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ACTUAL PROBLEMS OF ANTIVIRAL CHEMOTHERAPY

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If one takes into account that of the rare favorable results obtained in the specific treatment of human, animal or vegetable viruses the subject of chemotherapy of infections with virus can be treated in several ways. On the other hand, more detailed articles on research effected with the help of a relatively simple experimental system, with the object of obtaining a selective inhibition of the multiplication of the virus and of its pathogenic effect owing to the action of diverse products, requirements of long development.

In spite of the fact that this research has not generally brought about practical applications some among them have afforded a useful contribution to the mechanism of viral infection. Appraising in a large sense, antiviral chemotherapy, therefore, constitutes in our time an important area of general virology.

Many articles, some very detailed, have reviewed, in the past 10 years, this question (1,2,3,4,5,6,7,8,9); in order to give an idea of the number of articles published in this area, we indicate that the magazine of Hurst and Hull (3) contains no less than 480 references.

In the general review which we are presenting in turn here, we will try not to duplicate those which we will mention, and we particularly want to indicate the most recent articles. On the other hand, our study does not pretend to be exhaustive, we will only cite those articles which offer an undisputed interest, either a point of view of theoretical ideas which permitted specification or those practical applications which eventually are considered. In spite of considerable importance that the study of selective inhibition of the multiplication of virus of bacteria (bacteriophages), of the virus of vegetables and of the virus of invertebrates presents, which often furnish the conceptual foundations essential to virology, we have limited ourselves to the chemotherapy of infections by the virus of human and animal diseases. We simply point out that the study of selective chemical inhibition of the multiplication of bacteriophages and of viruses of vegetables has been reviewed in detail by Matthewes and Smith (1), it is approached likewise in a book dealing with bacteriophages and of vegetable viruses in a general way (10). We allow likewise one side of the problem of chemotherapy of infections caused by the agents of the group Bedsonia (psittacose, lymphogranulomatose, trachoma). By certain aspects of their intracellular multiplication (presence of an eclipse in the course

of the cycle of multiplication and reproduction by assemblage of non-infectious elementary units) these infectious agents connect to the virus. But they connnect with the bacteria due to the fact that they possess metabolic activities independent of the cellular metabolism: for example, the existance in some among those of 1 cytochrome C reductase (11) and of an enzyme capable of synthesizing again the folic acid (11 bis) and thus causing that the muramicacid -an amino sugar which characterizes the bacterial membrane- is present in its limiting membrane (12,13).

It is, therefore, not surprising that laboratory research (14,15) and clinical observations have shown that the agents of the Bedsonia group are able to be inhibited or inactivated by the products used in antibacterial chemotherapy, such as the sulfamides and certain antibiotics. Because of analogous reasons, these antibiotics with antibacterial action have been useful with respect to infections caused by rickettsia (16) which we will likewise exclude from our article.

I. Nature and Multiplication of Viruses

A review of the problems of antiviral chemotherapy can not be put aside as a brief account of the present knowledge of the nature of the virus and its mechanisms by which they infect the cells and reproduce themselves. This misjudgment of the fundamental differences existing between the bacterias and the virus is a responsible effect of an attitude sufficiently spread which consists in seeing antiviral chemotherapy as a simple extension of antibacterial chemotherapy and of thinking that the same concepts and the same methods are applicable in both cases. As a matter of fact, the viruses represent a category of biological entities entirely distinct from those which form microorganisms on the one hand and cellular organs on the other hand (17).

1.) From the point of view of their structure and their chemical composition, the viruses consist essentially of a shell of protein substance (or: capside) surrounding a central genetic material, bearer of the information necessary of the specific reproduction of viruses; this genetic material is either a ribonucleic acid (A.R.N.) or a desoxyribonucleic acid (A.D.N.). The capside is composed itself of elementary units (capsomers) whose name and the spatial disposition are without doubt specifics of a group of determined viruses: the whole capside and internal genetic material constitute a complete viral particle, or viron. Certain viruses for example those of poliomelitis, of influenza, of mumps, aphtous fever, contain A.R.N., others (for example those of vaccine, of smallpox, herpes, adenovirus) contain A.D.N. No viruses known contain both types of nucleic acids at the same time. The total chemical composition is so much more complex that their dimensions are very important, the smallest contain only nucleic acid and

protein, the largest contain, in addition, lipids, and carbon hydrates, even (like the vaccinal virus) biotine and copper. The amino acids of the proteins of the viruses and the purine and pyrimidine bases of their nucleic acids do not distinguish themselves at all from those present in the proteins and cellular nucleic acids. Likewise, the mucoproteins which are part of the capside of myxovirus (for example, the influenza virus) are probably identically composed to those of the mucoproteins of the cell membrane (18). With two exceptions, the enzymatic equipment of viruses is non-existant: the vaccine virus contains a flavine-adenine-dinucleotide (which is a co-enzyme of the respiratory chain) and the myxovirus possess a neuraminidase (19) capable of decomposing the mucoproteins of the cell membrane with the formation of N-acetyl-neuraminic acid (20,21).

2.) Viral multiplication.- It is, therefore, clear that the intracellular synthesis of constituent polymers of viruses (nucleic acids and proteins) is used while borrowing the constituent materials as the enzymatic and energentic reactions of the infected cell. This narrow relation between the normal process of the cell metabolism and those necessary to the viral multiplication explain the difficulty possessed by a selective inhibition of the latter. It is, however, not excluded that the enzymes which are normally present in the non-infected cell can there appear as a consequence of the viral infection and play a role in the synthesis of polymers of viruses, however, that may be these enzymes do not form an integral part of the viral particles themselves.

From the point of view of the dynamics of virus multiplication, which is unfolded in the midst of the infected cell, one can distinguish four phases, whose consideration is important to tne study of antiviral chemotherapy, since this is one or the other phases which selectively are inhibited: a) the adsorption into the cell; b) a phase of eclipse characteristic of the multiplication of all viruses and during which at least 90% of the viral particles having penetrated the cells are not evident by any distinct biological activity; c) a phase of multiplication during which the number of infectious viral particles which one cannot extract form the infected cells increases the exponentially; d) a phase of liberation in the course of which the viral particles newly formed release the infected cells and are adapted to infect the other cells. The mechanisms of penetration of viruses in the cell are again poorly understood; it is probable that they vary according to the type of viruses considered. In the case of myxovirus, the enzyme neuramidinase without doubt plays a role in attacking the mucoproteins which are part of the cell membrane. A process similar to that of the phagocytose (viropexie) owing to which the cell membrane surrounds the viral particle in order to make it penetrate into the cytoplasm, has been demonstrated in the case of

myxovirus (22,23), of the vaccine (24) and of adenovirus (25); furthermore, it is not certain that the totality of the viral particle penetrates intact into the cell. The phase of eclipse which immediately follows the penetration of the virus into the cell and lasts 3 to 8 hours according to the virus considered, corresponds to the dissociation of the constituent elements of the viral particle where the most important - the nucleic acids - then take possession of the cellular biosynthesis mechanisms which are necessary for their multiplication. During this period which is the most important in the series of reactions developed between the genetic viral material and the centers of biosynthesis activity of the cell, again it is impossible to isolate the infected cells of the viral particles completely formed and infectious from the other cells, but certain preliminary essential events have been observed toward the end of the eclipse. It is thus that the cells infected by influenza virus of viral A.R.N. are synthesized on the surface with a cell nucleus and appears afterward in the cytoplasm under the form of a ribonucleoprotein particle whose dimensions are much smaller than that of the viral particle itself (antigen S); at this moment, the mucoproteins which constituted the capside of the virus are synthesized in the cell cytoplasm; it is not until the end of the period of eclipse, by collection of the ribonucleoprotein, of mucoproteins and of certain lipides, that the whole viral particle is made up. It is probable that this phenomenon took place on the surface of the cell membrane immediately before going out of the virus. Under certain conditions the joining between the viral A.R.N. of nucleous origin and the mucoproteins of cytoplasmic origin is not produced and the latter accumulate around the microsomes of the cells, this then leads to the formation of particles which possess the antigenic specificity of influenza virus but have a particular morphologic aspect and are not infectious (28,29). In the case of adenovirus (which contain A.D.N.) the preliminary events essential to the biosynthesis of the viral nucleic acid develope entirely on the surface with the cell nucleus such that in the case of the virus of the vaccine (which likewise contains A.D.N.), the first manifestation of the biosynthesis of viral A.D.N. appears in the cell cytoplasm (30, 31).

