that cancer may be either concordant or discordant in MZ twin pairs, should be viewed in the light of the following observations: (1) concordance generally includes specificity (i.e., the same form of

cancer is generally found in both twins); (2) concordance generally includes age of onset when young twins live in the same environment; (3) early degradation of *ergon* is not considered by us as the sole agent in neoplastic disease: it represents rather the favourable condition on which exogenous influences may act, thus explaining cases of discordance.

The importance of the *E/C system* in pathology exceeds the simple " to be or not to be " of a disease (due to *ergon* damage) and its age of onset (due to the termination of *chronon*). The imprint of *E/C systems* permeates the morbid process: it is reflected in each of the symptoms that make up the disease. The variability in the symptomatology of each disease has long been considered by medical science as codified in the textbooks of special pathology. Such a qualitative and quantitative variability of the response to the same *noxa* may be considered as a function of the different relative or absolute efficiency of the *E/C systems* of the corresponding genotypes, revealing their weakness on the occasion of extreme stress such as disease.

In fact, disease may hasten the degradation of *ergon*, endangering the stability of information of certain genotypes. There follows a " selection of symptoms ", corresponding to the different nature and function of those genotypes whose *E/C system* is comparatively less stable.

For many years we have stressed the concept of a " *genius familiaris morbi* ", i.e., of a familial imprint (as to times and symptoms) in any endogenous or exogenous disease. The mechanism of the *genius familiaris morbi* is ascribed, on the one hand, to the common variability of *ergon* and *chronon* of genotypes that are shared by family members, and on the other, to the epistatic environment they also share. These mechanisms result in a familial imprint in the response to disease.

Clear proof of the existence of such familial concordance as to symptoms is provided by MZ twin pathology: these twins often show superimposable morbid patterns both in the occurrence of hereditary diseases in different environments and in the occurrence of exogenous diseases in the same environment.

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RIASSUNTO

La concordanza dei tempi fisiologici e patologici in coppie di gemelli umani identici ha indotto gli autori a postulare l'esistenza di un tempo biologico ereditario. Avendo formulato l'ipotesi che il tempo durante il quale ogni gene controlla la propria informazione specifica sia un carattere genetico, gli autori riferiscono su cinque diversi studi sperimentali intesi a verificare la loro ipotesi.

Nella prima ricerca (cf II.1) l'ipotesi è verificata attraverso uno studio gemellare dell'età ossea e dell'età dentaria. Lo studio cronologico, in 40 coppie gemellari umane (20 MZ e 20 DZ), della comparsa dei nuclei di ossificazione nelle ossa carpali e della mineralizzazione delle gemme dei denti permanenti dimostra che questi noti calendari biologici sono controllati dall'eredità nella misura di circa il 70%.

Per mettere in chiaro se il controllo del tempo biologico sia una funzione globale del genotipo o una proprietà del singolo gene, gli autori hanno condotto uno studio sperimentale sulla durata della vita in *D. mela-*

nogaster, in ceppi diversi a struttura genica nota (cf II.2). I loro risultati indicano che la specifica informazione di certi geni controlla la durata della vita. Ne consegue la deduzione che la persistenza della propria informazione specifica sia un attributo di ogni singolo gene. Questa dimensione cronologica del gene viene chiamata « *chronon* », che gli autori definiscono anche come « il periodo durante il quale l'informazione originale del gene rimane immutata, sia che venga usata per trascrizione o duplicazione, o che rimanga allo stato potenziale ».

La determinazione dell'attività della fosfatasi alcalina nei medesimi ceppi di *D. melanogaster* (cf II.3) permette di studiare il quantum di informazione genica (intensità del singolo carattere) e la sua variazione durante il *chronon* del gene. Gli autori ritengono che il quantum dell'informazione decresca gradualmente durante il *chronon* del gene, seguendo il graduale esaurimento di una data energia specifica. La diminuzione nella quantità dell'informazione nello studio longitudinale del *chronon* conduce gli autori a individuare un'unità parametrica fondamentale del gene a cui essi danno il nome di « *ergon* », il quale viene definito come il « quantum di stabilità dell'informazione del gene ».

