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Chronology of the Gene

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SUMMARY

The switching of genes from the silent to the operating condition following a rigorous temporal sequence may be called " chronology of the gene ". The succession of times and its biocybernetic mechanism are peculiar to each species, but the duration of information exhibits wide intraspecific variations between populations, families, and individuals. The highly isochronic behavior of MZ twins is proof of the existence of an individual temporal constitution.

Our previous studies have proved that the duration of each information is a property of the correspon-ding genotype, depending on its potential time of activity (*chronon*), which depends in its turn on the same genotype's energy of stability (*ergon*).

In terms of molecular genetics, the stability against mutation of each gene, governing its Ergon/Chronon (E/C) system, is a function of three factors:

(1) Code degeneracy, i.e., the different stability of synonyms coding for the same information through different ratios of GC and AT bases, the former being the more stable.

(2) Redundancy, i.e., the number of repetitions of the DNA molecule representing a given gene.

(3) Repair, i.e., the efficiency of the enzymes and cofactors conditioning the repair of a gene's specific molecular structures.

The E/C system of a given gene, established in the zygote, when the gene is activated according to a species-specific sequence, exhibits wide normal variability (in the phenomena of development, homeostasis, regeneration, and senescence) through variations in the quantity, and thus the duration, of information. The phenotypical variability of chronological phenomena results from the interaction between genetic and ecological variability.

Disease results from a damaged E/C system: hereditary diseases are the consequence of reduced stability of the corresponding specific genotypes, causing shortened duration of their information. In other words, disease is due to an abbreviated informatic chronon, due to the reduction or absence of genic ergon. A damaged E/C system means earlier cessation of specific information. This explains the phenomenon of characteristic times of onset of diseases, described in clinical medicine as an appointment of the predisposed individual with his disease.

The inheritance of biological time explains variability of normal and pathological times in individuals, families, and populations; it also explains the hereditary imprint in the rhythms that physical time determines in vital phenomena.

1. Genetic Determination of Biological Time
(The Ergon/Chronon System)

1.1. The Times of the Gene

An observation that every geneticist may make is that each gene works according to a schedule of times that are long or short, single or rhythmic, simple or compound, individual or of the species, normal or pathological. Genic information in every living being is controlled by a complex and detailed timetable confronting the research worker with the question of *why* and *how* the gene works with this exactitude.

This problem is fundamental to human chronobiology which up to now has developed on the phenotypical plane, emphasizing especially the influence of external synchronizers on biological times.

The problem is, however, also fundamental to genetics, which observes many chronological phenomena demonstrating close links with heredity. Sufficient to mention, on the plane of normal human genetics the impressive and significant phenomenon of the biological synchrony of MZ twins, and on the plane of medical genetics the fact of many hereditary diseases having preferred ages of manifestation.

Some hereditary diseases are produced in endouterine life and are present at birth; others appear during the first months or the following periods of development. Of the ages preferred by hereditary diseases, e. g., schizophrenia prefers 18 and 35 years; familial peptic ulcer the 3rd, 4th, and 6th decade; psoriasis the 2nd and 3rd decade for males, the 3rd and 4th for females; whilst tumours prefer the " third " age; etc.

1.2. A Scientific and Medical Problem

The problem of the impact of disease on man's life is important for preventive medicine (to combat its symptoms at the right time) and for curative medicine (to foresee the course and prognosis of the hereditary disease as to times).

Often, too, the age at which death occurs, if for natural reasons, bears the imprint of heredity. The chronological impact of disease is also of great importance for eugenics, providing as it does basic elements in foreseeing the health curriculum of offspring born of parents with a chronological anamnesis of familial diseases.

Obviously, however, at this time, the greatest interest in this appointment of man with his disease is scientific, that is to say, it regards the still unknown doctrinal aspect of the problem: chronobiology in its turn questions genetics as to the hereditary imprint of observed rhythms.

Alain Reinberg, director of the Rothschild Foundation's Physiological Laboratory in Paris, confirmed this in March 1971, writing as follows: " One may today affirm that rhythmic activity is fundamentally proper to living matter and that, at every organization level, as much for the vegetable as for the animal kingdom, it shows hereditary character. It is no exaggeration to admit that we are born with a certain temporal constitution, just as we are born with a certain anatomy (spatial constitution). Why? I am often asked this, and have to confess I am unable to give a satisfactory answer without having recourse to a whole host of hypotheses."

1.3. Physical Time and Biological Time

Before tackling the problem of the hereditary mechanism of time, it will be useful to refer briefly to physical time and biological time, which coexist as separate, integrated entities.

By the term *physical time* we mean the monophasic and polyphasic periods affecting the perennial becoming of nonliving matter. For instance, we call monophasic the continuous degradation periods of radioactive substances; polyphasic the rhythms of solar and lunar rotation and revolution, etc. This universe of the rhythms of inert matter represents time in the first place, not only because it is the " time " humanity records by means of clocks and calendars, but also and above all because physical time constitutes a reference system for biological times that are far more complex and variable.

By the term *biological time* we mean the immense proliferation of times which is repeated in every living being, producing the succession of forms and functions in its life span.

The temporal phenotype of every living being proceeds in physical time and it is no wonder it receives continuous impulses reflected in characteristic ways in every class of plants and animals, producing rhythms which are obviously shared by the human species. In the life of man, for example, such impulses reflect physical time; circadian rhythms of sleeping and waking, of temperature, pressure, secretion and

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incretion, the monthly rhythm of menstruation, circannual rhythms of the hair and epidermis, the equinoctial rhythms of certain diseases, etc.

On the other hand, it is astonishing that living beings, though subject to the same influence of physical time, maintain biological times highly characteristic and variable from one species to another, one population to another, from family to family, and individual to individual. These are biological times of which physical time cannot be the cause — at least, not the sole cause. We have to seek their origin elsewhere.

Tab. I. Time variations in developmental stages [From Altman and Dittmer, 1964]

	Man	Monkey Rhesus	Sheep	Rabbit	Rat	Chick
Morula 4 cells	48 hr	36 hr	34 hr	11 hr	48 hr	3¼ hr
Beginning of implantation	6½ da	9 da	10 da	7 da	6 da	—
Somite embryo	27 da	25 da	17 da	9 da	10½ da	3 da
End of embryonic period	36 da	28 da	21 da	10 da	12½ da	5 da
Open eyelids	140 da	—	84 da	42 da	38 da	21 da
Birth	267 da	164 da	150 da	32 da	22 da	22 da
Life span	70 yr	30 yr	20 yr	—	34 yr	30 yr

Tab. II. Life spans of plants, seeds, and pollens [From Altman and Dittmer, 1964]

Plants		Seeds		Pollens	
Sequoia gigantea	2000/3000 yr	Nelumbium nelumbo	150 yr	Malus pumila	1465 da
Quercus alba	300/600 yr	Trifolium pratense	100 yr	Prunus domestica	1278 da
Fagus grandifolia	300/400 yr	Triticum aestivum	10 yr	Lycopersicon esculentum	1095 da
Magnolia grandiflora	80/120 yr	Zea mays	9 yr	Cucumis melo	30 da
Salix nigra	50/125 yr	Lycopersicon esculentum	312 da	Betula lutea	30 da

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1.4. The Hereditary Imprint of Biological Time

But, first, it is necessary to demonstrate the hereditary imprint of biological time. We need only glance through a manual such as Altman and Dittmer's " Biology Data Book " (1964) to reap a harvest of data regarding the variability of certain animals, showing conspicuous species variation in development chronology (Tab. I), and regarding the average life of plants, seeds and pollens, also demonstrating heredity-related chronological programming (Tab. II).

Bünning (1963) and Rensing (1968) observed rhythmic times having a hereditary imprint respectively in beans and in insects.