Following the phase of eclipse is the phase of multiplication, during which the nucleic acids and the specific proteins of the virus infecting are synthesized at an exponential rhythm in diverse parts of the cell and joined rapidly to form new viral particles. As soon as the specific information has been provided to the cell by the viral genetic material, one can consider that the cell devotes all or part of its metabolic activity to the synthesis of the constituent materials of the virus. There is no doubt that the elementary structural

parts (amino acids, purines, pyrimidines, sugars) used for this synthesis are of cellular origin and that the enzymatic energetic mechanisms are likewise mechanisms proper to the cell. The role of the infectious virus reduces itself therefore essentially to a role of information and one knows that this information is contained entirely in the A.R.N. and A.D.N. of the virus.

In effect, following the work of Gierer and Schamm (32) and of Frankel-Conrat (33), showing that A.R.N. isolated from the virus of tobacco mosaic by extraction with phenol or with the help of a detergent (sulfate of dodecyl sodium) have for the Nicotina leaves an infectiousness wholly comparable to that of the entire viral particle, a series of research has obtained beginning with the animal virus (generally by the method of extraction with phenol) the preparations of A.R.N. which appear to be capable of penetrating living cells (in cultures in vitro or in the whole animal) and of causing the synthesis of viral particles identical to those from which the A.R.N. had originally been isolated.

In other words the infectiousness of a virus, that is to say, its property of releasing in a sensitive cell the process leading to its reproduction, resides entirely in the nucleic acid (A.R.N. or A.D.N.). The molecules of A.R.N. exempt from contamination by the proteins and endowed with infectiousness, have been isolated, the poliovirus (34,35), the encephalitogene virus (34,36,37), the virus of aphthous fever (38), probably also the influenza virus (39) whereas the infectious A.D.N. has been isolated from a virus of tumoral origin in a mouse, the virus of polyome (40). In the case of virus of very large dimensions and of a very complex organization, like those of the vaccine, again it has been impossible to isolate a nucleic acid which is only infectious but this has not been possible due to technical difficulties. The infectious nucleic acids isolated from animal virus never distinguish themselves from complete viral particles, from the point of view of their infectiousness for the cell, which by the fact that this infectiousness is rapidly destroyed by the nuclease (ribo or desoxyribonuclease) which are without activity on the intact viral particles. One then sees that the proteins which form the capside of the virus essentially have a protective role and assure a very effective penetration of the cell, but they do not seem to play a direct role in the multiplication of virus in the interior of the cell. The selective inhibition of this multiplication would therefore be essentially an inhibition of the biosynthesis of viral A.R.N. or A.D.N. But it is clear that an inhibition of the biosynthesis of viral proteins leads among others, to the formation of infectious particles imperfectly protected would decrease the possibility of propagation of the infection from one cell to the other.

Nevertheless, antiviral chemotherapy can offer other objectives than selective inhibition of intracellular biosynthesis of

constituent elements (nucleic acids and proteins) of viruses: it can aim at inhibiting the collection of these elements in a integrated particle, or block the departure of the viral particle outside of the cell or again diminish or suppress the pathogenic effect which it exercises on the cell the multiplication in its interior of the virus polymers. Different mechanisms change with the type of virus and sometimes the experimental conditions seem to intervene in order to render possible the departure of virus particles newly formed in the infected cell: in certain cases one assists in the explosive liberation of a great number of viral particles, but more often the liberation of these viral particles is a process which last many hours during and after the exponential phase of intracellular multiplication, for this liberation ordinarily does not result from having undergone lysis of the infected cell, contrary to what is observed in the case of bacteriophages. The cell membrane plays a primary role in the mechanism of liberation of certain viruses, for example in the case of myxovirus which leave the infected cell by inserting into the protuberances in the shape of a glove finger formed by the membrane (41) or in the case of poliovirus which causes a local disintegration of the cell membrane at the point of departure (42).

3.) The pathogenic effects of the viral infection to the cell scale can be described in biochemical terms and in morphological terms. These effects are so varied and numerous according to the type of virus and the cell considered that it is impossible to describe it generally. These biochemical alterations of cells infected by the virus have been the object of excellent articles (43,44) as well as the morphological alterations (45,46). Biochemically, the cell infected by a virus is the object of a deviation of its biosynthesis activities in behalf of the production of specific virus polymers, but it does not necessarily entail the suppression of all normal metabolic activities of the cell: in particular the cell respiration and glycolysis are not deeply affected during the first stages of viral multiplication.

Morphologically, the multiplication of a virus in a cell can entail a more or less brief maturity, its destruction; but a case exists where the production of the cell takes place without the life of this cell nor its capacity for division being notably altered (case of latent infections) and in other cases where the result of the viral infection is the transformation of the infected cell into a cancerous cell (case of onocogene virus). The scale of the entire animal organism, the alteration and the destruction of a considerable amount of cells following the viral infection contain the pathological manifestations whose nature varies according to the virus and the tissue, habitat of the infection. The relative clinical syndromes, (for example pulmonary, neurological etc.) can be produced by different viruses. There seems to exist a threshold for the number of infected and altered cell be-

yond which the viral disease manifests itself clinically (this is the case of a poliomelitic infection). Without going into the details of the pathogens of viral infections we add that a series of phenomena enter into play in order to provoke the symptomatology of viral diseases and specifically linked to the multiplication of viruses in a tissue or a determined organ.

4.) Methods used for the study of the inhibition. This is because it is necessary to distinguish now the tests of antiviral chemotherapy effected with the help of cells cultivated in vitro or in embryonic chicken eggs (in ovo) and those in which the experimentation concerns the entire animal. In the first case the study of the antiviral activity carried out in the intracellular multiplication of the virus, whose degree of inhibition can be measured with precision, or on the process of adsorption and the liberation of viral particles so that on the alterations which the viral infection causes in the cell (cytopathogenic effect, is expressed by a destruction of the formation of specific inclusions in the cytoplasm of the nucleus, lesions of embryonic membrane or death of the embryo). In this type of experimentation (cell cultures or in ovo) the product can be placed directly in contact with infected cells at a determined concentration. In the second case (animal experimentation) the study of the antiviral activity consists essentially on observing the modifications that a determined treatment can offer to the evolution of the viral disease and to its symptomalology (mortality, paralysis, histopathologic lesions) the product being introduced by diverse means but they do not always without transformation at the point where the infection is. These two ways of experimentation complement each other, but the activities eventually observed in an experimental model are not necessarily ascertained in the other, for the mechanisms used are not necessarily the same.