Nella quarta ricerca (cf II.4), il test gemellare è applicato allo studio dell'indice di associazione dei cromosoni in subcultura di linfociti umani prelevati da gemelli MZ e DZ di 6 e 60 anni. Questo studio permette una valutazione parallela del *chronon* (cioè, durata dell'informazione) e dell'*ergon* (cioè, stabilità dell'informazione). In questo modo, si può constatare che *chronon* ed *ergon* sono correlati, essendo considerati come variabili di un'equazione dimensionale del gene. Pertanto viene postulata l'esistenza del *sistema Ergon/Chronon* (*E/C*).

Nove parametri dello sviluppo e della senescenza (primo sorriso, prima parola, primi passi, comparsa dei peli al pube, menarca, primo capello bianco, prima caduta di un dente permanente, inizio della presbiopia e menopausa nelle femmine), studiati su 666 coppie gemellari MZ e DZ, consentono agli autori di ricavare delle conclusioni particolari intorno alle caratteristiche del *sistema E/C* (cf II.5).

L'interpretazione dei risultati ottenuti conduce gli autori a considerare l'energia di stabilità, o *ergon,* come la risultante della stabilità di tutti i nucleotidi nella sequenza di DNA corrispondente ad un gene. Poiché è noto che il grado di stabilità dei legami AT è maggiore di quello della combinazione GC, e che differenti combinazioni di codon diversi (differenti per almeno un nucleotide) possono fornire la medesima informazione, è chiaro che delle catene polipeptidiche identiche possono essere controllate da *ergon* geneticamente diversi risultanti in *chronon* conseguentemente diversi.

Su questa base, gli autori affermano i due principi: « un gene, un quantum di stabilità » (o *ergon*) e « un gene, un tempo » (o *chronon*).

Il modello del *sistema E/C* viene utilizzato dagli autori per interpretare il tempo ereditario, lo sviluppo, la senescenza, l'omeostasi e la malattia.

RÉSUMÉ

La concordance des temps physiologiques et pathologiques chez des couples de jumeaux humains identiques a conduit les auteurs à postuler l'existence d'un temps biologique héréditaire. Ayant formulé l'hypothèse que le temps pendant lequel chaque gène contrôle sa propre information spécifique soit lui-même un caractère génétique, les auteurs réfèrent sur cinq différentes recherches qu'ils ont conduit afin de vérifier leur hypothèse.

Dans la première recherche (cf II.1), l'hypothèse est vérifiée moyennant une étude gé-

mellaire de l'âge osseux et de l'âge dentaire. L'étude chronologique, chez 40 couples de jumeaux humains (20 MZ et 20 DZ), de l'apparition des noyaux d'ossification dans les os du carpe et de la minéralisation des bourgeons des dents permanentes démontre que ces bien connus calendriers biologiques sont sous le contrôle de l'hérédité en mesure de 70%.

Dans le but d'éclaircir si le contrôle du temps biologique est une fonction globale du génotype ou bien une propriété du gène lui-même, les auteurs ont conduit une recherche sur la durée de la vie en *Drosophila melanogaster* chez des souches différentes à structure génique connue (cf II.2). Le résultats indiquent que l'information spécifique de certains gènes contrôle la durée de la vie. La déduction en suit que la persistence de sa propre information spécifique est un attribut de chaque gène. Cette dimension chronologique du gène est appelée par les auteurs « *chronon* » — aussi défini comme « la période pendant laquelle l'information originaire du gène demeure inchangée, qu'elle soit usée pour transcription ou duplication, ou bien qu'elle demeure à l'état potentiel ».

