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ASSOCIATION

EUROPEAN ATOMIC ENERGY COMMUNITY - EURATOM
CONSIGLIO NAZIONALE DELLE RICERCHE - CNR
COMITATO NAZIONALE PER L'ENERGIA NUCLEARE - CNEN

REPORT OF ACTIVITIES OF THE INTERNATIONAL LABORATORY OF GENETICS AND BIOPHYSICS

July 1, 1963 - June 30, 1964

1965

Work performed at the
International Laboratory of Genetics and Biophysics, Naples - Italy

Association No. 012-61-12 BIAI

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SUMMARY

A general report is given of the activity from July 1, 1963 to June 30, 1964 of the International Institute for Genetics and Biophysics of Naples. Most of the work being carried out within the framework of the Association Euratom-CNR-CNEN 012-61-12 BIAI, the report also serves as the annual progress report of this Association. The fields covered include genetics and biophysics of bacterial viruses, human, animal and biochemical genetics, the biochemistry of nucleic acids and of the nervous system, the radiobiology, genetics and physiology of mammalian cells in culture and of oncogenic viruses.

During the period under review the Institute has grown from some 44 to some 52 research workers and some 29 papers have been published in the scientific literature.

The Institute has maintained a very active programme of international collaboration, notably through its courses and seminars.

CONTENTS

INTRODUCTION

The International Laboratory of Genetics and Biophysics (LIGB) which came into being in 1962 after an agreement between the Italian National Research Council (CNR) and the Italian Committee for Nuclear Energy (CNEN), has been established in Naples as a Laboratory governed by CNR on ground owned by this Agency. Under a five years contract EURATOM has become associated with CNR and CNEN for the development of LIGB.

The Research Groups working at LIGB are the following. Those marked (C) are under Contract.

(C) Biophysics of Bacterial Viruses
(C) Genetics of Bacterial Viruses
(C) Animal Genetics
(C) Biochemical Genetics
(C) Biochemistry of Nucleic Acids
Biochemistry of the Nervous System
Oncogenic Viruses
(C) Human Genetics
(C) Mammalian Cells in Vitro.

The highlights of this second year of activity have been the following.

(a) These persons have agreed to serve as scientific advisors to the Laboratory:

Prof. C. Barigozzi, Institut of Genetics, University of Milan.

Prof. D. Bovet, Istituto Superiore di Sanità, Rome.

Dr. S. Brenner, MRC Unit of Molecular Biology, Cambridge.

Prof. C. Castagnoli, Institute of Physics, University of Turin.

Prof. R. Dulbecco, Salk Institute for Biological Studies, La Jolla, Calif.

Dr. A. Hollaender, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Prof. F. Jacob, Institut Pasteur, Paris.

Prof. E. Katchalski, Weizmann Institute of Science, Rehovoth.

Prof. J. Lederberg, Department of Genetics, Stanford University School of Medicine, Palo Alto, Calif.

Prof. S. Luria, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.

Prof. A. Monroy, Institute of Comparative Anatomy, University of Palermo.

Manuscript received on August 16, 1965.

Dr. R. Revelle, University of California, Berkeley, Calif.

Prof. C.H. Waddington, Institute of Animal Genetics, Edinburgh.

(b) Two international advanced courses have been organized and partially subsidized with funds from EURATOM; one on the genetics and physiology of bacterial viruses, the other on embryology and epigenetics.

(c) Professor Jean Brachet has been appointed a member of the Scientific Direction and has started a program of molecular embryology in cooperation with other staff members;

(d) The new research group on oncogenic viruses has been established. It in financed with funds other than those of the EURATOM-CNR-CNEN Contract.

(e) Two additional courses have been organized: one on viral carcinogenesis; the other on cytoplasmic control of protein synthesis. These were attended by the staff as well as by a number of research workers from other Italian laboratories.

The general organization of the Laboratory has progressed according to the availability of more space, but the realization of the building plans have unfortunately slowed down markedly, due to local administrative difficulties. However, the Laboratory is now almost completed.

Personnel of the Research Groups has increased during the period July 1st, 1963, to June 30th, 1964, by 27 persons (from 79 to 106). Of the total, 78 work under EURATOM-CNR-CNEN Contract. Twelve guests of the Laboratory have spent periods of the order of a month. Six members of the Laboratory now abroad are not included in the EURATOM Contract while 22 others belong to the Research Groups but are not under Contract. Some of the administrative and maintenance personnel listed on page 5 are not included in the Contract with EURATOM.

The members of the "Comite de Gestion" of the EURATOM Contract are the following:

<u>representing EURATOM</u>

R.K. Appleyard
M. Carpentier
J. Coursaget

<u>representing CNR</u>

R. Ceppellini

<u>representing CNEN</u>

E. Citterio

representing CNR and CNEN

L. Gedda

The Scientific Direction of the Laboratory consists of 5 "directors of research" and 15 "researchers"

Its executive is as follows:

A.A. Buzzati-Traverso	Director
L.L. Cavalli-Sforza	Vice-Director
F. Graziosi	Vice-Director

Details of the progress of research and other aspects of the life of the Laboratory are found on the following pages.

PERSONNEL OF RESEARCH GROUPS

	Under Contract		Others in Naples		Others Abroad	
Directors of Research	5		-			
Researchers	12		3		1	
Research associates	14		2		5	
Fellows	6		3			
Guests	6		1		-	
		43		9		6
Research Assistants	4		4			
Technical Assistants	2		-			
Laboratory Technicians	18		6			
Secretaries	2		-			
Clerks	9		3			
		35		13		-
		78		22		6

PERSONNEL OF ADMINISTRATION AND MAINTENANCE

Directorate	
1 Secretary	
3 Typists	Total 4
Library	
1 Librarian	
1 Assistant	Total 2
Photo Lab.	
1 Photographer	
2 Assistants	Total 3
General Store	
1 Technician	
1 Clerk	Total 2
Workshops	
2 Engineers	
1 Assistant	Total 3
<u>Administration</u>	
Administrative Director	Total 1
Personnel Office	
2 Secretaries	Total 2
Accountancy Office	
2 Accountants	
1 Typist	Total 3
Computer Center	
1 Assistant	Total 1
Purchasing Office	
1 Head	
1 Assistant	
1 Typist	Total 3
Grounds Maintenance	
1 Head	
4 Workers	Total 5
Guardians	Total 2
	Total 31

RESEARCH GROUPS UNDER CONTRACT

BIOPHYSICS OF BACTERIAL VIRUSES

Research workers

1.	Graziosi F.	Director of research
2.	Aurisicchio S.	Researcher
3.	Coppo A.	"
4.	Donini P.	"
5.	Gaeta F. S.	"
6.	Terzi M.	Research associate
7.	Epstein H. T.	Guest

Laboratory personnel

1.	Giuliano I.	Secretary
2.	Baldi M. I.	Research assistant
3.	Eremenko T.	" "
4.	Perna G.	Technical assistant
5.	Mattoccia E.*	Technician
6.	Santonastaso V.	"
7.	Urbani C.	"
8.	Esposito B.	Clerk
9.	Migliaccio C.	"
10.	Vespa T.	"

* on military duty

GENETICS OF BACTERIAL VIRUSES

Research workers

1.	Calef E.	Researcher
2.	Di Girolamo M.	"
3.	Fischer-Fantuzzi L.	"
4.	Arditti R.	Research Associate
5.	Bendicenti Di Girolamo A.	" "
6.	Guerrini F.	" "
7.	Marchelli C.	" "
8.	Hinckley E.	Fellow
9.	Dahl D.	Guest*
10.	Gaetani S.	"

Laboratory personnel

1.	Busiello V.	Technician
2.	Del Giudice L.	"
3.	Menna A.	"
4.	Cacace M.	Clerk
5.	Cacace S.	"
6.	Urbaniello P.	"

* partially supported by LIGB

BIOCHEMICAL GENETICS

Research workers

1.	Baglioni C.	Researcher
2.	Felicetti L.	Research associate
3.	Colombo B.	Fellow

Laboratory workers

1.	Campana T.	Research assistant
2.	D'Alise F.	Technical assistant
3.	Sansone G.	Clerk

BIOCHEMISTRY OF NUCLEIC ACIDS

Research workers

1.	Brachet J.	Guest Director or Research
2.	Scarano E.	Director of research
3.	De Petrocellis B.	Research associate
4.	Geraci G.	" "
5.	Iaccarino M.	" "
6.	Tocco G.	" "
7.	Rossi M.	Fellow
8.	Winkelmans D.	"
9.	Bibring T.	Guest
10.	Bocchini V.	"
11.	Curci M.	"

Laboratory workers

1.	Behrend Ruth	Secretary
2.	Grippo P.	Research assistant
3.	Garofano F.	Technician
4.	Granieri A.	"
5.	Sepe S.	"
6.	Vaccaro C.	"
7.	Cardone A.	Clerk
8.	Limongelli V.	"