In short, taken in a wide sense, antiviral chemotherapy resides in the research of chemical products capable of modifying, with a given mechanism, the complex series of events which accompany, in the nucleous of the cell and of the nucleous of the whole animal, the development of the viral infection, which is a question of the adsorption of the virus on the cell, of its multiplication, of its liberation, of its propagation from one cell to the next or the lesions and the symptoms which this infection causes directly or indirectly.

Whatever it is, this which we have mentioned above about the nature and the mode of multiplication of the virus suffices to show that antiviral chemotherapy, in contrast to antibacterial chemotherapy has as its essential objective not only the infectious particle itself but also the association virus-cell, the viral infection being, in last analysis, only an alteration of the normal cell function by the introduction of a strange genetic material capable of deflecting for its own benefit certain synthetic activities of the cell.

The most logical way of presenting the chemical properties whose antiviral activity has been demonstrated experimentally would be

to classify them according to the mode of action, that is to say according to the events of the cycle of viral development which they inhibit specifically. Unfortunately, in a number of cases, it is not possible to determine with precision the phase of viral infection which is modified by the addition of a determined product and that is particularly true in the case of activities which have been ascertained in the whole animal and not in cell cultures. We will therefore adopt for this article a classification based essentially on the chemical nature and the probable chemical activity of the products considered.

II. Inactivation of Viruses (Products called "VIRUCIDES")

We will make only a brief allusion to the products capable of inactivating the virus in vitro by direct contact by a non-specific mechanism (denaturation of the proteins, oxidation, alcoylation of nucleic acids) and in which the formaldehyde and beta-propiolactone provide the most recent examples. The question has been considered in detail by Gard and Maale (47). These products have an interest evident by the inactive vaccines and eventually by the disinfection of objects contaminated by the virus but the nonspecific character of the denaturing action excludes them from the category of products have a selective chemotherapeutic activity; they also are more toxic for the living cell than for the viral particle.

The study of certain of these products is still useful for the knowledge of the chemical structure of the virus, thus the study of the inactivation of enterovirus by mercuric parachlorobenzoate has permitted the conclusion that the sulfhydryl groups present on the surface of the virus playing an essential role in their mechanism of adsorption on the cell (48). The disintegration of myxovirus by ethyl ether has permitted us to state exactly our knowledge about the architecture of the particles of this virus (49,50). Wallis and Melnick (50 bis) used a differential sensitivity at a temperature of 50° in the presence of $MgCl_2$ of poliovirus and the virus present in a latent stage in certain monkey kidney cultures in order to eliminate the last of the lot of weakened living poliomelitis vaccine.

III. Analogues and Metabolites

Being given that most research relative to the inhibition of the multiplication of viruses by analogous structures of amino acids has been effected with cells cultivated in vitro, it would be wise to recall the work of Eagle (51) which demonstrates that 13 amino acids should be present in the culture medium in order to allow the growth in vitro of the animal cells. These are lysine, tryptophane, phenylalanine, threonine, valine, methionine, leucine, isoleucine, arginine, cystine, glutamine, histidine and tyrosine (of which only the first 8 are indispensable for the nitrogen balance of mammals) (52). It is necessary therefore to rely that the addition to the cell medium of the

cells of analogues of the essential amino acids interferes with the cell anabolic reactions, their addition to the cell cultures beyond the cell survival while inhibiting the biosynthesis of viral proteins, for example: the simple omission of glutamine in culture mediums of HeLa cells, after their growth suffices to diminish the production of poliovirus for these cells (53).

The DL-ethionine inhibits the multiplication of poliovirus type 2 (54) and some influenza viruses in cell cultures (55) and in both cases this inhibition is suppressed by the addition of L-methionine to the cultures. The inhibition of the multiplication of polio virus by DL-ethionine moreover is obtained only with a concentration of this product likewise decreasing the cell respiration (56).

DL-methoxinine (DL-α-amino-γ-methoxybutyric acid) inhibits the multiplication of vaccine virus (57) and of influenza virus (55) in cell cultures, when the product is added to cultures during the phase of eclipse; the inhibiting effect is suppressed by the addition of L-methionine.

β-2-thienylalanine inhibits the multiplication of vaccine virus (58) and of poliovirus type 2 (59) in cell cultures, this inhibition is suppressed in the presence of phenylalanine. As in the case of Dl-ethionine the inhibition of the multiplication of polio virus is obtained with a concentration of β-2-thienylalanine which has a depressing effect on cell respiration (56).

The threo isomer of β-phenylserine as well as its methyl ester inhibit the multiplication of influenza virus type A in cultures of chorioallantoic membranes of chicken embryos, at a concentration close to 10 times less to that inhibiting the cell growth, with the condition that it is added to the cultures at the beginning of the phase of eclipse of the virus (60). The inhibiting action is suppressed by the addition of phenylalanine. The β-phenylserine likewise inhibits the multiplication of mumps virus, of vaccine virus, of ectromelia and of encephalomyocarditis murine virus. Its methyl ester prevents the multiplication of influenza virus on the allantoic cavity of the embryonic chicken egg. But β-phenylserine and its methyl ester administered to the mouse subcutaneously or in aerosol form have no prophylactic or curative activity on the infection of this animal with the influenza virus inoculated intranasaly. In the mice subject to a 4 to 5 day fast, in order to decrease their hepatic protein reserve, the methyl ester of β-phenylserine, administered subcutaneously, has inhibited the hepatic multiplication of the ectromelia virus, but this action remains minimal, without doubt, by reason of the normal presence in the liver of an enzyme capable of decomposing β-phenylserine (61). These results illustrate some of the reasons for which an analogue having antiviral activity in the cell cultures is not necessarily active in the whole animal.

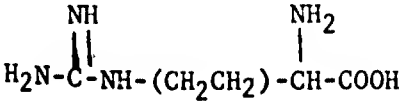
Still, β-phenylserine exercises an interesting activity in significantly diminishing the mortality rate of rats infected with a strain of rabies (62). This activity has been observed also when the treatment with this analogue, intraperitoneal, only started the day after infection. The concomitant administration of DL-phenylalanine or DL-tyrosine suppressed the prophylactic and therapeutic activity of β-phenylserine. The daily active dose of the analogue (60 to 100 mg/kg) is only slightly less than the lethal dose of the product for a rat (140 mg/kg). The rats have resisted the rabies infection owing to treatment with β-phenylserine, they manifested no immunity with respect to a subsequent re-infection with the same virus, which would indicate that the treatment has completely suppressed the multiplication of the virus in their tissues; this is likewise proved by the verification that the xanthine-oxidase content of the cerebral tissue remained unchanged, while the rate of the enzyme increased to a pronounced height in the brain in the course of the multiplication of the rabies virus and other neurotrope viruses in this organ (63).

β-phenylserine has likewise inhibited the multiplication of vaccine virus and of myxomatosis in the dermis of the rabbit (62). The activity of β-phenylserine is also suppressed by phenylalanine as well as by tyrosine, it is possible that this analogue hindered the incorporation of tyrosine in the viral protein blocking in the rat, the activity of the phenylalanine-hydrolase, hepatic enzyme which catalizes the transformation of phenylalanine into tyrosine.