La détermination de l'activité de la phosphatase alcaline chez les mêmes souches de *D. melanogaster* (cf II.3) permet d'étudier le « quantum » d'information génique (intensité du caractère) et sa variation durant le *chronon* du gène. Les auteurs observent que le quantum de l'information diminue graduellement durant le *chronon* du gène suivant l'épuisement graduel d'une energie spécifique donnée. La diminution de la quantité de l'information dans l'étude longitudinale du *chronon* conduit les auteurs à l'individuation d'une unité paramétrique fondamentale du gène, qu'ils appellent « *ergon* » et définissent comme le « quantum de stabilité de l'information du gène ».

Dans la quatrième recherche (cf II.4), le test gémellaire est appliqué à l'étude de l'index d'association des chromosomes en subcultures de lymphocytes humains de jumeaux MZ et DZ de 6 et 60 ans. Cette étude permet une évaluation parallèle du *chronon* (c'est-à-dire, durée de l'information) et de l'*ergon* (c'est-à-dire stabilité de l'information). Il est ainsi possible de constater que *chronon* et *ergon* sont corrélés, étant considérés comme des variables d'une équation dimensionnelle du gène. L'existence d'un *système Ergon/Chronon* (*E/C*) est par conséquent postulée.

Neuf paramètres du développement et de la sénescence (premier sourire, premier mot, premiers pas, premiers poils pubiens, ménarche, premiers cheveux blancs, première perte d'une dent permanente, début de la presbiopie, ménopause), étudiés chez 666 couples de jumeaux MZ et DZ, amènent les auteurs à des conclusions particulières concernant les caractéristiques du *système E/C* (cf II.5).

L'interprétation de ces résultats conduit les auteurs à considérer l'énergie de stabilité, ou *ergon,* comme le résultat de la stabilité de tous les nucléotides dans la séquence de DNA correspondant à un gène. Puisqu'il est bien connu que le degré de stabilité des liaisons AT est plus élevé de celui de la combinaison GC, et que différentes combinaisons de codon différents (tout au moins pour un nucléotide) peuvent fournir la même information, il est clair que des chaînes polypeptidiques identiques peuvent être contrôlées par des *ergon* génétiquement différents, qui résultent par conséquence en des *chronon* différents.

Sur cette base, les auteurs expriment les deux aphorismes: « un gène, un quantum de stabilité » (ou *ergon*) et « un gène, un temps » (ou *chronon*).

Le modèle du *système E/C* est utilisé par les auteurs pour interpréter le temps héréditaire, le développement, la sénescence, l'homéostase et la maladie.

ZUSAMMENFASSUNG

Die Konkordanz der physiologischen und pathologischen Tempi bei identischen menschlichen Zwillingen veranlasste Verfasser zur Behauptung, dass es ein erbliches biologisches Tempo gibt. Verfasser nehmen an, dass die Zeitspanne, in der ein jedes Gen seine spezifische Information kontrolliert, ein Erbmerkmal sei und berichten nun über fünf verschiedene experimentelle Untersuchungen, die den Zweck verfolgten, ihre Hypothese zu erhärten.

In der ersten Untersuchung (cf II.1) wird die Annahme erstmalig durch eine Zwillingsforschung über das Knochen- und Zahnalter bestätigt. Aus einer chronologischen Untersuchung über das Erscheinen der Ossifikationskerne in den Fussknochen und die Mineralisierung der Zahnkeime der zweiten Zähne bei 20 EZ- und 20 ZZ-Paaren ergbit sich, dass diese bekannten biologischen Daten zu ca. 70% erbbedingt sind.

Um zu klären, ob die Erbbedingtheit des biologischen Tempos eine globale genotypische Funktion oder nur die Eigenschaft des einzelnen Gens ist, nahmen Verfasser einen Tierversuch über die Lebensdauer der *D. melanogaster* bei verschiedenen Stämmen mit bekannter Genstruktur (cf II.2) vor. Ihre Ergebnisse weisen darauf hin, dass die spezifische Information gewisser Gene die Lebensdauer bedingt. Daraus lässt sich schliessen, dass das Beharren auf der eigenen spezifischen Information eine Eigenschaft jedes einzelnen Gens ist. Diese chronologische Dimension des Gens wird " *chronon* " benannt, und Verfasser bezeichnen sie auch als die " Periode, während der die Ursprungsinformation des Gens unverändert beibt " (unabhängig davon, ob sie zu Transkription oder Duplikation gebrauch wird oder in ihrem potentiellen Stadium verbleibt).