1.5. Variability among Species, Populations, Families, and Individuals

A variability at population level may be observed in the chronology of the human species. For example, growth speed during the various periods of human development differs with different populations (Lester and Millot, 1939). (See Fig. 1).
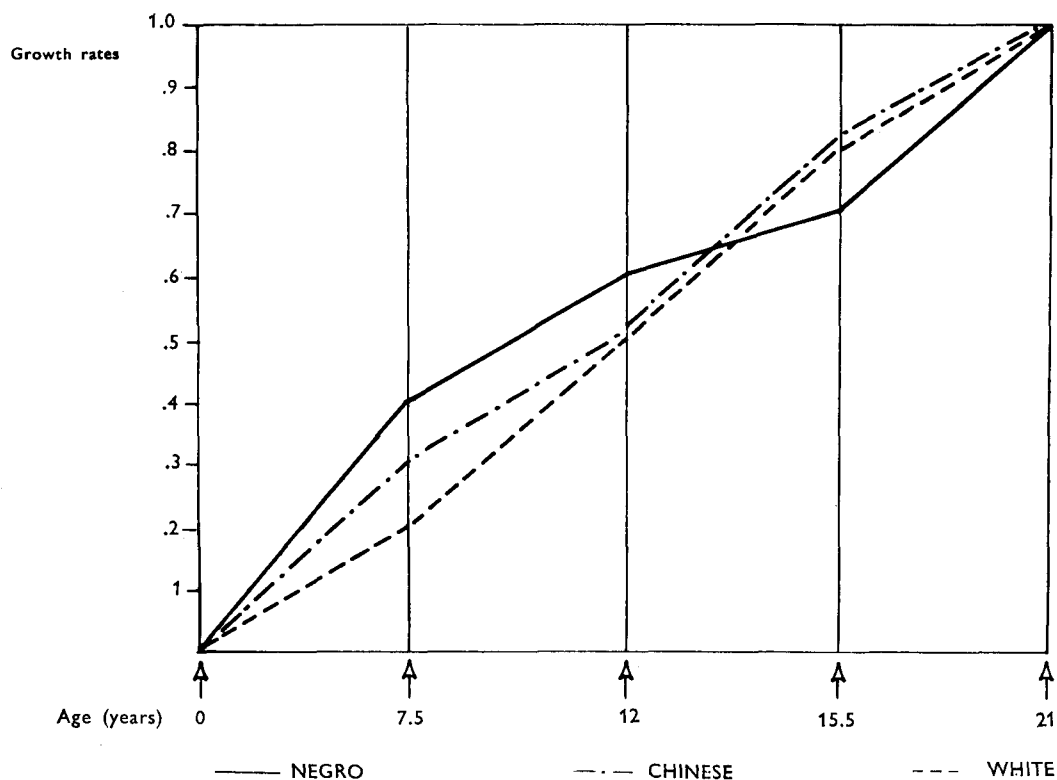

Fig. 1. Growth rates in different human populations

In the pathological field, Burch (1968) and ourselves (Gedda and Brenci, 1970a) have demonstrated the existence of populations with differing chronological models in the same disease. For instance, the population study of diabetes reveals the existence of three modal groups corresponding to diabetic populations with onset of the disease respectively in youth, maturity, and old age (Fig. 2).

Within each such subpopulation we find, in turn, a characteristic delimitation of chronological variability in families. Genealogical analysis of diabetes, for example,
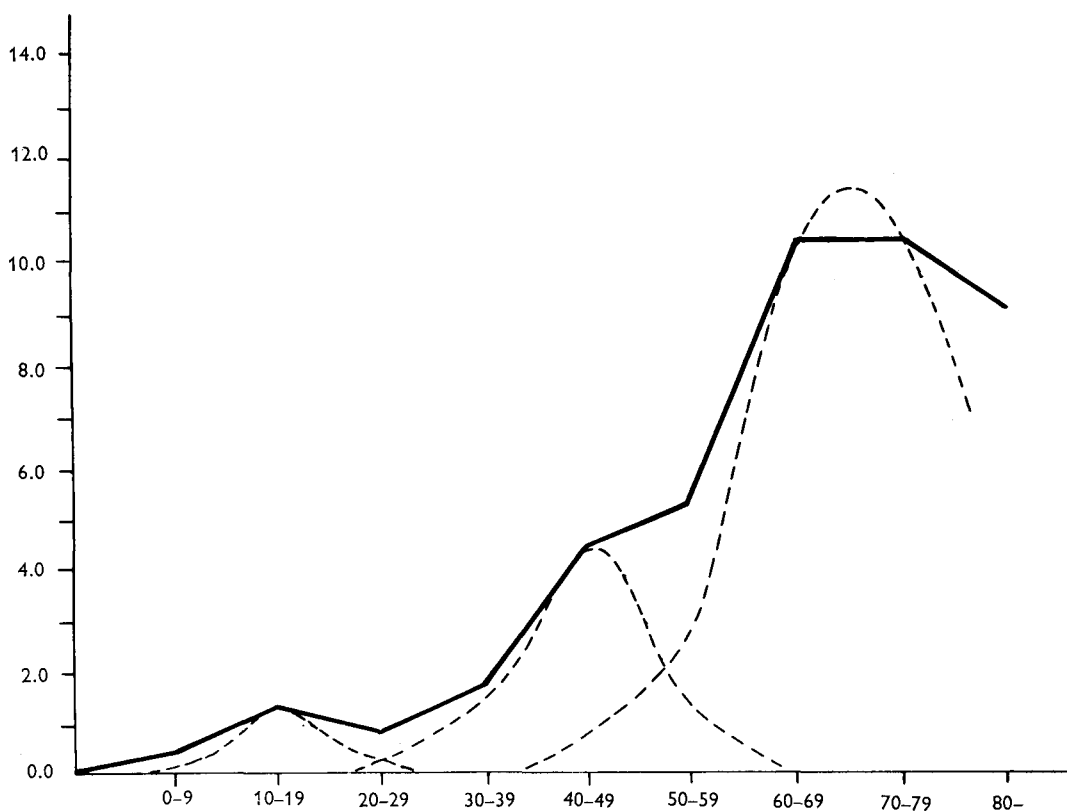
Fig. 2. Different modal groups in a diabetic population classified by age.

shows that onset of the disease in the offspring is determined by the chronological type of the disease in the affected parents. Admitting diabetes to be inherited with a polyallelic recessive mechanism, and three different alleles to correspond to different stability of information, we may establish the diagram presented in Fig. 3, in which the gravity of the disease is proportionate to its precocity.

We may also mention a family (Fig. 4) in which we find the phenomenon of anticipation described by Penrose (1948) for diabetes and other diseases. The *propositus*, macrosomic at birth, shows severe juvenile diabetes, whilst the father is a mild

Staub-negative prediabetic, and the grandfather shows moderate adult diabetes (Gedda et al, 1967). Basing our conclusions on what is indicated in the previous diagram (Fig. 3), the hypothesis may then be formulated (Fig. 5) as to how the grandfather and father affected by adult diabetes, mated to nonaffected carrier women, may have transmitted juvenile diabetes to the *propositus*. As we can see, the
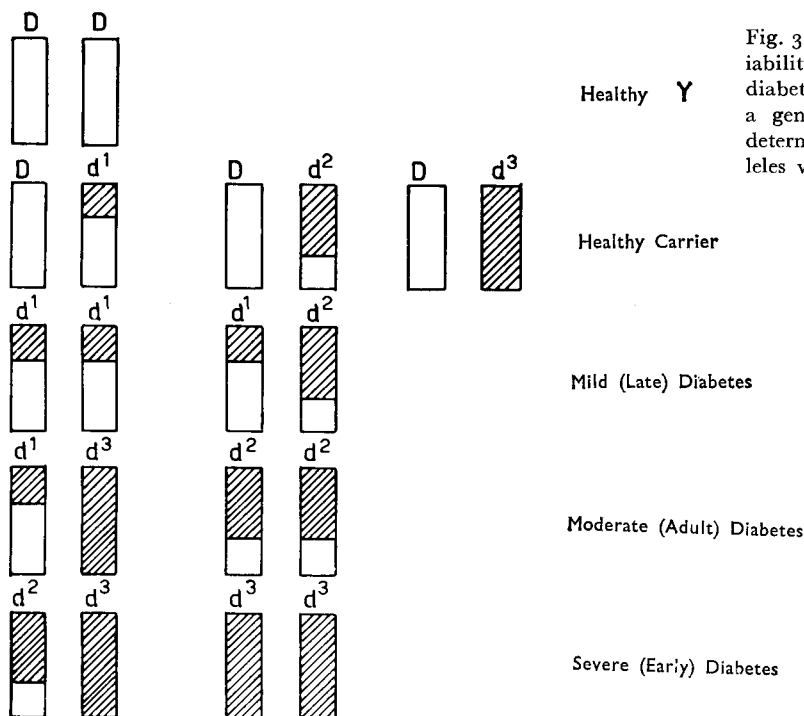
Fig. 3. Chronologic variability in the onset of diabetes, interpreted as a genotypic variability determined by polyalleles with different stability.