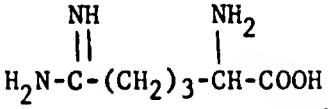
Para-fluorophenylalanine inhibits the multiplication of poliovirus type 3, without hindering the cytopathogenic effect, in cultures of HeLa cells (64) which is added to the cultures at the beginning of the phase of eclipse of the virus, at a cytotoxic concentration: this inhibiting effect is suppressed by the addition of phenylalanine. The same analogue permitted Zimmerman and Schafer (65) to make a remarkable study of the dynamics of the virus development of the aviain pest (from the myxovirus group) in cultures of chicken embryo cells. When the analogue is added to the culture at the time they are infected none of the constituent polymers of the virus (A.R.N. of antigen S, mucoproteins of hemagglutinin) is synthesized, although the virus penetrated the cell normally, and there undergoes a phase of eclipse. When the analogue is added to cultures 2 hours after infection, the synthesis of the ribonucleo protein of the antigen S (which is in the cell nucleous) is effected normally, but the production of hemagglutinin is suppressed and the antigen S accumulates in the nucleous instead of passing to the cytoplasm; consequently the production of complete and infectious viral particles is suppressed. When the product is added 3 to 6 hours after infection, the production of hemagglutinin and of infectious virus does not decrease, but these are doses different from the analogue which hinders the

formation of one and the other. The inhibiting effect of the analogue is reversible at different stages of the cycle of multiplication, by addition of phenylalanine. In conclusion, p-fluorophenylalanine inhibits at the same time the biosynthesis of mucoproteins of the hemagglutinant antigen and of the protein necessary to transport the viral A.R.N. from the nucleus to the cytoplasm. In addition, the research of Scholtissek and Rott (66) showed that the inhibition by p-fluorophenylalanine of the synthesis of viral A.R.N., is observed when the analogue is added to the culture soon after infection, it is explained by the incorporation of this analogue into a protein necessary to the biosynthesis of A.R.N., incorporation which renders the "preliminary" protein nonfunctional. In summary, the p-fluorophenylalanine interferes with the synthesis of the proteins of the hemagglutinant antigen, with that of the protein necessary for the reproduction of viral A.R.N. and with that of the protein necessary to transport the A.R.N. into the cytoplasm and its association with the hemagglutinant antigen.

L-canavanine, amino acid that has a structure analogous to that of arginine, present in certain haricots (jack beans)



CANAVANINE



ARGININE

inhibits the multiplication of the influenza virus (type B stain Lee) in the allantoic cavity of the embryonic chicken egg and in cultures in vitro of the chorioallantoic membrane (67); that inhibition is suppressed by the addition of arginine. It is interesting to verify that canavanine does not inhibit the multiplication of influenza virus type A (strain PR8) and of mumps, which would indicate that the synthesis or utilization of arginine is only necessary to certain myxovirus: on the other hand, the canavanine inhibits the multiplication of poliovirus muris (Theiler GD VII) in cell cultures (68).

Kundin and his collaborators (69) recently effected a study of the inhibition of the multiplication of influenza virus (types A and B) in lung cell cultures of chicken embryoes and observed significant activity of nor-leucine (reversible by methionine) β-phenylserine (reversible by phenylalanine), of canavanine (reversible by arginine), of N-allylglycine,

and of S-ethyleysteine (both reversible by cysteine). Any of these analogues only augment the survival of the mice infected intranasally with the viruses which they inhibit in cell cultures.

Finally it is proper to note that the presence in cell cultures of certain amino acids, natural in excess (for example o to 5 mg/ml) can without causing the toxic effect evident by the cells, inhibit the multiplication of certain viruses, thus arginine, lysine and ornithine vis-a-vis of myxovirus (70), lysine, tryptophane and histidine vis-a-vis of poliovirus muris (71). In addition, an enzymatic hydrolase of lactalbumin, containing an indefinite mixture of amino acids and of peptides, exercises an inhibiting effect on the multiplication of influenza virus (72) and of respiratory virus ECHO 28 (JH-2060) (73) in cell cultures.

It would be interesting to examine the eventual antiviral activity of polypeptides in particular those containing analogues of amino acids.

2. - SUGARS

The presence of glucose (in certain cases replaceable by pyruvic) in the medium of cell cultures is indispensable to the production of virus by those cells (44,53). Kilbourne (74) showed that an antimetabolite of glucose, D-2-desoxyglucose, inhibits the multiplication of influenza virus (type B) in the allantoic cavity of the embryonic chicken egg, this antimetabolite does not hinder the multiplication of this virus in the lungs of mice.

3. - FOLIC ACID

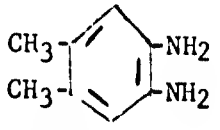
Aminopterin (4-pteroyl-L-glutamine) and amethopterin (4-methyl-10-pteroyl-L-glutamic), antimetabolites of folic acid interfere with the formation of cofactors necessary for the biosynthesis of purines and pyrimidines and have therefore an inhibiting effect on the biosynthesis of A.D.N. and A.R.N. Aminopterin exercises an inhibiting effect on the multiplication of vaccine viruses in cell cultures but in practice has no effect on the multiplication of poliovirus and of the Newcastle disease viruses (75); meanwhile the activity of this antimetabolite is not limited to viruses with A.D.N., since it inhibits the multiplication of reovirus type 3, which is a virus with A.R.N. (76).

The role of folic acid in the evolution of lymphoyctic choriomeningitis in the mouse has been brought to light by Haas and his collaborators (77,78,79.80): The mice receiving a diet deficient in folic acid or treated with amethipterine survived the intracerebral infection by the lymphocytic choriomeningitis virus. Still the virus multiplies in their brain as well as in that of the test mice. Therefore it does not seem, in this case, that the activity of the antimetabolite power is explained by an inhibition of the biosynthesis of the virus; it is more

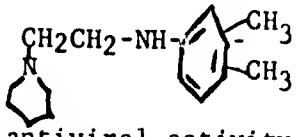
probable that the amethopterine acts, like irradiation with X-rays (81) in depressing certain immune reactions of the mouse, which in the case of lymphocytic choriomeningitis, have a fatal influence on the evolution of the disease.

4. - VITAMIN B_{12} and RIBOFLAVIN

Clark and collaborators (82) examined the inhibiting activity on the multiplication of influenza in cultures of chorioallantoic membranes of chicken embryos by derivatives of 4,5-dimethyl-o-phenylenediamine in which the radical is present in the molecule of cyanocobalamine (vitamin B_{12}) and of riboflavin (vitamin B_2).



Of these derivatives, 1-[2-(3,4-xylidino)ethylamino]piperidine showed itself to be the most active in inhibiting the formation of viral hemagglutinin, without hindering the formation of antigen S (A.R.N.), at a concentration noticeably less than that toxic for the cells.



The antiviral activity of this product has been suppressed by the addition of 4,5-dimethyl-o-phenylenediamine to the medium, but not for that of cyanocobalamine or of riboflavin. The product is devoid of activity on the multiplication of influenza virus in the allantoic cavity of embryonic chicken egg and in the lungs of mice (83).

5. - PURINES and PYRIMIDINES

The nucleic acids of viruses which contain the specific information necessary for their intracellular multiplication include, like cellular nucleic acids, 2 purine bases and 2 pyrimidine bases (adenine, guanine, cytosine, uracil in the case of A.R.N.; adenine, guanine, cytosine, thymine in the case of A.D.N.). A selective inhibition of the biosynthesis of purines and pyrimidines or of their incorporation into the viral nucleic acids realize one of the essential objectives of antiviral chemotherapy since it blocks the synthesis of the molecule which confers on the virus it infectiousness. The relations existing in the interior of the infected cell, between the biosynthesis of the cellular nucleic acids and those of the viral nucleic acids are in addition not known precisely and they vary, doubtless with the virus concerned, (in particular according to the nucleic acid it contains - A.R.N. or A.D.N. and according to the localization of the biosynthesis of the viral nucleic acid in the cell - nucleus or cytoplasm). It is in any case highly probable that the synthesis

of the viral nucleic acid borrows the same metabolic process that the cellular nucleic acid and that it uses certain preliminary materials already present in the cell, for example, the purine and pyrimidine bases of nucleosides and nucleotides. This would therefore be essentially quantitative differences of compositions (for example relative proportions of purine and pyrimidine bases) or of speed of biosynthesis between the nucleic acids of the virus and those of the cell than a selective inhibition could exploit.