ANIMAL GENETICS

Research worker

1.	Buzzati-Traverso A. A.	Director of research
2.	Pulitzer J. F.*	Fellow

Laboratory personnel

1.	Della Volpe N.	Technician

* NIH Fellow

PAVIA UNIT

HUMAN GENETICS

Research workers

1.	Cavalli-Sforza L. L.	Director of research
2.	Barrai I.	Researcher
3.	Edwards A. W. F.	"
4.	Moroni A.	"
5.	Scudo F.	Research associate
6.	Zei M. G.	" "
7.	Lunghi G.	Fellow

Laboratory personnel

1.	Anghinetti G.	Technician
2.	Attanasio E.	"
3.	Biondini M.	"
4.	Gorreri G.	"
5.	Mocenni G.	"
6.	Signifredi G.	"
7.	Zanardi E.	"

MAMMALIAN CELLS IN VITRO

Research workers*

1.	De Carli L.	Researcher
2.	Benerecetti Santachiara A.S.	Research associate
3.	Nuzzo F.	" "

* Technicians of this group are not under Contract

PERSONNEL ABROAD NOT UNDER CONTRACT

Cremona, T. (New York, N.Y., USA)

Di Prisco, G. (New York, N.Y., USA)

Falaschi, A. (Palo Alto, Calif., USA)

Gonano, F. (Baltimore, Md., USA)

Ritossa, F. (Urbana, Ill., USA)

Tocchini-Valentini, G. (Chicago, Ill., USA)

CURRENT RESEARCH ACTIVITIES

RESEARCH GROUP ON THE BIOPHYSICS OF BACTERIAL VIRUSES

The interest in the temperate phage α of B. megatherium began when this phage was observed to have ahigh rate of curing (spontaneous loss of the prophage) for lysogenic cells and a high sensitivity to disintegration of assimilated P^{32} and to X-rays. Phage α is similar in this respect to the single stranded DNA virus (γ X174).

Starting from these observations chemical and physico-chemical investigations were undertaken in order to study the molecular structure of the DNA contained in this phage. These investogations led to the discovery of a DNA showing a remarkable new property; a double stranded structure with complementary strands differing in base composition. In this DNA the usual rule A = T and G = C holds true but the relative amounts of the four bases in the two strands are different. Column chromatography on methylated albumin allowed to purify the complementary strands and their relative composition in bases gave the following result:

	Strand α_1 (light)	Strand α_H (heavy)
A	30.1	24.0
T	24.5	32.1
G	24.1	19.9
C	21.3	24.2

It is difficult to say whether this property is restricted to few DNAs of phage origin or it is a normal character of all the DNAs; on one hand density gradient centrifugation reveals this property only in favourable occasions. On the other hand evidence is now accumulating that DNAs from other sources may also show different base composition of the complementary strands.

The discovery of this new virus particles has led us in different directions:

1) Study of the DNA-RNA relationships in vivo
2) Study of the interaction between α prophage and the host cell
3) Study of the mechanisms of lethality induced by ionizing radiations.

As to the in vivo DNA-RNA relationships it has been recently shown (in collaboration with the group of P. Geiduschek of the University of Chicago) that the synthesis of RNA is asymmetric, only one strand of α DNA being copied (α_H). This finding is in contrast with what has been observed in vitro using many DNAs(including α) as primers for the Weiss RNA polymerase. It has been shown that in vitro both strands of DNA are copied by the RNA poly-

merase. Research are underway to elucidate the nature of the control system, present in vivo and absent in vitro, which is responsible for the asymmetric synthesis. On one hand we try to interfere with the synthesis of RNA through the use of proper chemical inhibitors to see whether in such conditions both strands are copied or not. On the other hand we now think possible that specific sites act as starting points on DNA for the activity of RNA polymerase. This hypothesis will be tested studying the RNA synthesized by irradiated B. megatherium cells infected by α, to ascertain whether this physical treatment, producing local lesions on the DNA molecules (local denaturations, breaks, etc.), makes the appearance of RNA material complementary to the light strand of DNA or not.

The study of the interactions between α prophage and the host cells has given interesting results. Arditti and Coppo have shown that acridine dyes are able to cure the lysogenic cells; moreover at different temperatures different rates of curing and different burst size have been measured. The instability of α in the intracellular stage can be attributed either to a high frequency of spontaneous detachment from the bacterial chromosomes (prophage hypothesis) or to a behaviour similar to that of a cytoplasmic-like particle. If the former hypothesis is correct curing can be attributed to a statistical distribution of the phage chromosomes among the cell progeny. The fact that lysogenic cultures grown in the presence of acridines show a higher rate of curing, suggests the cytoplasmic-like hypothesis as the more plausible. Moreover the different base composition of phage and cell DNA seems also to indicate a lack of homology between these genetic structures.

With this interpretation in mind the problem arises of the number of copies of phage α genomes that could be present in the host cell. This number would have to be greater than one as a consequence of the relative independence between host and phage DNA. Further research is necessary to elucidate this problem, which is directly connected with the mechanisms of cellular control of DNA replication.

As to the mechanism of X-rays lethality in phage α, Dr. Terzi in collaboration with Dr. Frontali of the Physical Laboratories of the Public Health Institute in Rome have seen that transverse cuts of the double helix of DNA are produced by X-rays with the same frequency in phage α and in phage T_2. Breakages in single strands appear to be non lethal in T_2 but lethal in α. These experiments have led us to think that the hypersensitivity of phage α to ionizing radiations and to assimilated P^{32} is due to a special biological behaviour of this phage; it may be that following infection one of the DNA strands is lost, the other then being responsible for the subsequent steps of transcription and replication.

Donini and Coppo have found that in α lysates a small fraction of particles is present which show a much lower buoyant density and which do not breed true. If the low density of these light particles were a consequence of a loss of DNA, application of the theoretical formula of Weigle, Meselson and Paigen relating buoyant density to DNA content would indicate that the light particles contain about half as much DNA as normal phage.
P^{32} suicide, X-rays sensitivity, autoradiography and paper chromatography of these purified particles will reveal if this hypothesis is correct. Another series of experiments makes use of chemical mutagens in an attempt to obtain genetic evidence for a difference in DNA content in normal and light particles.

Another line of work is the one studying the action of the repressor in lysogenic systems. For many reasons the system that have been chosen for study of repressor action is that of coliphage lambda. The first reason is that it is possible to infect cells with free DNA (Kaiser and Hogness experiment) and therefore it is possible to form heterozygous (hybrid) molecules in vitro. The second is that for this phage, at least three immunity systems are known, namely i^{b5} i^{λ} i.

The experiment we are making is the following: two stocks of λ with different immunity are prepared. The two DNA's are extracted and are denatured together. In these conditions 50% of heterozygous molecules are formed. By infecting with these hybrid molecules (separated in a cesium chloride gradient, having been one of the two DNA's labeled with heavy isotopes) cells lysogenic for one of the two alleles for immunity, the possible results are a priori three:

1. The repressor doesn't interfere with the heterozygous and blocks only the new daughter molecule with its same immunity (relative efficiency of the hybrid =1).
2. The repressor blocks the heterozygous (relative efficiency =0).
3. The repressor blocks the transcription of the strand with its same immunity. The information is in one strand and only in half of the cases the blocked strand in the good one (relative efficiency =1/2).

Dr. F.S. Gaeta is busy perfecting a new viscometer based on electromagnetic action, which has already proved to be a usable tool since its first and very primitive model. Its inherent superiority over other existing viscometers lies on one hand on the possibility of having much smaller gradients of shear velocity, in much better defined geometrical conditions. This last point is of the highest importance in dealing with non-newtonian liquids.

A preliminary study of the bahaviour of suspensions of solid particles in liquids, subjected to the action of moderately intense ultrasonic standing waves, has shown the existance of a phenomenon of separation (and concentration) of particles in the nodal planes of the standing wave pattern. This

application to the field of biology is under study.

Dr. Gaeta and Dr. Graziosi are collaborating with Prof. Brachet and his associates in a study of the cytoplasmic DNA of sea urchin eggs.

RESEARCH GROUP ON THE GENETICS OF BACTERIAL VIRUSES

The activities of the group for the genetics of phage have been mostly devoted to problems concerning the relation between temperate phage λ and its host, the bacterium Escherichia coli K 12; and to the organization of genetic structure of syngenotes.

The first problem deals with formal genetics of prophage. A temperate virus such as the temperate bacteriophage λ is adequately described as a dispensable genetic element of a bacterial cell. However, the phage may be found in a cell in a orm which in many respects resembles any other genetic element of the cell. I' one examines by experiments of formal genetics the genetic configuration of λ while it is in the bacterial cell, its determinants are found to share the same linear structure that carries the bacterial determinants.