Healthy Y

Healthy Carrier

Mild (Late) Diabetes

Moderate (Adult) Diabetes

Severe (Early) Diabetes

Fig. 4

Mild (Late) Diabetes

Moderate (Adult) Diabetes

Severe (Early) Diabetes
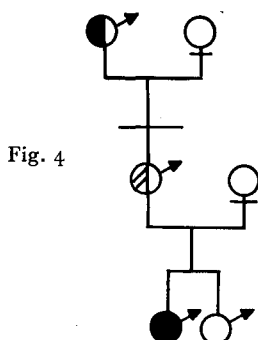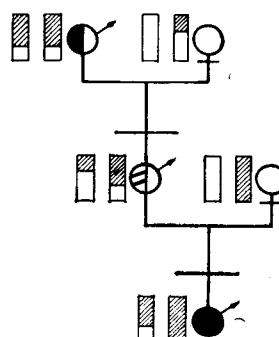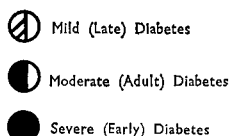
Fig. 5

Figs. 4 and 5. Pedigree with chronologically different diabetic phenotypes (4), and with their corresponding genotypes (5).

times of onset of the disease are strictly correlated within the family, so that a coherent hereditary explanation may be given.

Individual hereditary chronological variability, i.e., the existence of *individual time*, is demonstrated by the twin test. In fact, twins originating from the same zygote are concordant as to physiological times and rhythms as well as to hereditary disease and to chronological type of the disease. This concordance proves the existence of a chronological program repeated in the cotwin and shows that the mechanism of isochrony resides in the identical genotype.

1.6. THE TEMPORAL DIMENSION OF THE GENE

On these bases, it is possible to ascribe the origin of biological time to the genotype and to localize in the genotype the mechanism of reception and response to the environmental physical time.

Fig. 6. Average life span in different parental strains, and in their offspring strains, of *Drosophila melanogaster*.

Since biological time appears of a genetic nature and seems to be inherited as a Mendelian trait, one may infer that the hereditary unit of biological time corresponds to the gene. Our ow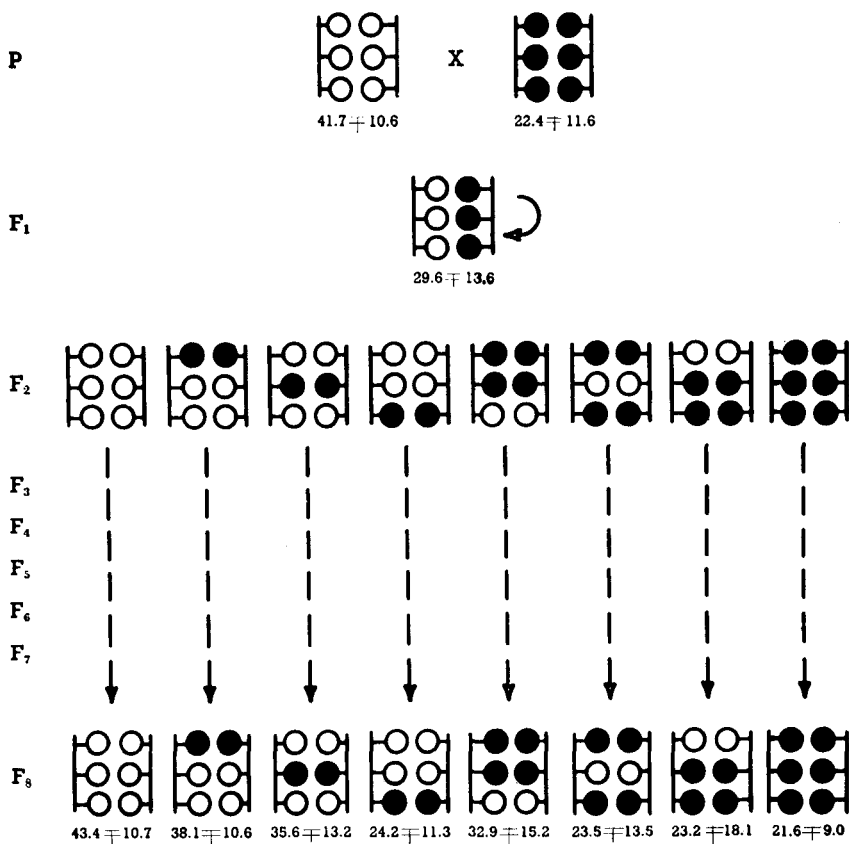n hypothesis is that the gene is responsible not only for the quality of the trait that corresponds to its information, but also for the duration in time of the information itself and of the corresponding phenotypic trait. In our hypothesis, every gene possesses a temporal dimension; biological times, whether autonomous or correlated to physical time, result from the simple or combined action of these temporal units.

The temporal dimension of the gene is the interval of time during which the gene retains its own information potential. We give the name *chronon* to the information time length potentially possessed by the gene.

1.7. Experimental Verification

In order to verify our hypothesis on an experimental plane, we have reported a series of researches which we consider to be demonstrative (Gedda and Brenci, 1969).

Research 1 – Making use of pure strains of *Drosophila melanogaster* we carried out factorial analyses of life span. The genotype and life span of every strain was known (Fig. 6). Crossing the *Oregon* strain with the *Bw, Cn, Vg* triple mutant strain we found in every descendant strain examined that homozygosis for a given locus produces a different and characteristic life span, and that heterozygosis for a pair of alleles produces a life span intermediate between those of the parent strains.

Our experiment thus confirmed (1) the chronological competence of the gene; and (2) the Mendelian behavior of the time trait.

Research 2 – By means of *Drosophila melanogaster* we made a longitudinal study of gene information, i.e., of its quantity in time (Fig. 7). As experimental trait we chose alkaline phosphatase, and as material *Drosophila* pupae of pure and hybrid strains. Our most instructive observation concerned the quantity of enzyme, which proved to be progressively decreasing during the larval stage. We therefrom deduced that the information flow depends on an information potential possessed by the gene in a determined quantity which becomes progressively exhausted and is proportionate to the information time length, i.e., to the chronon of the gene.

Research 3 – We further studied the " acrocentric chromosomes association " phenomenon occurring in the karyogram of leukocytes in vitro; we interpreted this phenomenon as indicating exhaustion of the information assuring the normal spacing of these chromosomes. Prokofieva (1968) already demonstrated that the phenomenon of association increases with the age of the *conceptus*. We extended our experiment to the lymphocytes of human MZ twin pairs of very different ages: 6 and 60 respectively (Fig. 8). We observed that the phenomenon of association increases with age. This demonstrates that the genotype responsible for nonassociation also shows progressive exhaustion, i.e., increasing loss of information potential (i.e., of ergon).

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1.8. THE ERGON/CHRONON SYSTEM

The experiment carried out on the *Drosophila* enzyme (alkaline phosphatase) and on an atypical mitotic process of human leukocytes shifted our studies from the phenotypical to the genotypical plane. On the latter we had to admit that the information potential of the gene corresponds to a " quantum " of energy (possessed by the gene itself) which becomes progressively exhausted.

The relationship of the gene to the chronon is therefore that of an energy potential which becomes actual in a period of time corresponding to the chronon. To this
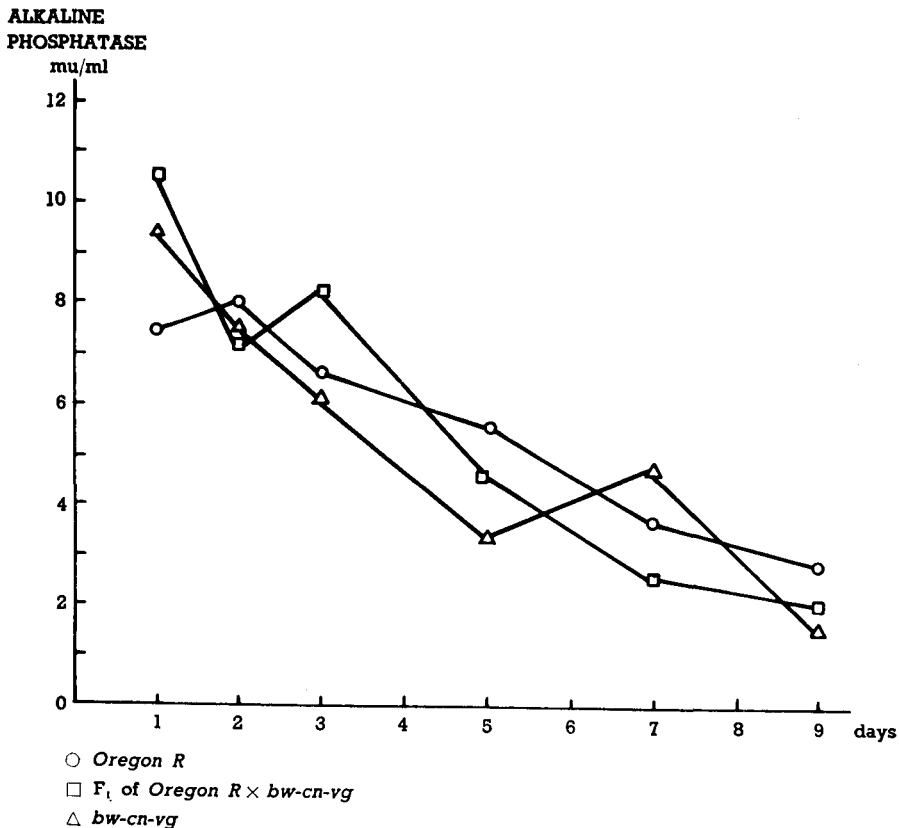
Fig. 7. Decreasing activity of alkaline phosphatase in *Drosophila* pupae of pure and hybrid strains.

quantum of energy we have given the name *ergon,* and since ergon and chronon are two aspects of one and the same phenomenon, we speak of the *Ergon/Chronon system* (E/C system).