The analogues of purines and pyrimidines whose antiviral activity has been studied can act by diverse mechanisms: a) incorporation of the viral nucleic acid instead of the normal base with the formation of a "fraudulent" nucleic acid devoid of infectiousness; b) inhibition of certain enzymatic reactions catalyzing the formation of precursors of nucleic acids, for example, of synthesized thymidine which catalyzes the methylation of uracil in thymine or of the decarboxylase of orotidylic acid, which catalyzes the transformation of the latter into uridylic acid.

The compound 2-6-diaminopurine(2-amino adenine) inhibits the multiplication of vaccine virus (84), of poliovirus type 2 (Lansing) (85) and of the virus of Russian encephalitis estivo-printaniere (86) on cell cultures. Its inhibiting action on the multiplication of poliovirus type 1, in cultures of HeLa cells, is observed only at a concentration which diminishes cell respiration (56). In the mouse, 2,6-diaminopurine administered i.p. increases the survival of the animal after infection with the Russian encephalitis estivo-printaniere (87). In cell cultures, the antiviral activity of 2,6-diaminopurine is suppressed by the addition of adenine.

Another antimetabolite of adenine, 6-mercaptopurine, has a therapeutic activity on leukemia caused in the mouse by the Fruend virus, but this activity is probably exercised more on the malignant proliferation of leucocytes than on the multiplication of the virus (88). This analogue exercises moreover a marked inhibition on the formation of antibodies in the rabbit (89) and an increasing effect on the infection of rhesus monkey by the smallpox virus, probably in diminishing the immunologic response of the animal (90). 6-thioguanine, an analogue of guanine decreases by a similar mechanism the resistance of the mouse to infection by the West Nile encephalitis virus (91).

The compound 2-thiouracil incorporates itself in the place of uracil (replace in proportion: 10 to 20%) in the A.R.N. in the tobacco mosaic virus and inhibits the multiplication of it. (92); this same analogue inhibits the multiplication of cold virus in the chorioallantoic membrane of the chicken embryo (93) and leads to the formation of incomplete particles, hemagglutinants but not infectious, since devoid of A.R.N.(94): This effect is suppressed by uracil. The 2-thiouracil and the 5-methyl-2-thiouracil decreases the multiplication of poliovirus in cell cultures (95).

The compound 4,6-dichloro-5-phenoxy-2-thiouracil exercises a protective influence highly significant in the mouse infected intracerebrally or intranasally with vaccine virus (96) and acts in synergism on this infection, with the thisemicarbazone of isatine which we will discuss later (97).

5-fluorouracil, an analogue of uracil, is an antimetabolite whose marked antitumoral activity has been demonstrated experimentally and clinically (98). It is incorporated instead of uracil in the A.R.N. of the virus of tobacco mosaic and the "fraudulent" A.R.N., thus formed is less infectious than the normal A.R.N. (99). This same analogue is incorporated to such a degree in 5-fluoro-uridylic acid, in the A.R.N. of poliovirus: nearly 30% of the uracil being replaced by the analogue; moreover the virus containing this abnormal A.R.N. does not differ from the normal virus by any biological properties (100). 5-fluorouracil can interfere, in different ways, with the biosynthesis of nucleic acids, it can be incorporated in A.R.N. instead of 5-fluoro-uridylic acid, but it can also inhibit the synthesized thymidilate which catalyzes the transformation of uracil into thymine and in thymidilic acid (101); by this last mechanism, it interferes with the synthesis of A.D.N.

Thus, it is the 5-fluorouracil that inhibits the multiplication of the vaccine virus (virus with A.D.N.) in a way reversible by thymidine, but has no effect on the multiplication of the Newcastle disease and of poliovirus (102).

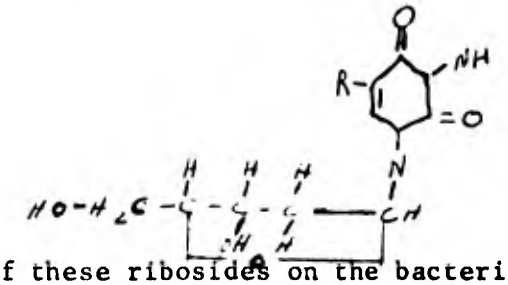
Kaplan and Ben-Porat (103) showed that 5-fluorouracil has 2 kinds of inhibiting effects on the biosynthesis of cellular A.D.N. by the kidney cells of rabbits cultivated in vitro: the first, which is produced immediately after addition of the analogue to the cultures, it is reversible by thymidine; the second, manifests itself only after the synthesis of a certain quantity of A.D.N. by the cell and is not reversible by thymidine: on the contrary, the inhibiting effect is suppressed when the cells are infected with pseudo-rabies virus (Aujeszky disease). These authors express the hypothesis that 5-fluorouracil is, in the second case, incorporated in cellular A.R.N. which leads to the formation of abnormal proteins and interferes with the production of enzymes necessary for the synthesis of A.D.N.; on the other hand, in the cells infected with pseudo-rabies virus, the formation of enzymes necessary for the synthesis would not be altered by the presence of "fraudulent" A.R.N. having incorporated the analogue, which leads us to think, in this case, that the viral infection modifies the reactions by which the synthesis of A.D.N. is produced, and the cell division. These considerations present a certain interest for the understanding of mechanisms possible of viral oncogenesis. On the other hand, the virus particles of pseudo-rabies produced by the kidney cells of a rabbit treated with 5-fluorouracil are not infectious because they are deprived of nucleic acid (104).

The compound 5-fluorouracil inhibits the multiplication of the encephalo-myocarditis murine virus (105) and of Rous' sarcoma virus (106): it acts, in both cases, on the virus with A.R.N. and it is probable that the inhibition is due to the incorporation of the analogue in viral A.R.N. It is said that in the case of other viruses with A.R.N. (poliovirus, Newcastle disease virus) (100,102), this incorporation does not alter their infectiousness. Any of these examples illustrate the diversity of possible effects of 5-fluorouracil on the cell metabolism and on viral multiplication.

Diazouracil has a chemoprophylactic effect, of non-elucidated mechanisms, on the infection of the mouse by poliovirus type 2 (Lansing strain), but does not protect the macaques rhesus against the oral infection by poliovirus type 1 (Mahoney strain) (107).

In recent years important observations have been made on the antiviral activity of nucleosides of synthesis incorporating in their molecule, a uracil halogeneated in the 5 position, in particular, 2'-desoxy-5-fluorouridine (II), 5-bromo-2'desoxyuridine (III), 2-desoxy-5-iodouridine (IV). These desoxyribosides are the analogues of thymidine (I) in which the methyl group of the thymine is replaced by a halogen;

(I) -R=-Ch_3(Thymidine)
(II) -R=-F (FDUR)
(III)-R=-Br (BDUR)
(IV)-R=-I (IDUR)



The study of the action of these ribosides on the bacterial metabolism has established that (II) is an inhibitor of synthesized thymidilate which catalyzed the biosynthesis of thymilic acid (108,109) while (III) and (IV) are incorporated in A.D.N. instead of thymidine (110).

Fluoro-5 desoxy-2' uridine (FDUR) inhibits the formation of vaccine virus in cell cultures, this inhibition is reversible by thymidine (111).