However, the virus determinants have a sequence that is not the same as that found in crosses between free virus (es. Calef and Licciardello have suggested that the change in marker order may be a general correlate of the lysogenization process). Later Campbell proposed a model for integration based on the idea that the viral chromosome has a circular configuration and integration is due to crossing-over between homologous portions of the virus and the host. The original finding was based on observation of three markers. To verify the model it was necessary to extend the observation to other genetic regions of the virus. Crosses between bacterial strains carrying genetically marked prophages have been made. The prophage gave a map which is in agreement with the previous finding, namely the vegetative map sus_1——h——cl becomes upon integration h—— sus_1——cl. A paper on this subject is in preparation and will be sent to Virology shortly.

Let us now go back to one of the main features of the temperate phages or better to the complex made by a temperate phage and its host bacterium, the lysogenic cell. The element added to the bacterial cell, the prophage, stays in the cells without showing its viral properties; it may be the temperate virus that manufactures some repressor. The presence of this substance confers immunity to the bacterium and makes it in susceptible to the attack of other viruses of the same kind. It is also thought that when a cell is cured or made non-lysogenic by means of some treatment, the loss of the prophage occurs in a single step and the entire prophage is removed. A case bearing on the concepts stated above but with some new features, has been found and studied this year and will be reported here. It concerns bacterial strains which carry viral genes and do not show any immunity.

The strains V160 and V173 are both defined as λ sensitive; they plate λ phage exactly as a strain which has been cured by it or which has never met this phage. Moreover, the strains do not show radiosensitivity, the usual feature of the λ lysogenic strain.

When these strains, however, are infected with a pure line of λ phage – for instance the mutant $\underline{sus}_1$ – the lysate obtained is not pure $\underline{sus}_1$ (or a mixture $\underline{sus}_1$ $\underline{sus}_1^+$ as the known mutation rate of the marker should produce) but contains 1/2 $\underline{sus}_1$ 1/2 $\underline{sus}_1^+$. The same situation is verified if one selects a lysogenic strain, again infecting with pure $\underline{sus}_1$. Most of the lysogenic cells appear to be double lysogenic and they release, upon induction, two types of particles about in equal proportion. The following results have been found:

1. The map shows the extension of the defect, estimated to cover about 1/3 of the entire λ genome.

2. The viral genes which can be rescued of that apparently cured V173 strain are genes of λ . Indeed we have shown that the gene for "host specificity" are λ genes.

3. The loss of genes determining immunity is associated with the loss of those genes where immunity acts.

The mode of transmission of the exogenotic fragment is a relevant question in the problem of the genetic structure of syngenotes. (This term indicates those partial diploids that can be obtained by transduction). The case of transduction by λ can be summarized as follows.

Phage can transfer a given piece of the chromosome from a donor to a recipient bacterium, this piece comprises the genetic determinants of galactose metabolism. The λ phage which performs this function is a defective phage unable to form plaques. Genetic analysis indicates that the gal region replaces a segment of the phage linkage map. This transducing defective phage is called λ -dg. Very recently it has been shown, however, that λ is also able to transduce the biotin character and in this case the transducing phage is less defective than λ -dg, since it is able to form plaques.

The great majority of the cells that have received the transducing phage are persistent diploids. This raises the question whether the added piece is attached to the chromosome or is free in the cytoplasm. In the second case one should imagine that its relative stability is due to some sort of centromerisation of the transduced piece. One possible way to solve this problem is to perform bacterial crosses between a male heterogenote and a haploid female. Some crosses of this type have been performed (Int. Congr. of Genet.) and one will be reported here in some detail. Other crosses with somewhat different parents gave exactly the same result.

The cross we use as an example was:

$$\text{Hfr } T^r_6 \ \underline{gal}^-_2 \ (\lambda^{h\,cl}) \ exo \ / \ \underline{gal}^+ \lambda \ \ \underline{d}^+$$

$$F^- \text{ Strepto } \underline{threo \ leu \ B1 \ gal}^-_{12} \ (\lambda^{++}).$$

With the Hfr used (Hfr Hayes) the gal region is transferred about 22 minutes after the mixture of the parents. In the cross performed gal colonies began at about the same time with minor variations among different crosses. This suggests that the exogenotic piece behaves as a gene inserted in the continuity of the male genome and located very closely to the gal region.

Furthermore, more than 90 per cent of the gal^+ recombinants were heterogenotes as shown by segregation of gal cells, production of both types of phage and ability to give high requency transducing lysates. Moreover there was a progressive increase with time in the number of male endogenotic phage markers among the recombinants. The data suggest the following sequence of markers:

$$\text{Hfr} \xrightarrow[\lambda \qquad \lambda\text{-dg}]{b \qquad\qquad a}$$

One does not know, however, wnere the gal bacterial region is now located; it might be in either a or b regions.

Summarizing these results: 1) there is a definite time in which the exogenotic piece is transferred; 2) there is a progressive increase in the amount of male endogenotic markers with time. The second fact indicates the sequence of the two pieces.

Having thus collected evidence for the attachment of λ-dg to the bacterial chromosome we are now preparing strains to perform a cross which will allow us to get more information on the subject. The male strain will be a heterogenote with two gal mutations. One will be in the endogenote (gal_2, a kinase), the other in the exogenote (gal_6, a transferase), and the viral fraction of λ-dg will also be appropriately marked. By crossing this male with a female double mutant for the two gal mutations one can hope to resolve the sequence of very closely linked markers by an experiment of interruption of mating. That is, in the male chromosome the region under study, will be present twice (exo- and endogenotic regions) and or any sequence of two adjacent markers of the female the alternative alleles in the male will be present in the two separate duplicated segments. The cross will be;

$$\underline{\text{Hfr}} \ \underline{gal}^-_2 \ \lambda \ \underline{\overset{sus}{\text{-}}11} \ / \ \underline{exo} \ \underline{gal}_6 \ \lambda \ \underline{d}^+ \ \underline{cI}^-$$

$$F^- \ \underline{threo}^- \ \underline{leu}^- \ \underline{B}^- \ \underline{\text{Strepto}} \ \underline{gal}^-_2 \ \underline{gal}^-_6 \ (\lambda^{h^-})$$

If we have a situation where exogenotic and endogenotic pieces can be separated by interruption-mating experiments (as indicated by the previous crosses) it will be possible to observe that the genetic distance between two alleles will be stretched by a factor proportional to the genetic length of the piece interposed between them. Moreover in this cross λ and λ-dg will be marked so that it will be possible to verify more accurately the previous observations.

Difficulties due to the low amount of recombination in the region under study have been predicted and it is hoped to overcome them by selecting a marker which will enter the zygote immediately before the relevant region.

A second major activity of the Group has been carried on by a section on cellular physiology, which since October 1963, has been associated with us. This section is headed by Mario di Girolamo. The experimental work reported below started at the beginning of February 1964.

Shlessinger recently found in B. megatherium a fraction of ribosomes bound to the cell membrane. This finding brings up some questions:

1. Are these ribosomes an exceptional event, or are they present also in other microorganisms?
2. Do these ribosomes have chemical and physicochemical properties different from those of the free ribosomes?
3. Do these ribosomes perform different functions?

As a partial answer to these questions it has been found that in E.coli K 12 there are also membrane-bound ribosomes which amount to 10-15 per cent of the total.

These ribosomes have an RNA/protein ration 3:1 instead of the 2:1 ratio typical of free ribosomes. It has been shown by centrifugation in a CsCl density gradient that this is not due to lack of homogeneity of the preparations. Moreover by lowering the Mg^{++} concentration the membrane-bound dissociate more easily than the free ribosomes into two subunits. Experiments are now in progress to examine further chemical and physico chemical properties of membrane-bound ribosomes and to determine whether they synthesize proteins different from those synthesized by free ribosomes.

Another problem which is being approached by this section is the study of early labeled RNA of liver nuclei and microsomes of protein-depleted rats. Previous work has shown that protein starvation produces a modification of the protein distribution in the rat liver, with a loss of some proteins and an increase of some others. It is interesting to know if this modification of the protein pattern is bound to a modification of the messenger RNA. Using C^{14} orotic acid as RNA precursor it has been found that the specific activity of the early labeled RNA is lower in protein-depleted rats. The radioactivity of RNA after sucrose gradient centrifugation from both nuclei and microsomes showed

lower activity for the protein-depleted animals in the 4-18 s region. The radioactivity curves of the RNA from depleted rats on sucrose gradients were shifted in the direction of heavier fractions.

The $\frac{28S}{18S}$ ratio is higher in the microsomal RNA of protein-depleted rats than in control, as a consequence of a preferential decrease of the 18S fraction. Moreover, by centrifugation in sucrose gradient it has been found that in the liver of protein-depleted animals there is a shoulder in the heavier part of the 28 S peak of the microsomal RNA. This shoulder is due to the presence of a heavier RNA.