Die Bestimmung der Aktivität der Phosphatase alkalina bei denselben Stämmen von *D. melanogaster* (cf II.3) gestattet es, das Quantum der Geninformation (Intensität des einzelnen Merkmals) und dessen Variation während des Gen-*chronon* zu untersuchen. Verfasser beobachten, dass das Informationsquantum dem langsamen Erschöpfen einer gegebenen spezifischen Energie folgend während des Gen-*chronon* allmählich zurückgeht. Die Verminderung der Informationsmenge bei longitudinaler Untersuchung des *chronon* führt Verfasser zur Ermittlung der Grund-Parametereinheit des Gens, welche sie " *ergon* " nennen und als " Stabilitätsquantum der Gen-Information " bezeichnen.

In der vierten Untersuchung (cf II.4) dient der Zwillingstest dazu, den Index der Chromosomen-Assoziierung in Subkulturen menschlicher Lymphozyten festzustellen, welche EZ- und ZZ-Paarlingen im Alter von 6 und 60 Jahren entnommen wurden. Diese Untersuchung lässt eine parallele Bewertung von *chronon* (d. h., Dauer der Information) und *ergon* (d. h., Stabilitität der Information) zu. Auf diese Weise ist zu bemerken, dass *chronon* und *ergon,* die als Variablen einer dimensionalen Gleichung des Gens angesehen werden, korrelativ sind. Daher wird das Bestehen des *Ergon-Chronon-Systems* (*E/C System*) postuliert.

Neun Entwicklungs- und Altersparameter (Zeitpunkt des ersten Lächelns, des ersten Wortes, der ersten Schritte, Auftreten der Schamhaare, der Menarche, Erscheinen des ersten weissen Haares, Verlust des ersten bleibenden Zahns, Beginn der Presbiopie und des Klimakteriums) die bei 666 EZ- und ZZ-Paaren untersucht wurden, gestatten es den Verfasser, eingehende Schlüsse über die Eigenschaften des *E/C Systems* zu ziehen (cf II.5).

Die Ausdeutung der Ergebnisse führt Verfasser dazu, die Stabilitätsenergie oder *ergon*

als Resultante der Stabilität aller Nukleotiden der einem Gen entsprechenden DNA-Sequenz zu betrachten. Da bekanntlich der Stabilitätsgrad der AT-Verbindungen stärker ist als derjenige der GC-Kombination und da einige der verschiedenen codon-Verbindungen (die sich wenigstens in einem Nukleotid unterscheiden) die gleiche Information erteilen können, so ist es klar, dass identische Polypeptidketten durch genetisch verschiedene *ergon* bedingt sein können, die sich dann in dementsprechend verschiedenen *chronon* auswirken.

Darauf gründen Verfasser folgendes Prinzip: " Jedes Gen ein Stabilitätsquantum " (*ergon*) und " Jedes Gen ein Tempo " (*chronon*).

Das Modell des *E/C Systems* wird von den Verfasser dazu verwandt, um die Begriffe Erbtempo, Entwicklung, Seneszenz, Homöostasis und Krankheit zu deuten.

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Explanatory note to the paper:

Human Genetics Studies in Areas of High Natural Radiation

I. Methodology

published in Acta Genet. Med. Gemellol. (1969), **18**: *175-212.*

In my paper published in *Acta Genet. Med. Gemellol.* (1969), **18**:175-212, Fig. 5 represents the final and better estimates of the mean levels of background radiation prevailing in the areas studied in Brazil. Although there is a reference to Fig. 5 in the text of page 189, this text refers exclusively to the data in Tab. I.

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