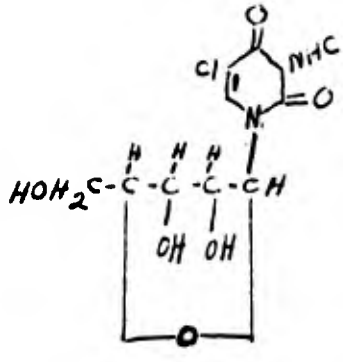
The same analogue suppresses the synthesis of the A.D.N. of adenovirus in cultures of HeLa cells and, consequently, the formation of infectious virus, likewise when it is added to cultures 10 hours after inoculation of the virus; this inhibition is reversible by thymidine (112). Kjellen (113) showed that when added to the cultures of cells inoculated with adenovirus type 5, the B.D. U.R., inhibits the formation of infectious virus, probably by being incorporated in the viral A.D.N., but only when this analogue is present at a concentration equal to that, also inhibits the cell proliferation, on the other hand, the cytopathogenic effect of the virus and the formation of its soluble antigen are not

suppressed by the presence of this analogue. On the other hand, the FDUR, at a concentration 100 times weaker than that necessary to hinder the cell proliferation suppressed the synthesis of viral A.D.N. and consequently the formation of the soluble antigen and the cytopathogenic effect. The FDUR does not have on the other hand, any effect on the multiplication of a virus with A.R.N., the virus of Rous' sarcomo, in cell cultures, when it is present at a concentration inhibiting the cell proliferation (114). The synthesis of viral A.R.N. is therefore independent of the synthesis of cellular A.D.N.

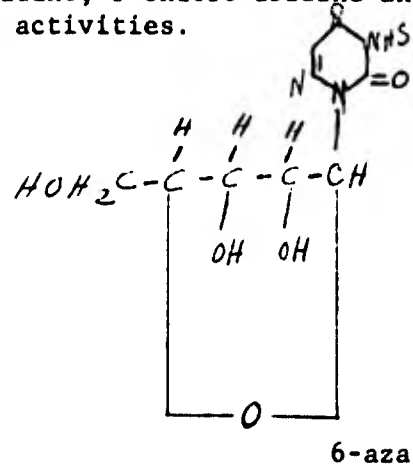
On the practical side, very interesting results have been obtained by Kaufman and his collaborators (115,116,117,118) who following Herman (114 bis), showed the inhibiting action of IDUR and BDUR on the multiplication of the vaccine virus and of herpes (virus with A.D.N.). The local administration of a collyrium with 0.1% BDUR hinders the multiplication of a herpetic virus in the cornea of a rabbit and the appearance of keratosis; the same when the local treatment is instituted during the 48 hours following the experimental infection of the cornea of the rabbit scarified with the herpes virus, it decreases very clearly the frequence and the severity of keratosis. Providing the collyrium is instituted every 2 hours, day and night, IDUR has been able to modify favorably the evolution of the herpetic keratosis of the rabbit for whom the treatment had started 5 hours after the infection. What is more, the clinical tests, conducted by Kaufman and his collaborators on a total of 155 patients infected with corneal herpes, showed that the collyrium with 0.1% IDUR administered repeatedly at close intervals effected the rapid cure (in 2 to 6 days) in the case of superficial dendrite lesions without reaching the corneal stroma. In patients where the corneal stroma is attacked, the cure is sometimes very slow and often less than complete. Relapses have been observed occasionally after the cessation of treatment, but often have been controlled by a repetition of the local administration of IDUR. On the other hand, the treatment with IDUR permits the local use of corticosteroids in the case of disciform keratosis or of herepetic irititis, in protecting the advantage of the anti-inflammatory action of the corticosteroids while preventing the well known aggravating effect which it exercises on the herpetic infection. IDUR is shown likewise effective on the treatment of vaccine keratosis symptoms which are sometimes produced following anti-smallpox vaccination. The works of Kaufman represent the first clinical application indubitably effective of a specific antiviral chemotherapy. It is clear that the local characteristic of the infection in corneal herpes is the main reason for Kaufman's success. In the case of a viral infection, necessitating the internal administration of the antimetabolite, the inhibition of the multiplication of the virus could not be obtained without the concomitant interference with the normal cell metabolism (in particular, the hemopoientinic system). The toxic phenomena of this product are analogous to those of the products used in anti-cancer chemotherapy.

The highly significant activity of IDUR on the experimental herpetic keratosis of the rabbit on the superficial forms of corneal herpes in man have already received many confirmations (119,120) and the encouraging results in the treatment of cutaneous herpes in man will be published, the resolution of the eruption were obtained 24 to 72 hours after the start of the local application (all times) of a solution of 0.1% IDUR (121). On the other hand, 5-iodo-2'-desoxycytidine, 5-iodo-2'-desoxy-3',5'-diacetyluridine and 5-iodo-2'-desoxy-diacetyl-3'-cytidine have proved an activity comparable to that of IDUR on experimental herpetic keratosis of the rabbit (122). The diacetyl derivatives, which are transformed to active metabolites on the surface of corneal cells by desacetylation, and by reason of their great solubility in lipids, would be of oral administration (in the case of a less localized infection such as cutaneous herpes). 5-fluoro-2'-desoxyuridine (inhibitor of synthesized thymidilate) is a very active inhibitor of the multiplication of the herpes virus in the chorioallantoic membrane of the chicken embryo - the same as in experimental herpetic keratosis of the rabbit (123) when it is added 24 hours after the inoculation of virus proves the therapeutic activity.

Among the analogues of uridine, 5-chloro uridine and 6-aza uridine manifested interesting antiviral activities.



5-chloro uridine



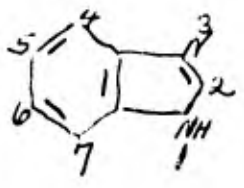
6-aza uridine

The compound, 5-chloro uridine, inhibits the multiplication of polio virus muris (Theiler disease, G. VII stain) in tissue cultures

in the presence of this analogue, the increase of cytoplasmic A.R.N., which is produced during the infection by the virus, is suppressed; uridine is antagonistic to this activity, it is probable that the analogue works in inhibiting the synthesis of viral A.R.N. (124).

The compound, 6-aza uridine (AzUr) is an inhibitor of the biosynthesis of cellular A.R.N.: converted metabolically in AzUr-5'-monophosphate, it blocks the activity of the decarboxylase of orotidylic acid, an enzyme which catalyzes the transformation of orotidylic acid into uridylic acid (125). This antimetabolite has been given clinical tests in anticancerous chemotherapy, where results are encouraging (126). In cell cultures, AzUr exercises no inhibiting activity on the multiplication of virus with A.R.N. but it inhibits significantly the multiplication of vaccine-virus (127) and of herpes virus (128) which are both A.D.N. viruses. This product has a certain chemoprophylactic activity on the intranasal infection of the mouse by the vaccine virus (129) and on the intradermic infection of the rabbit by the same virus (130). The embryonic cells of mice cultivated in vitro in the presence of non-cytotoxic concentrations of AzUr are resistant to the infection by the vaccine virus and of herpes, the same when the culture medium is replaced before the inoculation by a medium no longer containing antimetabolite (128). In all stages, the inhibiting activity of 6-aza uridine on the multiplication of the vaccine virus is suppressed by the addition of uridine, but not by that of 2'-desoxy uridine; uridine only partially inhibits the activity of AzUr on the multiplication of the herpes virus (128). The fact that AzUr is an inhibitor of the biosynthesis of A.R.N. and of its activity on the herpes virus appears in embryonic cell cultures of mice but not in embryonic cell cultures of chickens, nor in monkey kidney cell cultures (128), suggesting that its inhibiting activity is concerned indirectly (and only for certain types of cells) with the synthesis of cellular A.R.N. in competition with the synthesis of viral A.D.N. It is interesting to verify that such an inhibitor of the synthesis of A.R.N. does not interfere with the multiplication of virus with A.R.N. but on the contrary, with that of virus with A.D.N.