The Group is now testing the stimulatory activity of the different fractions of the RNA separated in sucrose gradient in an in vitro system of E. coli (Nihrenberg and Matthaei).

RESEARCH GROUP ON BIOCHEMICAL GENETICS

This Group has been studying the action of amino acid analogs on hemoglobin synthesis in rabbit reticulocytes. Phenylalamine, histamine and tryptamine have been found to inhibit hemoglobin synthesis. The action of tryptamine has been studied in detail. The relationship between analog concentration and inhibition of hemoglobin synthesis has been established. It has been observed that the polyribosome pattern of rabbit reticulocytes is modified after the addition of tryptamine. The modification of the polysomes pattern is evidentiated by pulse labelling nascentpeptide chains with C^{14} amino acids, adding the analog and chasing afterwards with cold amino acids. Only the chains whose synthesis has been interrupted by the addition of the analog, remain bound to the ribosomes. After prolonged incubation with tryptamine, the polysomes disappear and only single ribosomes are observed. Since tryptophan occurs in the α chain of rabbit hemoglobin only one near the N-terminus and in the β chain twice, also near the N-terminus, these results suggest that the growing peptide chains are blocked by tryptamine at the point where tryptophan should be incorporated.

The reticulocytes were examined for the presence of incomplete peptide chains released from ribosomes in the presence of amino acid analogs; no evidence of such release has so far been obtained. The genetical and biochemical significance of these observations has been discussed in a paper presented at the Cold Spring Harbor Symposia on Quantitative Biology (June 5-11, 1964).

Investigations on the late stages of hemoglobin synthesis in rabbit reticulocytes have been made. Reticulocytes were labeled by short incubations with C^{14} amino acids; the polyribosomes were then isolated by sucrose density gradient centrifugation. The polyribosomes were digested with ribonuclease and trypsin after the addition of carrier rabbit globin. The hemoglobin peptide chains were then separated by column chromatography. It has been consistently found that a relevant fraction of completed or nearly completed peptide α chains are associated with the polysomes, while less or none complete β chains are attached to polyribosomes. These findings have been interpreted as suggesting a control mechanism by which α chains are released from polyribosomes after combining with β chains only.

In addition to the work described above, human hemoglobin variants of particular genetic interest have been investigated by different techniques, such as chromatography, electrophoresis, fingerprinting, etc., in order to identify the amino acid substitutions present. Abnormal hemoglobins which are present in heterozygotes in reduced amount have been investigated in detail; one of the abnormal hemoglobins investigated was found in a child affected by severe anemia and in some of his relatives. This hemoglobin,

which shows a reduced rate of synthesis (10 per cent in heterozygotes), shows the amino acid substitution aspartic to glycine in residue 47 of the α peptide chain.

A group of abnormal hemoglobins obtained from Bulgaria has been studied. It has been discovered that a hitherto rare abnormal hemoglobin - hemoglobin O_{Arab} - is present with relatively high frequency in one region of Bulgaria. The ethnological and genetic significance of this hemoglobin in relation with the diffusion of malaria are discussed in a manuscript to be published in Nature.

RESEARCH GROUP ON THE BIOCHEMISTRY OF NUCLEIC ACIDS

1. Studies on the regulatory properties of deoxycytidylate aminohydrolase. The main line of investogation by this group has been devoted to studies on the regulatory properties of the enzyme deoxycytidylate aminohydrolase. This enzyme catalizes the hydrolytic deamination of deoxycytidylate (dCMP) to deoxyuridylate (dUMP). It acts at a branching point in the pathway leading to deoxycytidine triphosphate (dCTP) and deoxythymidine triphosphate (dTTP) which are the immediate precursors of pyrimidine deoxynucleotides in DNA biosynthesis. The activity of dCMP aminohydrolase is strongly inhibited by dTTP with a multimolecular kinetics with respect to the inhibitor and this inhibition is completely reverted by dCTP. dCTP has no effect on the inhibition of the enzyme by competitive inhibitors like dUMP and deoxythymidylate (dTMP).

At present, using donkey spleen as source of the enzyme, we have obtained a preparation 50 per cent homogeneous as judged by starch gel electrophoresis. By sucrose gradient centrifugation a molecular weight of $1.2 \cdot 10^5$ has been determined. Studies on the dependence from pH of the inhibitions of several deoxynucleotides demonstrate a pattern for dTTP and deoxyguanylate (dGMP) - allosteric effectors - which is different from that of dTMP and other competitive inhibitors.

Organic mercurials such as p-Cl-mercuribenzoic acid, p-Cl-mercuriphenylsulfonic acid and o-Cl-mercuriphenol mimic the action of the allocteric inhibitor dTTP, and their inhibition can be prevented by the allosteric activator dCTP. Thus the hypothesis may be advanced that sulfhydril or imidazole groups are of importance in the allosteric transition of dCMP aminohydrolase.

Kinetic experiments have been performed in order to understand the molecular basis of the changes in catalytic properties of the enzyme. All the experiments are in agreement with a model of the enzyme with multiple substrate sites and multiple regulatory sites.

E. Scarano was invited to read a paper on the regulatory properties of dCMP aminohydrolase at the symposium on "Regulation of Enzyme Activity" at the VIth International Congress of Biochemistry, New York, July 26-August 1, 1964.

2. Studies on the control of enzyme synthesis during the early embryonic development of the sea urchin. The effect of actinomycin on dCMP aminohydrolase content of sea urchin embryos has been studied. In normal embryos the enzyme content decreases during development (E. Scarano and R. Maggio, Exptl.Cell Res. 18, 333, 1959). We have shown that embryos grown in 2.5

mg/l of actinomycin stop development at gastrula stage and embryos grown in 5 mg/l of actinomycin stop at blastula stage. In both cases the dCMP aminohydrolase content of the treated embryos is higher than in the control and increases with actinomycin concentration. At the concentration of 40 mg/l (the highest tested) the enzyme content is higher than in the unfertilized eggs.

These results have been described in a preliminary note in Biochim. Biophys.Acta 87, 174, 1964, in which the authors put forth the hypothesis of inhibition by actinomycin of the synthesis of a specific repressor, which in turn is responsible for enzyme decrease in normal development.

We have used also another approach to the problem of control of enzyme synthesis during embryonic development by studying cells from disaggregated embryos. If eggs of sea urchins just after fertilization are deprived of the membrane and cultured in Ca-free sea water, the eggs divide into separate cells and a culture of single cells is obtained. A single-cell culture can be obtained also from later stages of development (blastula or gastrula) by gentle homogenization of the embryos in Ca-free sea water. The enzyme content of such cells is being studied. Preliminary results indicate that dCMP aminohydrolase in cells obtained from eggs deprived of the membrane does not decrease but remains constant at the level found in eggs. In the case of cells obtained by disaggregation of blastulae or gastrulae the enzyme remains at the same level found in the embryo at the time of disaggregation.

3. Studies of methylated bases in the sea urchin embryo. The techniques of extraction and analysis (both chemical and chromatographic analysis) of nucleic acids and their derivatives have been standardized to study the methylated bases in the sea urchin embryo. Some new thin-layer chromatographic separations have been performed.

Upon incubation with radioactive precursors it has been possible to demonstrate that:

a) the methyl group of 5-methyl-cytosine is formed by direct transmethylation from methionine;

b) in the pyrimidine ring there is almost no radioactivity;

c) the methyl group of thymine at least in part is formed probably by a hydroxymethyl intermediate from the CH_3 of methionine;

d) radioactivity of guanine is in the purine ring and is formed via a "one carbon" intermediate;

e) the results concerning adenine and possible methylated analogues of this base are still uncertain.

Also, in vitro experiments were performed in order to demonstrate the presence of a DNA-methylating enzyme or of a dCMP-methylating enzyme.

The experimental conditions were similar to those used by Gold M. et al., Biochem.Biophys.Res.Comm., 11, 107, 1963. In preliminary experiments it has not been possible to demonstrate a methylation of the DNA or of the dCMP added.

In preliminary work we have demonstrated that, when embryos of Paracentrotus lividus are incubated at the blastula stage with $^{14}CH_3$- methionine and the RNA is purified according to Nemer (Proc.Nat.Acad.Sci., 50, 230, 1963) the ribonucleotides separated by ionophoresis are radioactive.

Preliminary experiments with wheat germ have been undertaken because of the high 5-methyl-cytosine content of the DNA of this organism.