The mechanism relating to the E/C system is typically Mendelian because it consists of the fragmentation of complex biological times into simple units and of the

attribution of each of them to a gene. We consider that the fourth dimension, i.e., the time length of the trait, must be referred to the gene which, in the Mendelian hypothesis, controls the qualitative variability. Bound to the
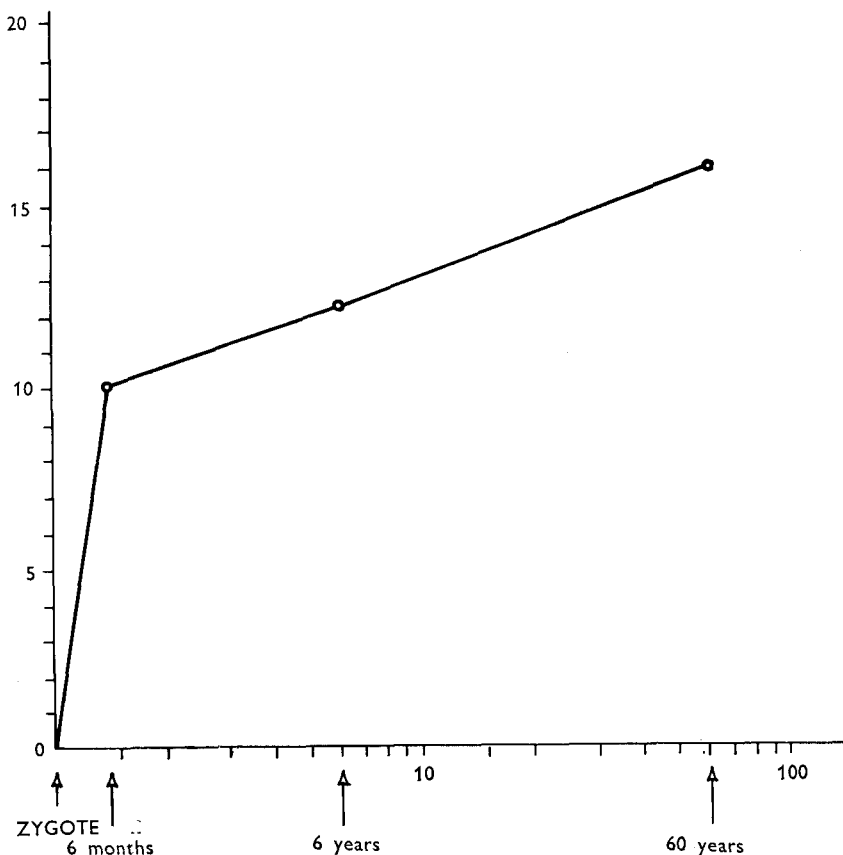
Fig. 8. Frequency of acrocentric chromosomes associations in phytohemagglutinin-induced metaphases in lymphocytes from donors of different ages. (The data for the age, 6 months, are drawn from Prokofieva, 1968.)

gene, the E/C system explains the endogenous source of biological time (Gedda and Brenci, 1970b).

At this point it becomes necessary to investigate the E/C system on the plane of molecular genetics, and for this we must refer to the concepts of stability and decay of the DNA molecule according to the principles of thermodynamics.

2. Models for the Study of the Ergon/Chronon System at the Molecular Level

2.1. STABILITY OF THE DNA MOLECULE

We consider as stability of a system its ability to resist the forces which tend to alter its state. Therefore, stability consists in the possibility of equilibrium between a system and the field in which the system is placed. The actual possibility of describing, from a thermodynamic point of view, "states far from equilibrium" of dissipative structures, i.e., of structures maintained through continuous exchanges of energy and matter, permits today the study of the stability of structures such as the DNA molecule.

Since the beginning of the studies on the helix structure (1950-1960), experimental researches were carried out, demonstrating how the molecules of this acid may have a different degree of stability according to the $AT/(AT+GC)$ ratio.

This led us to focus our attention on the different value of GC and CG pairs with respect to AT and TA ones. In the course of our research in the last few years we based ourselves on the presence of a different number of H bonds in the two cases (i.e., three for GC and CG, and two for AT and TA) and have now eventually come to the following conclusion: the higher the excess of GC and CG pairs with respect to AT and TA ones, the higher the stability of the information. Now, on the basis of studies on DNA denaturation (through variations of temperature, pH and molarity) the existence of a relationship between the degree of denaturation and the base-ratio of the DNA molecule has been demonstrated. Analogous experimental researches have pointed out the existence of a decreasing stability of the hemimolecules according to whether they were composed of ATGC or AT or A polynucleotides. Further evidence of a differential stability of the information is found in the fact that DNA duplication and transcription are handicapped by the formation of pyrimidine dimers, whose probability is different according to whether they are TT or CC. Finally, various mutagenic agents (free radicals, alkylating agents, etc.) operate in a differential manner according to the base-ratio of the DNA molecule.

These and other similar reports in the literature have been considered nonconclusive. For example, Watson (1965) observes: " 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," and he adds that the variation in base-sequences is sufficient to produce genic differences.

We maintain that the different $AT/(AT+GC)$ ratios possible for the same information have a causal motivation in the differential stability of the DNA molecule.

2.2. DEGRADATION OF THE DNA MOLECULE

We intend degradation as the irreversible phenomenon by which highly complex structures change into less organized ones.

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At the level of " dissipative structures, " such as the DNA molecule, the maintenance of a given state of organization is characterized by minimum entropy production compatible with bond conditions.

If we introduce unidirectional as well as fluctuating perturbations, however minimal, the degradation curve becomes exponential.

This kind of degradation (D), similar to the decay of radioactive nuclei, may be described by the formula $D = N\lambda$, as a function of the initial number of repetitions of the information (N) and of the degradation constant of the same information (λ), expressing the probability of degradation of a given information structure in the time unit.

Of course, different repetitions of the same information may become degraded independently one from the other, but, since the different degradation modalities are characterized by partial constants, the final probability of degradation results from the total of partial probabilities, i.e., $D = N\lambda_1 + N\lambda_2 + ... + N\lambda_n$.

If we accept the hypothesis of a random degradation, the life span of any given repetition, i.e., the length of its degradation interval, may vary between o and ∞. If we introduce the concept of average information life span, i.e., the average degradation of the single repetitions of the information (V_i), we obtain a definite value, which may be calculated each time. In the hypothesis of exponential degradation, it is possible to demonstrate that $V_i = 1/\lambda$, i.e., the inverse of the degradation constant.

The existence of degradation phenomena in biology may be experimentally proved at the following three levels.

The first level concerns the phenotypic degradation. Out of a wealth of supporting evidence we may pick a table by Strehler and Midvan (1960) illustrating the decline in time of several functional indexes (Fig. 9).

On a second level we find degradation phenomena in the hereditary material. Various progenetic studies, on the material transmitted from one generation to another, indicate that exhaustion of gametic efficiency is correlated to parental age. We ourselves had the occasion to verify the modalities of this phenomenon in *Drosophila*, studying the times of ontogenetic development with respect to parental age, development being as longer as older a parent (Tab. III).