IV. DERIVATIVES OF BENZIMIDAZOLE



The inhibiting activity of benzimidazole on the multiplication of vaccine virus (131) and poliovirus (132) in cell cultures have been observed for more than ten years. The same product caused a slight increase of the survival of mice infected with poliovirus type 2, Lansing

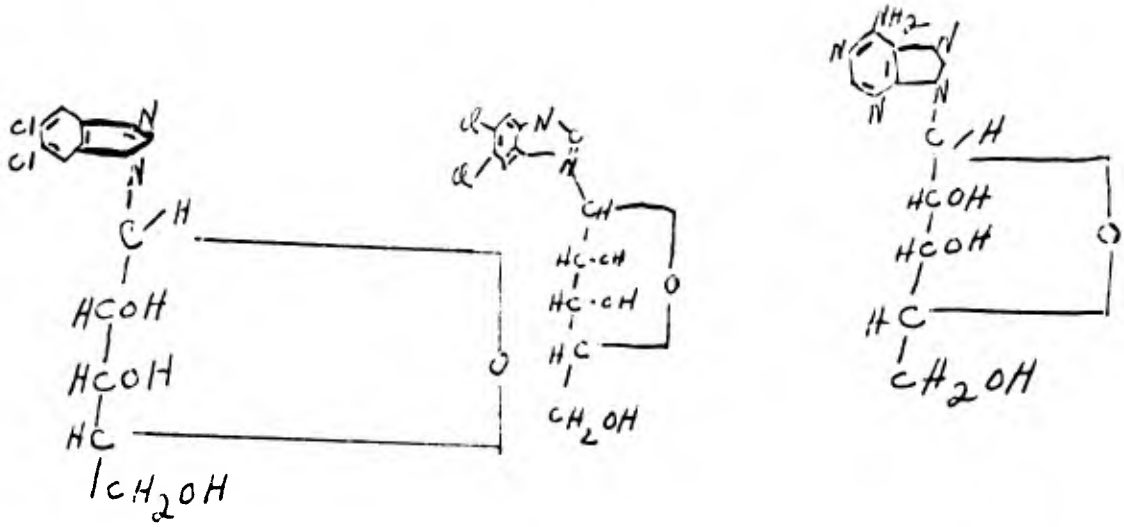
strain (133). But it is to Tamm, Horsfall, Folkers and their collaborators, who received the credit for having shown the interest in and the diversity of specific antiviral activities of benzimidazole derivatives in a series of articles ranging from 1952 to the present and of which we can only summarize the most important points. The compound 2,5-dimethyl-benzimidazole, added to the cultures in vitro of chorioallantoic membranes of chicken embryos during the phase of eclipse of the influenza virus, inhibits the multiplication of this virus by diminishing the production of hemagglutinin and that of antigen S (134,135). The product does not inactivate the virus in vitro and, at effective antiviral concentrations, does not affect the cell respiration.

The compound 5,6-dimethyl benzimidazole exercises an inhibiting activity on the multiplication of influenza virus (136). One would note that this configuration exists in the molecule of vitamin B_{12} (cyanocobalamin) in the form of halfi-(α-D-ribofuranosyl)-5,6 dimethyl benzimidazole (which we abbreviate as RDB) which exercises a highly significant inhibiting activity in the multiplication of influenza virus (136). RBD at the concentration of 4×10^{-5}M inhibits the multiplication of influenza viruses types A and B and of mumps virus without having discernable toxic effect on the cell metabolism (no alteration of the morphologic aspect, no effect on the incorporation of L-alanine in the proteins); a toxic effect is observed only in the presence of a concentration of RBD 5 times higher than that inhibiting 75% of the production of virus. In this respect, RDB is not only more active than 5,6 dimethyl benzimidazole but considerably more selective (137).

Other N-glucosides of 5,6 dichloro benzimidazole have been synthesized by Tamm and his collaborators but they appeared less active and less selective than RDB (136). This last product, administered to the mouse 2 hours after intranasal infection by the grippe virus, exercises an inhibiting action on the multiplication of virus in the lungs of the animal, and slightly prolongs the survival of the mice. It inhibits the multiplication of poliovirus type 2 in cultures of monkey kidney cells. The relationship, already observed in the case of influenza virus, between structure and activity in a virus of N-glucosides of halogen derivatives of benzimidazole, are likewise valid for poliovirus; however the action of RDB on the multiplication of poliovirus is less selective with regard to the influenza virus, the concentration of RDB necessary to inhibit poliovirus is close to the cytotoxic concentration (138).

The fact that RDB is active on the multiplication of influenza virus only when it is present during the first half of the phase of eclipse (3 hours after the infection) and it does not hinder the adsorption nor the penetration of the virus on the cells, suggests that this product works on the level of the synthesis of viral ARN. On the other hand, the chemical structure presents an analogy to that of adenosine,

riboside of the adenine [β-D-ribifuranosyl-9-adenine]: it is therefore interesting to note that R.D.B. decreases the incorporation of adenosine, marked in 8 by C^{14} in cell A.R.N. (139) and that of orotic acid, marked in 6 by C^{14} in A.R.N. of nuclei isolated from calf thymus (140).



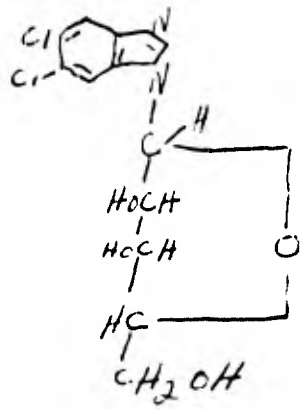
RDB Adenosine

Adenosine exercises, besides, a certain antagonistic activity vis-a-vis of the inhibition of the viral multiplication of R.D.B.: 400 μM of adenosine suppress the inhibition of the multiplication of influenza virus in cultures of chorioallantoic membranes caused by 40 μM of R.D.B.; the guanosine is devoid of antagonistic effect (141). Tamm and Overman (142) likewise studied the activity of a series of benzimidazole derivatives on the multiplication of vaccinia virus in cultures in vitro of chorioallantoic membranes of chicken embryos. The antiviral activity of benzimidazole already observed by Thompson (131) is considerably increased by the presence in the molecule, in position 2, 5 and 6, of methyl substituents- or Cl: the most active product is 5,6-dichlorobenzimidazole itself. But unlike that which happens with infleunza virus, the N-glucosides of this derivative, in particular R.D.B. and no more active on the multiplication of vaccinia virus than 5,6-dichlorobenzimidazole itself. The conversion to riboside, which increases the activity and the selectivity of benzimidazole disubstituted vis-a-vis of influenza virus (virus with A.R.N.) and without effect with respect to the vaccinia virus (virus with A.D.N.).