4. Nucleic acid and protein metabolism in nucleated and anucleated sea urchin eggs. A group led by professor J. Brachet has pursued a research program on nucleic acid and protein synthesis in nucleate and anucleate fragments of sea urchin eggs. Previous work by Brachet and Tencer (Exptl.Cell Res., 32, 168-170, 1963) has shown that anucleate fragments of sea urchin eggs respond to activating (parthenogenetic) treatments by increased incorporation of an amino acid, leucine, into acid-insoluble material. The purpose of the present experiments was to confirm these preliminary findings (which were based on autoradiographic observations only), and try to understand the mechanisms of the stimulation of protein synthesis in the anucleate egg fragments. Since this stimulation cannot be the result of a synthesis of messenger RNA, one is left with two possibilities (which do not exclude each other): a) the anucleate fragments might contain a store of inactive messenger RNA, which would be released upon parthenogenetic activation; b) these fragments might contain cytoplasmic DNA, which could conceivably serve as a template for the synthesis of cytoplasmic messenger RNA.

The experiments have led to a number of conclusions: first, there is little doubt that the activated anucleate halves are sites of a true protein synthesis; they incorporate several amino acids in their proteins and this incorporation is inhibited by puromycin. It is worth mentioning that the anucleate fragments are more active in this respect than are the nucleate ones (after parthenogenetic activation).

The reverse situation occurs in the case of the incorporation of nucleic acids precursors such as uridine and thymidine: the nucleate fragments are more active than the anucleate ones (after parthenogenetic activation). But it is important to note that anucleate fragments of sea urchin eggs are not completely inactive in nucleic acid synthesis: they are capable of limited DNA and RNA synthesis (or turnover).

Chemical estimations have shown that both RNA and DNA are almost equally distributed between nucleate and anucleate halves. This finding gives, for the first time, definite proof of the existence of cytoplasmic DNA in sea urchin eggs.

Attempts have been made to isolate, by phenol extraction, the DNA and the RNA present in the anucleate halves. The former is being studied with the analytical centrifuge, in close collaboration with F. Graziosi and F. Gaeta. The first results strongly suggest that cytoplasmic DNA has the same base composition as nuclear DNA. The RNA extracted by the phenol method will be tested for messenger activity in the in vitro system of Nirenberg and Matthaei.

RESEARCH GROUP ON ANIMAL GENETICS

This Group has been studying puff formation, a phenomenon which requires a brief explanation.

In the polytene chromosomes of Drosophila and other Diptera one observes an ordered banding pattern of Feulgen-positive material which, as shown by cytogenetic methods, reflects the ordered sequence of genetic loci in the chromosomes (for instance, the delection or duplication of a genetic determinant is paralleled by the delection or duplication of band: N, 307-w, /C2-3).

It has also been observed that certain bands can undergo dilution-swelling, to the prior of losing their capacity to stain by Feulgen's reagent. Which band swells depends on the developmental stage of the larva and on the organ (salivary gland, mid-gut, malpighian tubule) concerned. This phenomenon goes under the name of puff formation.

The appearance of the puffing pattern of a later period can be anticipated experimentally by treatment with ecdysone (Clever, 1961); whereas the reversion to an earlier pattern occurs on treatment with the juvenile hormone secreted by the corpora alata (Kroeger, 1963). Specific puffing patterns are induced by transplantation of nuclei into egg cytoplasm. In one case the presence or absence of certain cytoplasmic granules in two species of Chironomus (C. tetans and pallidivittatus) has been correlated to the presence or absence of a specific puff (Beerman).

This in essence is the circumstantial evidence supporting the theory that puffing is the primary manifestation of differential gene activation during development.

The major concern of our Group during the past year has been the experimental induction of puffing at loci 2L 14, 15 and 20 of D. busckii. In Pavia, Ritossa (1963), now at Oak Ridge, found that induction is effected by means of various agents: DNP, heat shock, dicumarol, salicylate, azide and anaerobiosis, all of which are known or supposed to uncouple oxydative phosphorilation. Beyond this we have, as of now, no knowledge of the metabolic variation responsible for puff induction.

Ritossa (1963) has also studied by autoradiography nucleic acid synthesis at the puff level. He found that puffs actively synthesize RNA and that under certain conditions RNA synthesis is exclusive of DNA synthesis.

Histochemical studies show that puffs contain RNA and protein. To find out which or what combination of these substances is essential for puff formation we have employed the antibiotics actinomycin (10^{-5}M) and puro-

mycin (10^{-3}M) which are known to inhibit respectively DNA-primed RNA synthesis and protein synthesis. We found that only actinomycin inhibits puff formation; moreover, it determines the regression of the puffs once they are formed (Ritossa and Pulitzer, 1963).

This picture of puffs as structures made up essentially of an RNA which is synthesized at a high rate is also consistent with the idea that puffs represent hyperactivated genes.

For the future, we plan to continue our work on puff RNA synthesis. We also plan to try our luck on some fundamental but technically recalcitrant problems. Is some specific protein synthesis correlated with the appearance of puffs? What is the genetic basis of puff regulation? These questions will have to be answered if the puff phenomenon is to prove a useful tool in the solution of the enigma of cellular differentiation.

PAVIA SECTION

RESEARCH GROUP ON HUMAN GENETICS

Since July 1963 the Research Group on Human Genetics has been chiefly concerned with the study of consanguinity in Italy and on the reconstruction of evolution.

An investigation of consanguineous marriages in Italy was started about then years ago and had been extended to the whole country. Advantage was taken of the fact that consanguinity dispensations have to be requested for catholic marriages (over 98 per cent of all marriages, in Italy). Copies of the dispensations for the whole country can be had from a single source.

Dispensation was asked for 520,348 consanguineous marriages in Italy, in the period 1910-1962. The records are being transferred to magnetic tape and ordered alphabetically by surnames of the spouses. Some partial statistics are already available.

A small fraction of the data collected earlier has been subjected to a more complete analysis. It concerns the dioceses of Parma, Piacenza and Reggio Emilia (centrally located in the Po Valley). In part of this region demographic and genetic data have also been collected. Dispensations usually permit us to distinguish several hundred types of consanguineous matings. The frequencies of these matings show very clear cut regularities which we thought would have permitted an explanation on the basis of demographic factors. We have investigated the possibility of predicting the observed frequencies of consanguineous marriages of the various kinds.

Two factors in particular were found of importance: migration, age at marriage and generation time. An analysis of age effects permitted us to obtain formulas for predicting age differences between relatives of any degree which fitted remarkably well the observed distributions collected for the purpose. The migration pattern in this area was also analysed. From these, with a suitable theory one can predict the expected frequency of consanguineous matings of the various types, on the assumption that there is no prejudice for or against consanguineous matings. The analysis has also shown that, when age effects are properly considered, the frequencies of consanguineous marriages give information on migration which can be of some use in defining population structure. So far the agreement with observed data leads us to believe that prejudice cannot be very important.

The availability of names and surnames of consanguineous mates permits

several approaches for the study of the effects of consanguinity. We will mention one pilot experiment.

From the psychiatric hospitals of the three towns mentioned above, the records on 4789 patients entering in the period (approximately) 1947 - 1960 were obtained, and the average inbreeding coefficients of these patients were computed. Expected inbreeding coefficients on the assumption that consanguinity and mental disease are independent, were also computed, on the basis of the age distribution of the patients and the frequency distribution with time of consanguineous marriages.

The second large-scale program of the Group has been to make an analysis of human evolution starting from the principle that evolution can be reconstructed where reasonably good data on genotypic differences between populations are available and a satisfactory model of evolution can be given; methods of reconstructing evolutionary thus have been set up and applied to human blood group gene frequencies, obtained for some 50 human populations thanks to the cooperation of Dr. Mourant and his Nuffield Center for Anthropological Research on Blood Groups, London. The results of the analysis now being completed, are impressive. It is the first time that such a synthesis has been attempted and we believe that the available data are now mature, or very largely so, for this type of work. Of course it would be desirable to extend the analysis beyond the human species; but the availability of a large number of gene frequencies data in our species makes it a good starting point.

RESEARCH GROUP ON MAMMALIAN CELLS IN VITRO

1. Studies on the clonal distribution of alkaline phosphatase variants in the EUE cell population. The general subject of the research work of this Group is the investigation of genetic mechanisms underlying somatic cell variation in mammals. Previous attempts have been made to find good genetic markers in cultured cell populations; we have now succeeded in finding a suitable character namely alkaline phosphatases. These enzymes are rather complex from a biochemical point of view and their genetic determination may not prove simple but they have the advantage of being easily recognisable in vitro by reactions which do not inactivate the cells. Cells with a high alkaline phosphatase content are revealed by a yellow color developing from hydrolysis of para-Nitro-Phenyl Phosphate (pNPP). Another staining method turns the positive cells blue while the deficient cells appear colourless or faintly yellow.