The third level identifies degradation in the gene. On the basis of studies concern-

Tab. III. Period of development (in days) from fertilization to emergence from puparium

Sex	Age (in days) of one parent[a]				
	2	4	6	8	10
♂	8.5	8.7	9.1	9.3	9.9
♀	9.2	9.5	9.3	10.3	10.6

[a] The age of the other parent is constant (6 days) in each case.
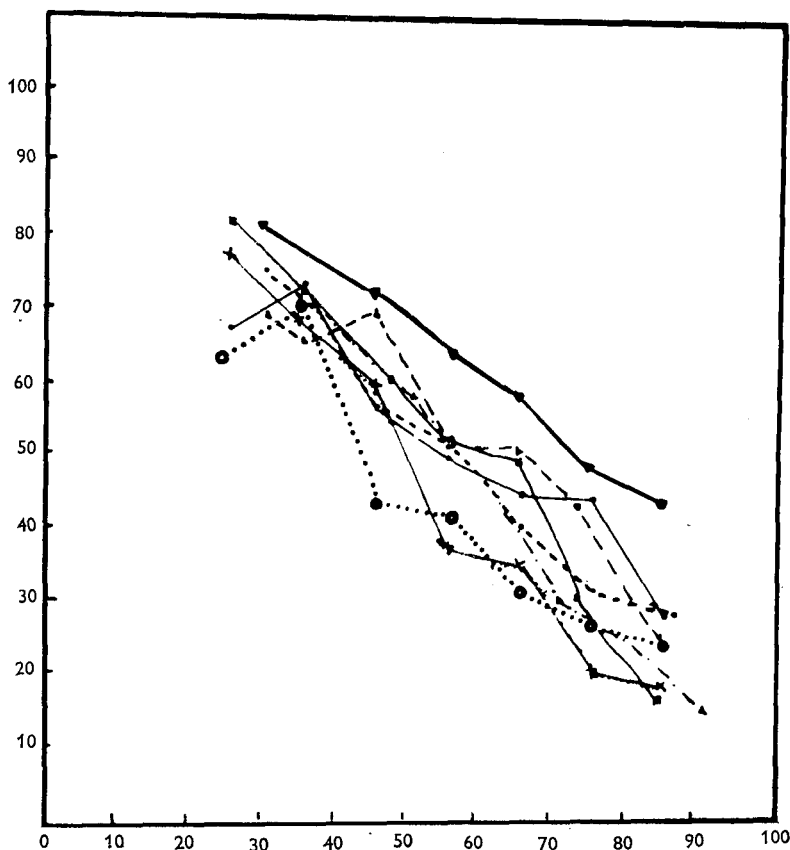
Fig. 9. Decline in time of a number of functional indexes:
·—·— nerve conduction velocity; ▲—▲— maximal breathing capacity;
●—●— standard renal plasma flow (Diodrast); ▲---▲ standard glome-
rular filtration rate inulin; · — · — · basal metabolic rate; ×—×— standard
cell water; ·----· vital capacity; o·······o·· cardiac index.

ing the phenomenon of rearrangement in long-term cultures of leukocytes from MZ twins, we could demonstrate that the exponential inactivation curve of disjunction genes is synchronous only in individuals with identical genic structure.

2.3. ANALYSIS OF GENE STABILITY

Having listed the definitions and evidence for the existence of (1) differential stability, and (2) degradation of the hereditary information, it is now possible to give an analytical description of both phenomena.

As far as the stability function is concerned, the sources of variability should be defined first.

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On the basis of the model we have adopted, i.e., the E/C system, genes codifying for the same information may differ as to stability according to the following parameters:

(1) An " a " parameter, determined by the stability of each of the triplets codifying an information, i.e., a function of the different stability of the synonymous codons (*degeneration*).

(2) A " β " parameter, determined by the number of repetitions of the same information (*redundancy*).

(3) A " γ " parameter, determined by the differential possibility of restoration of the information (*repair*).

The field of existence of the " a " parameter may be defined considering that an average DNA molecule includes 1,500 nucleotides, i.e., 500 codons, and that in every codon the first two bases are highly specific, while the third one is relatively indifferent.

Stability being greater in the GC than the AT pair (as has been proved in already-cited studies), the field of existence of the parameter is limited by the possibility of GC (or CG) pairs in triplets third position,[1] thus varying between 0 and 500.

The field of existence of the " β " parameter may be calculated considering that chemical estimates give 1,200,000 DNA molecules in the human nucleus, while biological estimates indicate an average of 60,000 genes in the same nucleus.

Thus the field of existence of the " β " parameter may be assumed to vary between 1 and 20 redundant DNA molecules.

Finally, the field of existence of the " γ " parameter is included between 0 (impossibility of repair) and 1 (ability of total repair).

2.4. MATHEMATICAL MODEL OF GENE STABILITY DEGRADATION

A single parameter is sufficient to define the degradation function of a gene. In fact, it is sufficient to postulate for each individual, in a given environment, a " μ " quantity (constant in time) corresponding to the intensity of alterations per gene per time unit.

The previous condition may be explained in terms of formal genetics, on the basis of the fact that in our model the mutation rate is constant in time for each gene, as long as the physico-chemical field is constant.

Now, indicating by $p(t)$ the probability that a gene may still have validity at the time t, and considering the exponential model of degradation as more likely, the analytical function representing the above phenomenon is:

$$p(t) = pe^{-\mu t}$$

On the basis of such parametric definition of both phenomena (stability and degradation) and assuming stochastic independence in the production of errors among the

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[1] The different chronobiological significance of triplet third-position bases in synonymous codons seems to be confirmed by the finding that, whenever one aminoacid is coded by two triplets only, a third-position A or T base in the one always corresponds to a G or C base in the other.

repetitions of the same information, it is possible to define mathematically a series of relations leading to an estimate of the average period of degradation of a gene.

In fact, h being the number of still efficient informations at time t, and N the initial number of informations, the distribution of probability $p_h(t)$, i.e., the distribution of the survival probabilities of a given number of repetitions of the information at the moment t is:

$$P_h(t) \; = \binom{N}{h} \; P(t)^h \; (I-P) \; (t)^{N-h}$$

Considering this as a random process with independent increments, it is possible to obtain, on the basis of this definition of the degradation function, the period of phenotypic degradation of each information.

Now, S being the minimum number of repetitions for normal development of the gene product, and N the number of initial repetitions (at time t_0), we have:

$$f(t)dt \; = N_s P_s \; dt$$

it follows that the time t of phenotypic degradation of each information is given by:

$$t \; = f(t)dt$$

and finally, solving by integration in parts, we have (k being the integration constant):

$$t_d \quad \frac{I}{\mu} \sum_{i=0}^{I-S} \quad k \quad \frac{Si}{Ni}$$

The possibility of calculating the average degradation period of a gene, i.e., its chronon, may thus be obtained.

3. Development, Senescence, and Disease in the Light of the Ergon/Chronon System

The definition of the E/C model allows to face the chronology of the gene in its two fundamental aspects: one essential or primary, the other induced or secondary.

(1) The essential aspect represents the gene's selfprogrammed time sequence (based on its inborn energy of stability) which is reflected in its information flow.

(2) The induced aspect represents the interference of physical time on the gene's essential chronology, whereby primary genic time sequences, much as they retain their hereditary nature and resulting variability, also bear the imprint of cosmic time.

This classification of the chronology of the gene opens a new perspective for the study of normal and pathologic phenomena in the human phenotype.

3.1. GENE STABILITY IN THE GAMETE

The resolving power of the E/C system may concern an individual even before conception, i.e., it may concern the parental gametes from which he will originate.

The differential duration of the pre-imago embryonic period in *Drosophila*, as related to parental age (Gedda and Brenci, 1970b), may explain what Turpin's school identified as " progénèse " (Turpin, 1955), i.e., the higher efficiency of conceptuses when born to younger parents. In the light of the E/C system this finding is explained as a consequence of the higher stability of genes in gametes produced in earlier age (their ergon has undergone less degradation with time).

At the Genetic Counseling Service of the Mendel Institute in Rome, with the cooperation of Del Porto, we have gathered many familial cases which seem to support our view. Here are some examples:

— Case 69/60 (Fig. 10): the first-born is normal; the second child is a mongol, followed by four abortions.

— Case 70/30 (Fig. 11): a stillbirth comes first, followed by abortions, respectively at 5, 3, and 2½ months.
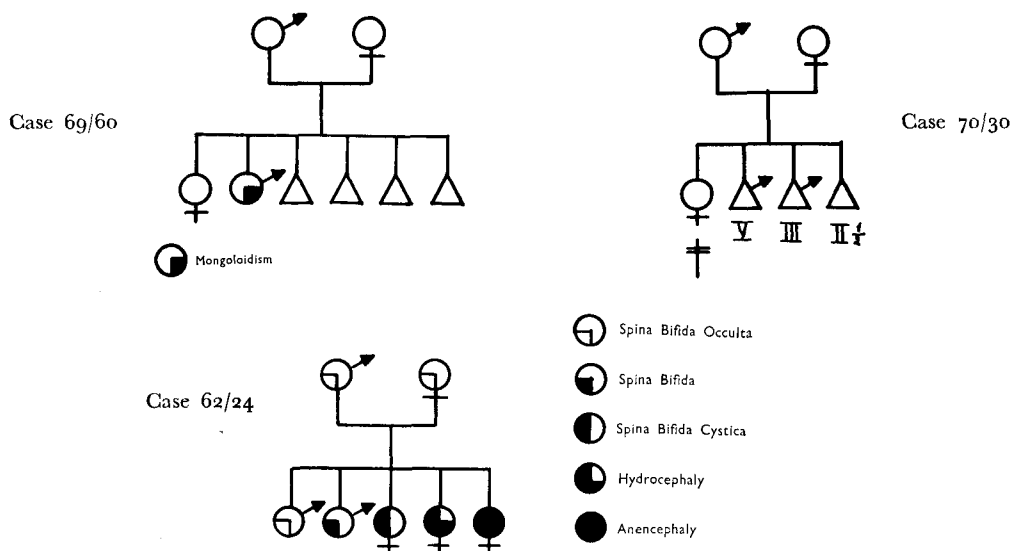
Figs. 10, 11, and 12.   Increasing expression of genetic damage by maternal age.