Using these methods we isolated a number of deficient clonal lines from a human cell strain with a heteroploid constitution. The genetic study of the alkaline phosphatase system depends on the study of the pattern of transmission of the enzyme character in the cell population. The study is almost completed thanks for the collection of much data on the clonal distribution of the deficient variants and the so-called variegated lines. At present, investigations of the same property are underway using lines with a high level of alkaline phosphatase in order to obtain a general picture of the dynamics of the EUE cell population with respect to AP. The preliminary observation of the pattern of variation of AP led us to the hypothesis that gross chromosomal changes were responsible for the enzyme differences.

2. Cytogenetical studies with alkaline phosphatase. A cytogenetic analysis has been undertaken and was intended to correlate the enzyme variation with karyotypes in different cell lines by analysing both the parental EUE strain and its clonal derivatives. In fact it was possible to reveal a specific loss of chromosomes in the lines with low enzyme activity. The chromosomes involved are those of group 21-22. The preliminary data on the association between enzyme levels and the number of small acrocentric chromosomes were reported in the Journal of the National Cancer Institute, December, 1963.

Further evidence on the cytological location of AP has recently been accumulated. From this it would be possible to construct a curve of AP - specific activity in relation to the number of small acrocentric chromosomes. This is equivalent to studying the gene dosage effect. We are currently collecting data able to decide which kind of function relates the number of chromosomes 21-22 to the enzyme activity. Karyotypes of several lines with various levels of AP are being analyzed. On the basis of the available information one can exclude the existence of a linear relationship between chromosomal dose and enzyme activity.

The deviation from linearity may be due to the interference of control mechanisms. These would postulate the existence of regulatory genes which may even be located on chromosomes other than 21-22. If this can be proved, an enzyme deficiency might then originate either from a loss of chromosomes 21-22 or from an increase in the number of chromosomes which carry regulatory genes - or possibly with. We are now concentrating on some chromosomal patterns in enzyme-deficient lines which might eventually give evidence in favor of this hypothesis.

3. Studies on enzyme induction by nucleic acids. The stability of AP variants makes this system suitable for studies of transfer mechanisms of genetic material. Several experiments on DNA transfer have been carried out, using cells with high AP activity as donors and enzyme-deficient cells as recipients and vice versa. So far no clear cut success has been realized.

Recently another series of experiments has been started on enzyme induction by RNA. The nucleic acids are extracted from AP-positive cultures and added to cell suspensions from enzyme-deficient lines. The treatment is performed under conditions similar to those described by Chu et al. (1962). These authors apparently succeeded in inducing synthesis of liver enzymes in hepatoma cells by RNA from fresh liver. If this result can be confirmed in the experiments now underway in our research group, the AP system will allow a better control of the induction dynamics, since the RNA-treated cells can be kept for indefinite periods in vitro. So far, the experiments clearly demonstrate that enzyme synthesis is induced in the cells treated with extracts containing nucleic acids. The induced activity is proportional to the optical density at 260 m/u of the incubation mixture. The induction factor varies from x 10 to x 100 after six hours of incubation following the treatment. After three days of incubation the induction no longer occurs. Nucleic acids extracted from recipient cells do not significantly induce the synthesis of the enzyme. Histochemical staining shows the presence of the enzyme in the treated cells. However, the distribution of the enzyme in the recipient cells has a characteristically discontinuous pattern.

One explanation of the observed facts we can rule out to some extent namely, that the products of degradation of Na induce enzyme synthesis. One must remember that AP is a phosphatase inducible by some organic phosphate esthers. However, the fact that nucleic acid extracts from negative cell lines do not induce AP we argues against this interpretation.

One cannot yet stable, however, that nucleic acids are incorporated and direct the synthesis of the enzyme. In fact, so far we have not obtained a method that permits us to keep the inducing activity of the extract without completely eliminating the enzyme itself. In standard deproteinization enough enzyme survives so that passive transport of the znyme cannot be ruled out as an explanation of the phenomenon.

4. Karyotype hybridization. Studies of genetic interactions are also being carried on at a more organized level of genetic material. We are attempting hybridization of karyotypes in experiments similar to those successfully performed by Ephrussi and Borel (1961). These authors maintain that hybridization takes place in a mixed culture of mammalian cells, but that this is a rare phenomenon which only becomes apparent after several months. In order to increase the frequency of cell fusion we are planning to use a viral agent, the Herpes simplex virus, which is known to produce polykaryocitosis. We will attempt to mix those cell lines which have already been selected for different levels of alkaline phosphatase activity.

In addition to the biochemical marker these lines must also be cytologically distinguishable. From an enzyme-deficient line a particularly stable clonal derivative has been selected, whose karyotypes is recognizable by a marker chromosome and which originated spontaneously in culture. In the lines with high alkaline phosphatase activity it was necessary to induce chromosome rearrangements by X-rays in order to detect chromosomal markers. A number of clones from irradiated cells which contain suitable chromosomal markers are being isolated. This will enable us to attempt the hybridization.

GUESTS

The following scientists, other than those listed in the Research Groups, have been guests of the Laboratory, either in conduction of research or for training periods:

Dr. E. Baltus, Laboratoire de Morphologie Animale, Universite Libre de Bruxelles, Bruxelles, Belgium (Group on the Biochemistry of Nucleic Acids)

Dr. L. Bernini, Department of Human Genetics, University of Leiden, Holland (Group on Biochemical Genetics)

Dr. A. Chersi, Centre de Recherches sur les Macromolecules de Strasbourg, Strasbourg, France (Group on Biochemical Genetics)

Dr. H.M. Cann, Department of Pediatrics, Stanford University School of Medicine, Palo Alto, Calif., USA (Group on Human Genetics)

Dr. G. De Francesco, Clinica Pediatrica, University of Naples, Naples, Italy (Group on Biochemical Genetics)

Dr. A, Ficq, Laboratoire de Morphologie Animale, Universite Libre de Bruxelles, Bruxelles, Belgium (Group on the Biochemistry of Nucleic Acids)

Dr. M. Kimura, Department of Genetics, Mishima, Japan (Group on Human Genetics)

Mr. J.R. Lance, Scripps Institution of Oceanography, La Jolla, Calif., USA (Group on Animal Genetics)

Dr. J. Quertier, Laboratoire de Morphologie Animale, Universite Libre de Bruxelles, Bruxelles, Belgium (Group on the Biochemistry of Nucleic Acids)

Dr. N. Six, Laboratoire de Morphologie Animale, Universite Libre de Bruxelles, Bruxelles, Belgium (Group on the Biochemistry of Nucleic Acids)

Dr. A. Steens-Lievens, Laboratoire de Morphologie Animale, Universite Libre de Bruxelles, Bruxelles, Belgium (Group on the Biochemistry of Nucleic Acids)

Dr. P. Strata, Institute of Human Physiology, University of Pisa, Pisa, Italy Group on the Biochemistry of Nucleic Acids)

C O U R S E S

During the second year of its activities in Naples, four specialized courses, two of which are international, have been organized by the International Laboratory of Genetics and Biophysics.

Course on the Genetics and Physiology of Bacterial Viruses

An international course on the Genetics and Physiology of Bacterial Viruses was held from September 17 to October 12, 1963 under the auspices of UNESCO and ICRO. The Course was directed by Prof. E.Kellenberger of the University of Geneva with the collaboration of Dr.R.S. Edgar of the California Institute of Technology, Pasadena, Calif.and Dr. R.H. Epstein, Dr. W. Arber and G. Kellenberger, all of the University of Geneva.

The Course was organized on the example of the courses held for many years in Cold Spring Harbor, the difference being in the manner of subsidy it. In Cold Spring Harbor the participants have to pay a tuition fee and bear subsistence expenses. They usually get money from their own institution, but only a few fellowships are available. In the Naples Course all participants had the benefit of a fellowship, 12 from ICRO for students coming from France, Germany, Holland, Czeckoslovackia, Hungary, India, Israel, U.K., USA, Switzerland, URSS and 4 for Italians.

The scientific program has dealt primarily with phage T_4 by doing experiments on growth, inactivation by serum and radiation, mutagenesis, recombination, reactivation of radiation damage, physiological genetics (with help of conditional lethal mutants). The second part of the course was devoted to phage λ in order to study the lysogeny induction by U.V., transduction, separation of different transducing phages by density gradient centrifugation, genetic determinants of the lysogenic condition and zygotic induction.

Some lectures covering some fundamental aspects of molecular biology have been delivered by invited scientists.

This course was supported in part ($ 6,000) by UNESCO and ICRO and in part (L. 21,657,190) under EURATOM Contract with LIGB.

Course on Viral Carcinogenesis

On the occasion of his visit to Naples on January 9-12, 1964, Dr. Renato Dulbecco, from Salk Institute for Biological Studies, La Jolla, California, delivered a course on Viral Carcinogenesis.

The first lesson dealt with cellular transformation by DNA viruses such as Polyoma SV40, rabbit, bovine and human papyllomatas, and adenovirus type 12 and 18. After the description of the molecular characteristics of the DNA of these viruses, Dr. Dulbecco gave particular attention to the problem of the circulatiry of the DNA of Polyoma and Papilloma and its implication to the mechanism of transformation.

The second lesson dealt with the cellular phenomena involved in the transformation by DNA viruses. The special characteristics of the transformed cells were discussed; morphological characteristics, ability to produce changes in tumor antigenicity, and resistance to metabolic inhibitors. The discussion was centered on the absence of vegetative virus and provirus in the transformed cells and the possibility of the permanence of the virus genoma in cells.

The third lesson dealt with the transformation of RNA virus particularly the Rous sarcoma virus and avian mieloblastosis virus. After description of the two experimental systems, the discussion was devoted mostly to the role of the helper viruses and the mechanism by which the cellular transformation is performed by the RNA viruses.

Because of the importance of the course, some research workers from outside were given the opportunity of attending the lessons. A total of 67 scientists, 42 of whom were from other Italian institutes, attended the course.

This course was supported entirely by the EURATOM Contract with IIGB (L. 243,615)

Course on Embryology and Epigenetics

An international Course on Embryology and Epigenetics was held from the 6th to the 18th of April, 1964, under the auspices of UNESCO and ICRO. The course was under the direction of Prof. C.H. Waddington of the University of Edinburgh, with the collaboration of Prof. A. Monroy (University of Palermo), Prof. G. Reverberi (University of Palermo), Dr. E.L. Elsdale (University of Edinburgh), Dr. J.L. Sirlin (University of Edinburgh) and Dr. L. Wolpert (King's College, London).

The course was rather unusual and there were few precedents to guide in its construction. The subject to be dealt with-experimental embryology and developmental genetics- are very wide in scope and have been actively pursued for half a century or more, during which period an immense store has been accumulated, both of factual information and of technical know-how. However, the field has not been characterized by the introduction of radically novel techniques, such as have been so rewarding in microbiology and molecular genetics. The course was not intended therefore to provide experience of new technical approaches; instead it attempted a quite different task namely, to explore how far it is possible to reformulate the classical problems of embryology and epigenetics in terms of recent theoretical advances in molecular biology. Further, it was intended to present this material not to trained embryologists or developmental geneticists but rather to students whose experience had been in molecular biology, biochemistry, microbiology, or other fields related to the aspects of subcellular biology which have been advancing most rapidly in the recent past. At the same time, the course was designed to give biological specialists of this kind some acquaintance with the types of biological systems (i.e. eggs and embryos of various groups of higher organisms) which are available at present for the study of differentiation.

Twenty-one students from European and non-European countries were admitted to the course. Thirteen fellowships offered by UNESCO-ICRO went to students coming from France, Belgium, United Kingdom, Hungary, Czeckoslivakia, URSS, Israel, Denmark and USA. Four Italians were awarded a fellowship offered by LIGB.

This course was supported in part ($ 5,000) by UNESCO and ICRO and in part (L. 4,394,999) under EURATOM Contract with LIGB.

Course on Repression and Suppression: cytoplasmic control of protein synthesis

A course on Repression and Suppression: cytoplasmic control of protein synthesis was held from April 27 to May 8, 1964, by Prof. Luigi Gorini (Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.) and Dr. Annamaria Torriani (Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass.).

Having defined structural genes and suppression, Prof. Gorini explained the mechanisms controlling the rate of protein synthesis. Repression and retroinhibition were discussed in more detail. He examined the repression due to substances present in the culture medium, the kinetics of derepression, the biosynthetical potential, the endogenous control of biosynthesis of some enzymes, the relations between repression and induction and between repression and retroinhibition; the allosterie, the aporepressor synthesis and its chemical nature.

Then Prof. Gorini explored genetic aspects of control of enzyme synthesis and proposed possible molecular models to explain them. In addition he discussed the factors affecting the meaning of the genetic code at the translation level, the suppression mechanisms, and the cytoplasmic factors activating suppression.

Fifty-two students attended the course, representing twenty-two different institutions. Free discussions integrated the lessons.

This course was supported entirely by the EURATOM Contract with LIGB (L.697,930).

S E M I N A R S

A total of 52 seminars was given at LIGB by scientists from other institutions or by members of the Laboratory, at a total cost of L. 2,903,267 borne by EURATOM Contract with LIGB.

List of seminars

Dr. P. Amati, Massachusetts Institute of technology, Cambridge, Mass., U.S.A.: "Moltiplicazione vegetativa dei fattori colicinogenici", "Alta interferenza negativa in lambda".

Dr. R. Baldwin, Stanford University, Department of Biochemistry, Palo Alto, Calif., U.S.A.: "Physical studies on the replication of DNA".

Prof. C. Barigozzi, Institute of Genetics, University of Milan, Milan, Italy: "Ereditarietà semimendeliana del carattere freckled (D. melanogaster) e sua interpretazione di tipo episomico".

Dr. G. Bernardi, Centre de Recherche sur les Macromolecules, Strasbourg, France: "Ricerche sulla DNA-ASI acida".

Dr. L. Bernini, University of Leyden, Department of Human Genetics, Leyden, Holland: "Studi sulla biosintesi delle emoglobine umane".

Prof. E. Bonavita, Institute of Neurology, University of Palermo, Palermo, Italy: "Proprietà molecolari di enzimi NAD-dipendenti e regolazione nel tessuto nervoso".

Dr. P. Bourgaux, Service de Bactériologie, Parasitologie et Immunohematogie, Faculté de Medécine et de Pharmacie, Université Libre de Bruxelles, Bruxelles, Belgium: "In vitro transformation by tumour viruses".

Prof. D. Bovet, Istituto Superiore di Sanità, Rome, Italy: "Dati recenti sulla farmacologia del condizionamento e dell'apprendimento: alcune considerazioni sui problemi del "coding" e dell'accumulo di informazione nella scienza del comportamento".

Prof. A. Carrelli, Institute of Physics, University of Naples, Naples, Italy: "Recenti ricerche sulla viscosità dei liquidi".

Dr. T. Cremona, International Laboratory of Genetics and Biophysics, Naples, Italy: "Conversion of DPNH dehydrogenase to cytochrome reductase".

Dr. R. Devoret, Centre des Faibles Radioactivite du CNRS, Gif-sur-Yvette, S. et O., France: "Radiactions et induction du prophage λ chez E. coli K12".

Miss L. Diamond, Institute of Virology, Glasgow, England: "Some characteristics of large plaques and small plaques polyoma virus".

Dr. P. Douzou, Laboratoire de Biophysique, Paris, France: "Some biophysical investigations in the field of biochemical and photobiological processes".

Dr. L. Eldjarn, Institute of Biochemistry, University of Oslo, Oslo, Norway: "Mechanism of action of the S containing protective agents".

Dr. A. Falaschi, International Laboratory of Genetics and Biophysics, Naples, Italy: "Fleomicina, un inibitore della DNA polimerasi".

Dr. J. J. Fox, Donald S. Walker Laboratory, Sloan Kettering Institute for Cancer Research, Rye, N. Y., U.S.A.: "Nucleic acid components: the chemistry and chemotherapeutic value of nucleosides".

Dr. M. Fox, Massachusetts Institute of Technology, Division of Biology, Cambridge, Mass., U.S.A.: "The fate of deoxyribonucleate following bacterial transformation".

Dr. M. Freed, California Institute of Technology, Pasadena, Calif., U.S.A.: "Temperature mutants of polyoma virus".

Dr. G. Giudice, Zoological Station, Naples, Italy: "Riaggregazione di cellule isolate da embrioni di riccio di mare".

Dr. F. Gonano, International Laboratory of Genetics and Biophysics, Naples, Italy: "Traduzione del codice genetico".

Dr. J. D. Gross, Institut de Biologie Moléculaire, Laboratoires de Biophysique et de Biochimie Génétique, Université de Genève, Genève, Switzerland: "The relation between DNA synthesis and chromosome transfer during bacterial conjugation".

Dr. L. Grossman, Graduate Department of Biochemistry, Brandeis University, Waltham, Mass., U.S.A.: "The molecular basis for the mutagenic and lethal effects of UV irradiation".

Dr. P. E. Hartman, Johns Hopkins University, Department of Biology, Baltimore , Md., U.S.A.: "Genetic control of histidine biosynthesis in Salmonella".

Prof. J. W. Hastings, Biochemistry Division, Department of Chemistry, University of Illinois, Urbana, Ill., U.S.A.: "Biological clocks: biology and biochemistry".

Dr. A. S. Kelus, Department of Experimental Pathology, Medical School, University of Birmingham, Birmingham, England: "Immunogenetics of plasma proteins".

Dr. W. E. Kisieleski, Department of Biochemistry, University of Oslo, Blindern, Norway: "Tritium in biological studies".