In other families a given systemic damage becomes increasingly serious with higher birth order, i.e., with gametic age.

In case 62/24 (Fig. 12) both parents exhibit spina bifida occulta. Their children were progressively affected as follows: I, spina bifida occulta; II, spina bifida; III, spina bifida with lumbosacral meningocele; IV, hydrocephaly; V, anencephaly.

The same phenomenon may be verified in population data, as illustrated by a

graph (Fig. 13) representing the frequency of stillbirths, malformations, mongolism, and multiple births with increasing maternal age.

These phenomena exhibit an exponential increase with increasing maternal age, in full agreement with our model, underlining gamete " senescence " and the gradual decline of generative normalcy in the woman; the latter phenomenon is interpreted, in our view, as due to loss of stability by the gene systems involved.

A Stillbirths   B Malformations   C Mongolism.   D Twins

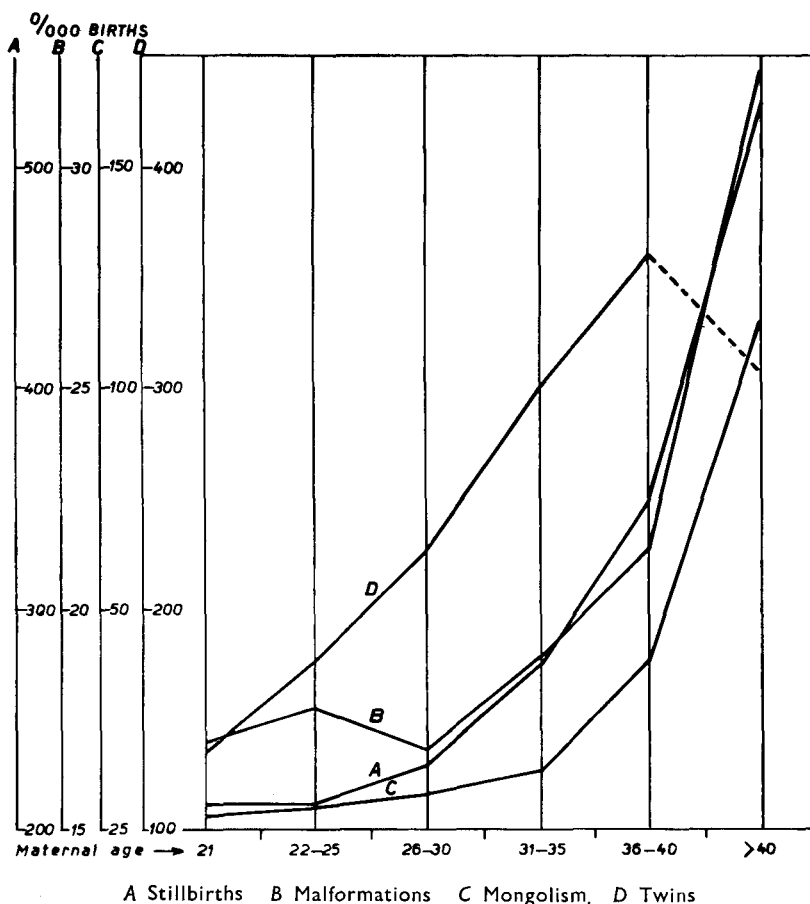Fig. 13.   Frequency of stillbirths, malformations, and twin births (ISTAT, 1956-58), and of mongolism (Oester, 1953), according to maternal age.

3.2. AMPHIMIXIS AND THE E/C SYSTEM

Amphimixis establishes the ergon and chronon of the approximately 60,000 genes of the conceptus on the basis of the stability of genetic alleles. A much simplified diagram may explain the situation as it occurs at the zygote level (Fig. 14).

The ergon (Er) of a normal gene is proportional to the biological requirements of its information (I). Through transcription (T) and translation (t) the latter produces a polypeptide (P) for a corresponding time, i.e., a normal chronon.

A mutated gene, i.e., one from which mutation subtracts a quantum of stability, has a reduced ergon, providing information for the corresponding polypeptide for a
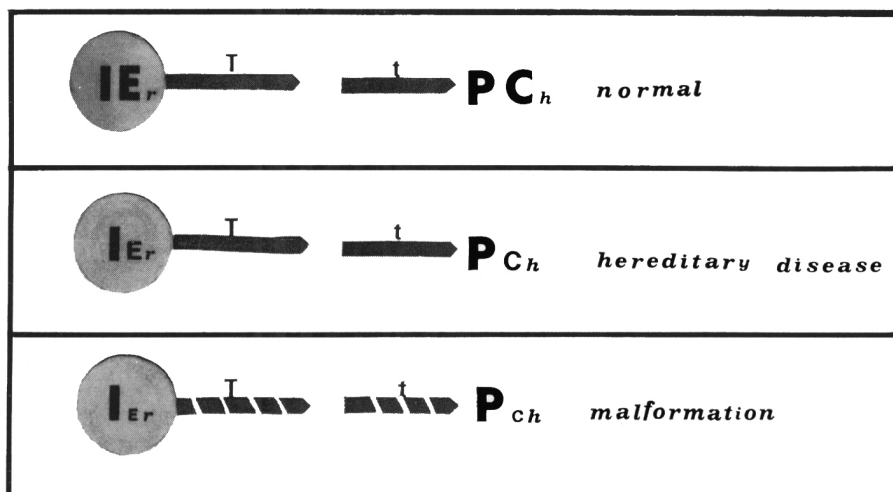

Fig. 14. Diagram of the E/C system. (See text, 3.2.)

time shorter than normal: when the reduced chronon ends, the hereditary disease begins.

A gene whose ergon was mutated below the expression threshold remains virtual; if its gene product is involved in ontogenesis, a malformation will result.

Following amphimixis a steady, generalized degradation occurs in the entire genetic material: the energy decline is slow and global. Such a gradual gene degradation is the participation of the genome in the entropic decline (*entropic degradation*). Individual genes, when they leave the " silent " state and repeat their specific information, also undergo further degradation, in direct proportion to informatic flow (*degradation by consumption*).

3.3. ONTOGENESIS AND THE E/C SYSTEM

On the phenotypical level, amphimixis marks the beginning of ontogenesis, i.e., the escalation of closely controlled biological times leading the individual organism from the zygote to its full development.

In the progression of developmental times a fundamental distinction must be made between " order " and " duration " of times.

The order, or sequence, of biological times repeats in the individual the ontogen-

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etic model of genic activation of the species. This is a fixed model, resembling an assembly line, exhibiting sequence variations only between species.

The duration of each developmental time reflects instead the stability of regulatory and structural genes in these cybernetic biomechanisms, thus offering a high level of individual variation. Since stability of information is inherited, developmental times bear the imprint of individual hereditary timing.

Thus minus- and plusvariant ontogenetic times exhibit, in fact, peculiar frequencies in different families, populations, and races. Until now, terms such as " timing " have been applied only to the first aspect of biological time, i.e., its order or sequence, ignoring the other aspect, duration of times, which represents the focusing parameter of hereditary chronological individuality.

The genetic nature of the variability in the duration of ontogenetic times is obvious for students of twins. Everyone mentions the concordance of both quantitative and qualitative traits, but many fail to mention that concordance is dynamically simultaneous, thus including the chronological aspect of the traits involved.

Although macroscopic, the dynamic simultaneity of development in MZ twins is a sensitive index of the synchronous, chronologically controlled operation of billions of identical genes (in so many body cells) concordant as to total result because they tread together the downward steps of molecular degradation. Such extensive isochronic beating of genic clockwork in MZ twins may continue for decades in spite of environmental modifying factors.

When carried out horizontally on a population of both MZ and DZ twins, the study of biological times proves how closely they are genetically controlled; when carried out longitudinally on one MZ pair it proves the stability of the chronological genotype, because both twins mark the same biological time like two clocks simultaneously wound.

MZ twins are also precious for studies on biological times because they provide information on which exogenous agents are able to modify the chronological sequence programmed in the zygote. Studies in the Mendel Institute in Rome have indicated that, when two MZ twins exhibit a weight difference at birth, this difference often extends into independent life, i.e., the heavier twin tends to reach earlier the subsequent ponderal deadlines (Gedda et al, 1971). In this case the intrapair difference is not strictly quantitative: both twins do reach the same weight, but at different times.

3.4. HOMEOSTASIS, REGENERATION, AND THE E/C SYSTEM

The period of development ends when the sequence of ontogenetic times is concluded and the body has attained the " optimum " established by its inherited constitution.

A conventional but useful definition identifies a subsequent " status " period, corresponding to adulthood, during which homeostasis is most obvious.

By homeostasis we mean the preservation of structure and function against the

variable and discontinuous environmental forces which tend to alter the genetic model. A special type of homeostasis, acting at all ages, is represented by regeneration. Homeostasis and regeneration are chronologically distinct, since homeostasis concerns all genes at all times, while regeneration concerns selected genes for sporadic activity. Both homeostatic and regenerative activity become gradually slower with increasing age, indicating that both are a function of gene stability.

When, in 1915, Carrel asked Lecomte du Noüy to conduct a mathematical study of wound scar formation, a phenomenon was proved (Lecomte du Noüy, 1939) whose explanation may now be found in the E/C system: the gradual exhaustion of gene stability causes a reduction in the information flow required for scar formation.

3.5. Constitution and the E/C System

The hereditary control of biological times tends to widen the medical concept of " constitution ", the latter encompasses not only the genotype and phenotype responsible for structure and function, an already admitted, but also an energy genotype and a time phenotype that change with age. In the light of the E/C system life may be viewed as an energy endowment parceled into the units of stability of individual genes: this endowment is inherited at amphimixis and then gradually expended according to the organism's normal and exceptional needs.

The study of the individual's inherited chronological constitution may help to appraise his performance, resistance, adaptability, i.e., his potential behaviour in work, sports, nutrition, climate, etc.

The organism retains its adult status as long as all necessary information is available, i.e., until some gene batteries, relevant for genic homeostasis, exhaust their ergon: the pattern varies from case to case, with occasional pathological results, without order or apparent explanation. This is the onset of senescence.

3.6. Senescence and the E/C System

The phenomenon of senescence has been widely investigated. Yet if we consider the reports submitted to the recent geriatric symposia at Leeds (1970) and Giessen (1971) we find that ageing has been investigated cytologically, histologically, biochemically, immunologically, etc., but always on the phenotypic level (Von Hahn, 1970; Referomed Bayer, 1971).

Many voices are calling for a global interpretation of the entire phenomenology of ageing. Several theories have been proposed, referring in part to genetic material, such as Szilard's theory of mutagenic " hits " (Szilard, 1959) causing random inactivation of a chromosome and its gene contents. Strehler relates instead such a random gene damage to the energetic fluctuation of the physicochemical environment (Strehler and Midvan, 1960), while Medvedev (1966) underlines the high probability that the chance mutagenic influences may affect regulatory genes. Burch (1968) admits

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that such chance actions may affect only predisposed individuals, while Sinex (1961) stresses, in the lability of DNA macromolecules, the receptive power of the gene under environmental mutagenic influences.

Such intuitions fail to reach the heart of the problem, represented by hereditary variability of genic stability, conditioning the genotype's response to environmental forces.

The solution the E/C system may give to the problem of senescence is as follows: ageing is neither primarily a phenotypical nor an environmental phenomenon; it is a genotypical phenomenon, interacting with environment and reflected in the phenotype. In other words, ageing is due to the increasing percentage of genes whose information flow has ceased because their ergon has become exhausted. Such cessation is inserted in the balance of inherited genic stability and follows the Mendelian law of independence.

Since this phenomenon occurs when the reproductive period is concluded, the species could hardly succeed, by natural selection, in programming senescence as it did ontogenesis.

The genetic nature of the E/C system explains how the phenomenon of senescence, however disordered, is related to heredity. In other words the absence of a senescence model common to all the species, as found for ontogenesis, does not mean that senescence is not genetically controlled: all such phenomena are controlled by the amount of energy allotted at amphimixis to the genotype.

Much as gene allottment to the zygote occurs at random, it is limited to parental assortment and to linkage. There follows a biological affinity among consanguineous individuals even as regards senescence. The late Italian clinician, Greppi, used to refer to this when stating that resemblance among relatives was closer in old age than at any other time during life.

Senescence represents an occasion for spontaneous breakdown of complex genotypes, identifying component genes differing for their informatic content. In the senescence of hair, for example, we may distinguish by their different chronon the information responsible for structure from that responsible for color, whenever hair turns white before falling off: the chronon of hair color genes was shorter than that of hair structure genes. Thus senescence analyses the different ergon received in the Mendelian lottery by different genes acting together.

Gene stability during ontogenesis and senescence does not trace the traditional arched pattern of life: it follows instead a more or less rapidly decreasing line.

3.7. Disease and the E/C System

The effect of a gametic mutation on the time of onset of a disease depends on the loss of stability it causes in the gene. If loss is such as to prevent information flow from reaching the threshold of specific effect, the disease strikes just when the information should have become expressed. This is how several congenital malformations may occur.

If loss does not cut off information, affecting gene stability only partially, onset of the disease is delayed as long as information flow is sufficient. Environment may affect an information deficit, causing an earlier onset of disease, by requiring a faster information flow resulting in faster degradation of gene stability and abbreviation of chronon. An example may be found in the accelerated senescence of sailor's skin, related to the increased " consumption of ergon " caused by longer exposure to sun rays.

The effect of genic decline below the critical level of its ergon differs according to whether a regulatory or a structural gene is affected.

In the case of regulatory genes different effects result from the mutation of a repressor or an operator gene. When a repressor gene is affected, the genes it controls are activated out of their programmed time; under such conditions the resulting information may be wrong and harmful. This may represent an oncogenic mechanism, especially when tumours follow a familial pattern, or for tumours of the elderly, when natural degradation of ergon may be equivalent to a mutagenic effect.

When an operator gene is affected, the accelerated exhaustion of its information will cut off the entire system of dependent genes, causing deficiency of one or more polypeptides (such as enzymes). Here again, deficiency may be total, even before birth, or it may appear during independent life following a measure of information flow. The former case may originate congenital enzyme defects; the latter may originate metabolic diseases in adult life.

3.8. Special Alterations of Informatic Flow

Many pathological effects may be viewed as resulting from partial deficiency of ergon. For example, enuresis may be interpreted as an ergon deficiency either in the genotype responsible for normal sphincter control or in the genotype of a conditioning derepressor mechanism.

In the case of protracted pregnancies recurring in certain families we may suppose either a minusvariance in the ergon of genes controlling oxytocin release or a delay in the reaction of the target due to plusvariance of an antagonist. Similar interpretations may be proposed for other so-called functional disorders.

Many diseases increase the information flow from genes more or less directly involved, resulting in higher consumption of ergon, i.e., reduction of chronon. This is why febrile diseases accelerate development but also, at the other extreme of life, hasten senescence.

In a study of silicosis among Sardinian miners (Gedda et al, 1964) we were able to prove the existence of a genetic factor in this disease. We showed that the time of onset of silicosis, after at least two years of exposure in superimposable environment, differs in different families: this chronological variability indicates differential stability in the genotypes responsible for the defense against silica particles.

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3.9. Information " Quoad Vitam " and " Quoad Valetudinem "

The amount of damage a disease inflicts to the life of an organism reflects the biological hierarchy of the E/C system involved in mutation: the shortened chronon may concern genes indispensable for survival (*quoad vitam*) or simply for health (*quoad valetudinem*). Hereditary cardiac diseases provide many examples of chronon reduction " quoad vitam ", while hereditary diseases of the skin provide many examples of chronon reduction " quoad valetudinem ". Life expectancy corresponds to the chronon of the least stable " quoad vitam " gene inherited by each individual.

The chronology of the gene does represent a new perspective in the study of normal and morbid phenomena in the organism. The qualitative aspects of information and the damage resulting from its absence are only part of the genetic picture. In order to be complete the picture must include the quantitative aspect of information, originating the information flow, i.e., the E/C system; information flow regulates biological times; the failure of informatic flow (a genetic trait) sets the onset of the specific disease.