Prof. A. Kolin, Department of Biophysics and Nuclear Medecine, School of Medecine, Los Angeles , Calif., U.S.A.: "Utilization of magnetic fields in methods of electrokinetics separation of molecules and biological particles".

Prof. A. L. Lehninger, Johns Hopkins University, School of Medicine, Baltimore, Md., U.S.A.: " Ion transport by isolated mitochondria".

Prof. S. E. Luria, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.: "Sulla relazione fra profago e geni cromosomici".

Prof. V. Luzzati, Laboratoire de Génétique Physiologique du C.N.R.S., Gif-sur-Yvette, S. et O., France: "Risultati recenti sulla struttura degli acidi nucleici in soluzione".

Dr. R. Maggio, Institute of Comparative Anatomy, University of Palermo, Palermo, Italy: "Attivazione delle sintesi proteiche nell'uovo di riccio di mare in seguito alla fecondazione".

Dr. A. Mattera, International Laboratory of Genetics and Biophysics, Section of Pavia, Naples, Italy: "Lo stato attuale della teoria del drift genetico".

Dr. S. L. Miller, Department of Chemistry, University of California, La Jolla, Calif., U.S.A.: "The origin of life".

Prof. A. Monroy, Institute of Comparative Anatomy, University of Palermo, Palermo, Italy: "L'attivazione dell'uovo di riccio di mare studiato dal punto di vista biochimico".

Dr. A. Moroni, International Laboratory of Genetics and Biophysics, Section of Parma, Naples, Italy: "Indirizzi generali e stato attuale di un vasto gruppo di ricerche sulla consanguineità umana".

Dr. D. G. Ott, Scientific Laboratory, Los Alamos, New Mexico: "Chemical synthesis of polynucleotides".

Prof. G. Pontecorvo, Department of Genetics, University of Glasgow, Glasgow, England: "Molecular genetics: the next step".

Dr. W. Schreil, Department of Microbiology, University of Rochester, Rochester, N. Y., U.S.A.: "Some remarks on bacterial nucleoplasms".

Dr. P. Siekevitz, Rockefeller Institute, New York, N.Y., U.S.A.: "Observations on the ribosome".

Prof. L. Silvestri e Dr. L. R. Hills, Institute "P. Stazzi", University of Milan, Milan, Italy: "Un'ipotesi generale sull'evoluzione dei batteri, basata su considerazioni tassometriche e molecolari. Esposizione e discussione".

Dr. T. P. Singer, Edsel B. Ford Institute for Medical Research, Detroit, Michigan, U.S.A.: "Reconstitution of the respiratory chain".

Prof. F. H. Sobels, Department of Radiation Genetics, State University, Leiden, Holland: "Differential radiosensitivity and repair in the male germ line of Drosophila".

Dr. A. Stretton, Medical Research Council, Laboratory of Molecular Biology, Cambridge, England: "Colinearity of the gene and protein of the head of bacteriophage T_4".

Dr. P. Strigini, Laboratoires de Biophysique et de Biochimie Genetique, Institut de Biologie Moleculaire, Universite de Genève, Switzerland: "Sul meccanismo della back-mutazione di mutanti rII nel fago T_4".

Dr. I. Takahashi, Central Experimental Farm, Department of Agriculture, Canada: "Transduction in bacillus subtilis".

Dr. S. W. Tanenbaum, Department of Microbiology, College of Physicians and Surgeous, Columbia University, New York, N. Y., U.S.A.: "Experimental induction of antibodies which cross-react with single-stranded DNA".

Prof. A. Wacker, Institut fur Therapeutische Biochemie, Universitat Frankfurt am Main, Frankfurt, Germany: "Molecular mechanism of radiation effects (X-ray and U.V.-light); "Photoinactivation and photoreactivation (visible light)".

Dr. W. E. C. Wacker, Biophysics Research Laboratory, Harvard Medical School, Boston, Mass., U.S.A.: "RNA metals".

Dr. R. C. Williams, Virus Laboratory, University of California, Berkeley, Calif., U.S.A.: "Some observations on a "lipovirus".

Dr. M. J. D. White, University of Melbourne, Melbourne, Australia: "Heterotic, epistatic and biometric effects of inversions in grasshopper populations".

Prof. S. Zamenhof, College of Physicians and Surgeons Columbia University, Department of Biochemistry, New York, N. Y., U.S.A.:
"Some studies on the chemistry of mutation".

P U B L I C A T I O N S

During the period July 1st, 1963 - June 30, 1964, members of LIGB were authors of 29 published papers and 28 are currently in press. Those marked with an asterisk are related to work which was supported by Contract with EURATOM. Others, marked (°°), were carried on independently of EURATOM Contract.

List of papers

°° Edwards A. W. F., 1963 - Migrational selection - Heredity 18: 101-106.

°° Scarano E., Geraci G., Polzella A. and Campanile E., 1963 - The Enzymatic Aminohydrolysis of 4-Aminopyrimidine Deoxyribonucleotides IV - J. Biol. Chem., 238: PC1556-PC1557.

°° Maio J. J. and De Carli L., 1963 - Induction and repression of alkaline phosphatase in human cultured cells by prednisolone, hydrocortisone and organic monophosphates - Biochem. Biophys. Res. Commun., 11: 335-342.

°° Giuditta A. and Strecker H. J., 1962 - NADH-Tetrazolio riduttasi del cervello di bue - Soc. Ital. Biol. Sper., 38: 1833-1836.

°° Di Prisco G. and Giuditta A., 1962 - Effetto dei barbiturici sulla Diaforasi I di cervello di bue: relazione tra struttura e inibizione - Boll. Soc. Ital. Biol. Sper., 38: 1836-1839.

°° Edwards A. W. F., 1963 - Human sex ratio and maternal immunity to male antigen - Nature, 198: 1106-1107.

°° Benerecetti A. S. and Salvati A. M., 1963 - Sulla presenza di alcune attività enzimatiche in una frazione cromatografica non emoglobinica di emolizzati umani - Giornale di Biochimica, 12: 154-158.

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NADH oxidation by brain mitochondrial fragments - Acta Chemica Scand., 17: S67-S78.

°° Edwards A. W. F., 1963 - Natural selection and the sex ratio: the approach to equilibrium - Amer. Nat., 97: 397-400.

°° Giuditta A. and Di Prisco G., 1963 - The inhibition of cerebral NAD(P)H: (Acceptor) oxidoreductase by barbiturates and nervous depressants - Biochim. Biophys. Acta, 77: 394-407.

°° Tocco G., Orengo A. and Scarano E., 1963 - Ribonucleic acids in the early embryonic development of the sea urchin. I. Quantitative variations and ^{32}P orthophosphate incorporation studies of the RNA of subcellular fractions - Exper. Cell Res., 31, 52-60.

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°° Baglioni C. and Weatherall D. J., 1963 - Abnormal human hemoglobins. IX. Chemistry of hemoglobin $J_{Baltimore}$ - Biochim. Biophys. Acta, 78: 637-643.

* Ritossa F. M. and Pulitzer J. F., 1963 - Aspects of structure of polytene chromosome puffs of Drosophila busckii derived from experiments with antibiotics - J. Cell Biol., 19: 60A.

°° De Carli L., Maio J. J. and Nuzzo F., 1963 - Alkaline phosphatase activity and chromosome variation in human cells in culture - J. Nat. Cancer Inst., 31: 1501-1509.

* Edwards A. W. F., 1963 - A linkage for drawing the normal distribution - Appl. Statistics, 12: 44-45.

* Graziosi F., 1964 - La biofisica nella sua relazione con le ricerche e l'insegnamento di biologia e di fisica - Ric. Sci., 4: 5-20.

°° Baglioni C. and Sparks C. E., 1963 - A study of hemoglobin differentiation in Rana Catesbeiana - Develop. Biol., 8: 272-285.

* Barrai I. and Barbieri D., 1964 - Drift in una popolazione stimulata - Atti Ass. Genet. It. 9: 233-245.

°°Aurisicchio S., Dore E., Frontali C., Gaeta F. and Toschi G., 1964 - Chromatographic purification of one of the complementary strands of DNA of phage - Biochim. Biophys. Acta, 80: 514-516.

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°° Giuditta A. and Casola L., 1963 - Effetto del Fenobarbital su enzimi flavinici e non flavinici - Boll. Soc. Ital. Biol. Sperim., 39: 1861-1863.

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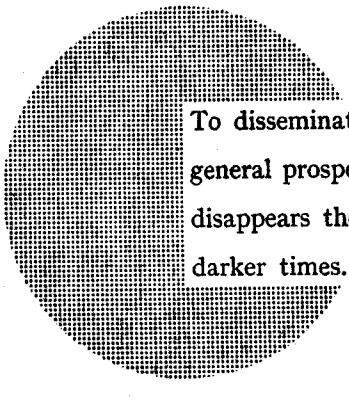

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Alfred Nobel

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