As the saying goes, there are no illnesses, but rather ill people; we would like to state that there are no diseases, but rather differentially shortened times of genic information. Disease is a dischronic phenomenon, i.e., the result of an altered relationship between reciprocally scheduled genic times.

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RIASSUNTO

Il passaggio dei geni dallo stato silente a quello operativo obbedisce ad un rigoroso ordine temporale che può essere chiamato « cronologia del gene ». La successione dei tempi ed il suo meccanismo biocibernetico sono caratteristiche della specie, ma la durata dell'informazione presenta un'ampia variabilità intraspecifica relativamente alla popolazione, alla famiglia e all'individuo. I gemelli MZ, che dimostrano un notevole isocronismo, provano l'esistenza di una costituzione temporale individuale.

Ricerche precedentemente condotte dagli Autori (1969), hanno dimostrato che la durata di ogni informazione è una proprietà del genotipo corrispondente, la quale dipende dal suo tempo potenziale di attività, o *chronon*, e questo dall'energia di stabilità del medesimo genotipo, o *ergon*.

Sul piano della genetica molecolare la stabilità (da cui dipende il sistema Ergon/Chronon) di ogni gene di fronte alle mutazioni, è funzione di tre fattori:

1. La degenerazione del codice, cioè la diversa stabilità dei sinonimi che leggono la medesima informazione in rapporto alla diversa presenza delle coppie di basi GC e AT, le prime più stabili, le seconde meno stabili.

2. La ridondanza, cioè il numero di ripetizioni della molecola ADN che rappresenta quel gene.

3. Il repair, cioè l'efficienza degli enzimi e dei cofattori necessari per la riparazione delle strutture molecolari specifiche del gene.

Il sistema Ergon/Chronon del singolo gene, impostato a livello zigotico, quando il gene viene attivato secondo una sequenza caratteristica della specie dimostra un'ampia variabilità normale nei fenomeni dello sviluppo, omeostasi, rigenerazione e senescenza, attraverso le variazioni della quantità di informazione e perciò della sua durata. La variabilità fenotipica dei parametri cronologici risulta dall'interazione fra variabilità genetica e variabilità ecologica.

La malattia è l'effetto di un danno arrecato al sistema Ergon/Chronon in quanto le malattie ereditarie sono conseguenza della riduzione di stabilità del genotipo specifico, quindi della riduzione della durata della informazione corrispondente. In altre parole, la malattia è dovuta ad una abbreviazione del chronon informatico prodotta dalla riduzione o assenza dell'ergon genico. Questo danno si traduce nella cessazione anticipata

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dell'informazione specifica. Così si spiega il fenomeno del tempo d'insorgenza delle malattie descritte dalla medicina clinica, come un appuntamento dell'individuo tarato con la sua malattia.

L'eredità spiega la variabilità individuale familiare e popolazionistica dei tempi normali e patologici, come pure l'impronta ereditaria dei ritmi determinati dal tempo fisico sui fenomeni della vita.

RÉSUMÉ

Le passage des gènes de l'état silencieux à l'état opératif obéit à un ordre temporel rigoureux que l'on peut appeler « chronologie du gène ». La succession des temps, avec son mécanisme biocibernétique, est caractéristique pour chaque espèce, mais la durée de l'information génique présente une vaste variabilité dans chaque espèce, aux niveaux des populations, des familles, des individus. Les jumeaux identiques prouvent, par l'extension de leur isochronisme, l'existence d'une constitution temporelle individuelle.

L'étude de plusieurs caractères temporaux, conduite par les mêmes auteurs, a prouvé que la durée de chaque information est une propriété du génotype correspondant. Cette propriété dépend du temps d'activité potentielle (chronon) du génotype, qui est à son tour une fonction de son énergie de stabilité (ergon).

Au niveau de la génétique moléculaire la stabilité (qui contrôle le système Ergon/Chronon) de chaque gène contre les mutations est une fonction avec trois variables:

1. La dégénération du code (c'est-à-dire la diversité de stabilité des synonimes codifiant la même information) en raison de la différente proportion de bases G-C et A-T, dont les premières ont plus de stabilité.

2. La rédondance, c'est-à-dire le nombre de répétitions de la molécule d'ADN représentant le même gène.

3. Le « repair », c'est-à-dire l'efficience des enzymes et des co-facteurs nécessaires pour la restauration des structures moléculaires spécifiques du gène.

Le système Ergon/Chronon d'un gène donné, établi au niveau du zygote, lorsque le gène est activé suivant une séquence charactéristique de chaque espèce, montre une vaste variabilité normale (dans les phénomènes de développement, d'homéostase, de régénération et de sénescence) liée aux variations de la quantité, et donc de la durée, de l'information. La variabilité phénotypique des paramètres chronologiques est le résultat de l'interaction entre variabilité génétique et variabilité écologique.

La maladie est le résultat d'un système Ergon/Chronon endommagé, puisque les maladies héréditaires sont la conséquence d'une réduction de stabilité du génotype spécifique, et donc de durée de l'information correspondante. En d'autres termes, la maladie est causée par un chronon informatique abrégé suivant la réduction ou l'absence de l'ergon génique. Un tel dommage se traduit par une cessation anticipée de l'information spécifique. Cela explique le phénomène du temps de manifestation des maladies, que la médecine clinique décrit comme le rendez-vous de l'individu taré avec sa maladie.

L'hérédité du temps biologique explique la variabilité individuelle (entre familles ou populations) des temps normaux et pathologiques, aussi bien que l'empreinte héréditaire des rythmes déterminés par le temps physique dans les phénomènes vitaux.

ZUSAMMENFASSUNG

Der Übergang der Gene vom stillen zum aktiven Zustand folgt einer rigorosen Zeitordnung, die man « Chronologie des Gens » nennen kann. Die Zeitenfolge und deren biokibernetischer Mechanismus sind artspezifisch, doch ist die Dauer der Information innerhalb einundderselben Art, d.h. bei den einzelnen Bevölkerungen, Familien und Individuen sehr unterschiedlich. Bei MZ bemerkt man einen erheblichen Isochronismus, der für die Existenz einer individuellen Zeitkonstitution spricht.

Verf. hatten sich zuvor (1969) mit einigen temporalen Merkmalen beschäftigt und dabei bewiesen, dass die Dauer jeder Information eine Eigenart des entsprechenden Genotyps ist, die durch seine potentielle Aktivitätszeit (= Chronon) und diese wiederum durch seine Stabilitätsenergie (= Ergon) bedingt wird.

Auf dem Gebiet der Molekulargenetik hängt die Stabilität (die das Ergon/Chronon-System bedingt) jedes Gens gegen Mutationen von drei Faktoren ab:

1. Degeneration des Kodexes, d.h. Unterschiede in der Stabilität der Synonime, welche die gleiche Information lesen, im Verhältnis zum verschiedenen Vorhandensein der Basispaare GC und AT, von denen ersteres mehr und letzteres weniger stabil ist.

2. Der Überschuss, d.h. die Zahl, wie oft sich das DNS-Molekül, das das betreffende Gen darstellt, wiederholt.

3. Der repair, d.h. die Wirksamkeit der für die Reparatur der spezifischen Molekularstruktur des Gens notwendigen Enzyme und Nebenfaktoren.

Das Ergon/Chronon-System des einzelnen Genotyps bei den Zygoten wird einer artspezifischen Reihenfolge gemäss aktiviert; dabei weisen Entwicklung, Homöostase, Regeneration und Altern durch Unterschiede in der Informationsmenge und demnach der Informationsdauer eine normal grosse Variabilität auf. Die phänotypische Variabilität der chronologischen Parameter ergibt sich aus der Wechselwirkung zwischen genetischer und ökologischer Variabilität.

Eine Krankheit ist die Folge eines Schadens am Ergon/Chronon-System, denn Erbkrankheiten sind die Konsequenz einer Stabilitätsverminderung des spezifischen Genotyps, d.h. einer Verminderung der entsprechenden Informationsdauer. In anderen Worten: eine Krankheit wird durch Reduktion oder Fehlen des informativen Chronons bedingt. Dieser Schaden wirkt sich in vorzeitigem Stillstand der spezifischen Informations aus. Auf diese Weise lässt sich das zeitgebundene Auftreten eines Leidens, so wie es die klinische Medizin beschreibt, wie eine Verabredung des erblich belasteten Individuums mit seiner Krankheit erklären.

Die Vererbung des biologischen Tempo erklärt die individuelle Variabilität der normalen und der pathologischen Tempi, in einundderselben Familie oder Bevölkerung, sowie den Erbeinfluss des durch die physischen Tempi bedingten Rhythmus auf die Lebensphänomene.

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