On the other hand R.D.B., on the multiplication of adenovirus on monkey kidney cell cultures, has an inhibiting effect comparable to that which it exercises on the multiplication of influenza virus in the

same type of cultures (141). Given that R.D.B. is certainly not an inhibitor of the biosynthesis of A.D.N. - in particular, it is without effect on the incorporation of thymidine in the A.D.N. of nuclei isolated from calf-thymus leads one to conclude that this product works indirectly at the level of cell A.R.N., on the multiplication of a virus with A.D.N. In the case of the inhibiting effect of R.D.B. on the multiplication of a virus with A.R.N., such as the influenza virus, it is probable that the very great selectivity of the product, by comparison to that of the derivatives of benzimidazole without a glucoside bond, is due to the quantitative differences between the biosynthesis of cell A.R.N. and that of viral A.R.N.

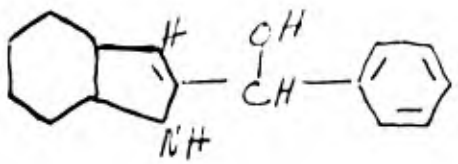
The study of the inactivity of derivatives of benzimidazole on the multiplication of poliovirus in cell cultures has led to observations of very great interest. Tamm and Menes (138) ascertained that the 1-(α-D-arabinopyranosil)-5,6-dichlorobenzimidazole (which we abbreviate as A.D.B.) exercises a clearly more selective activity than that of R.D.B. on the multiplication of poliovirus type 2 in monkey kidney cell cultures.



1-(α-D-arabimopuranosil)-5,6-benzimidazole

R.D.B. is active only on the multiplication of poliovirus at a concentration slightly toxic for the cells and only when it is present at the start of the phase of eclipse of the virus, it has no effect on the synthesis of cell proteins. On the other hand, A.D.B. inhibits the multiplication of poliovirus at a concentration clearly less than the cytotoxic concentration, it is active during all phases of eclipse of the virus and later stages and it inhibits protein synthesis (143). On the other hand, it is no more active than the nonsubstituted benzimidazole on the multiplication of the influenza virus (138). Since this virus and the poliovirus are both viruses with A.R.N. underline the differences existing between their mechanisms of multiplication and show well the selectivity of the action of these inhibitors.

There is nevertheless anot er derivative of benzimidazole 2-(α-hydroxybenzyl)-benzimidazole (which we abbreviate as H.B.B.) which has manifested vis-a-vis of poliovirus a most interesting activity.



2-(α-hydroxybenzyl)-benzimidazole

Tamm and his collaborators (144) showed that this product and its 5-chloro-derivative inhibit the multiplication and cytopathogenic effect of poliovirus type 2 in monkey kidney cell cultures when added to the cultures at a concentration less than the cytotoxic effect, and that this inhibition had acted the same when the products were added during the phase of exponential multiplication of the virus. These products do not inactivate the poliovirus in vitro and in cell cultures, they do inhibit the multiplication of influenza virus. In later works, Eggers and Tamm (145) showed that H.B.B. has, at a non-cytotoxic concentration of 200 to 500 M and inhibiting activity on the multiplication and the cytopathogenic effect of the virus following: poliovirus types 1, 2 and 3; Coxsackie virus type A9 and B1 and B6: ECHO virus types 1 to 9, 11 to 21, 24 to 27 while it is inactive vis-a-vis of the following viruses:Arbovirus B and C; reovirus type 1 to 3, mumps, parainfluenza types 2 and 3, influenza type B, adenovirus types 2, to 4 herpes and vaccinia. The product herefore has a highly selective activity since it only inhibits the viruses appertaining to the group of enterovirus, and which even in the midst of the group, it does not inhibit the multiplication of Coxsackie virus types A7, A11, A13, A16, A18, and of certain ECHO viruses (types 22, 23, 28). Among the viruses sensitive to inhibition by H.B.B. one observes the difference of degree of sensitivity, certain viruses or even certain strains of the same virus are inhibited by much weaker concentrations of H.B.B. than others. On the other hand, following the passage of strains of enter virus s nsitive in cell cultures, to subinhibiting concentrations of H.B.B., variants more resistant than the original strain have been

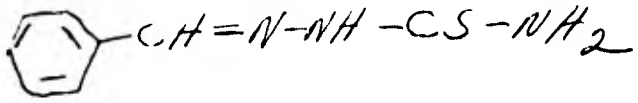
obtained. At concentrations active on the multiplication of enterovirus. H.B.B. has no effect on the cell morphology, on the cell respiration, on their utilization of glucose, their production of lactic acid, or on the incorporation of alanine in the cell proteins. In summary, this product seems to work in an absolute selective manner on the multiplication of certain enteroviruses, and this not only in the course of the phase of eclipse but also in an advanced stage of the reproduction cycle of this virus. In effect, when added to the infected culture with ECHO virus type 12 during the phase of exponential multiplication of this virus, the product has entirely stopped the production of new infectious particles in the space of 45 minutes after its addition (146); the duration of the process of viral multiplication sensitive to inhibition by H.B.B. is about 4 hours, the process begins 2 hours after inoculation and continues beyond the first half of the phase of exponential multiplication. On the other hand, the experiments of Eggers and Tamm (146) indicate that the action of H.B.B. is located essentially at the level of the biosynthesis of viral A.R.N., either that the inhibitor combines specifically with A.R.N. in rendering the virus incapable of using the A.R.N. following its synthesis, or that it interferes with one or more specific enzymatic reactions necessary for the synthesis of viral A.R.N. This interesting inhibitor has therefore revealed not only the quantitative but qualitative differences between the biosynthesis of A.R.N. of certain enteroviruses and that of cellular A.R.N. It has, on the other hand, revealed differences of the same order between the synthesis of A.R.N. of enteroviruses sensitive to its action and that of A.R.N. of enterovirus which are resistant to it. Obviously, the reactions which are specifically inhibited by H.B.B. can be substituted other biosynthetics with which H.B.B. does not interfere, since it shows the emergence of variants resistant to this inhibitor. For the first time, a certain analogy appears between antiviral chemotherapy and antibacterial chemotherapy. The appearance, in cell cultures, of variants resistant to the inhibition by H.B.B., beginning with viruses originally sensitive to that inhibition, furnish the most plausible explanation for the lack of activity of H.B.B. in the animal, particularly on mice infected with poliovirus type 2 (147). On the other hand, Eggers and Tamm (148) isolated a variant of Coxsackie virus type A9, which only multiplies in optimal fashion in monkey kidney cell cultures, which in the presence of 100 µM of H.B.B., infectious A.R.N. extract of this virus requires the presence of H.B.B. for its optimal reproduction. The nonsubstituted benzimidazole can replace H.B.B. in allowing the multiplication of this dependent variant. This observation likewise suggests an analogy to antibacterial chemotherapy and shows well that H.B.B. works directly on the level of the multiplication of the virus and not by an intermediary of a cell reaction. One could observe that Tamm (149) previously observed that 2-(β-D-ribofuranosyl)-5-methyl benzimidazole exercises a stimulating activity on the multiplication of influenza virus in cultures of chorioallantoic membranes of

chicken embryos, but, in this case, it is not a question of a selective activity on the reproduction of viral A.R.N.

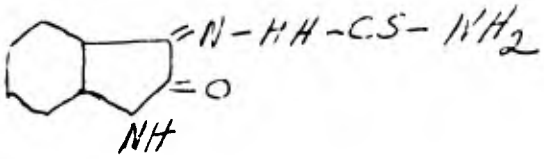
In summary, it is striking to see that, up to the present, the only example of rigorously selective inhibition of the biosynthesis of viral A.R.N., example furnished by 2-(α-hydroxybenzylbenzimidazole (H.B.B.), has been observed with certain enteroviruses which by reason of the similarity of its chemical structure and of the smallness of its particles, are theoretically those for which one has little reason to expect a possible analogy with the mechanisms of antibacterial chemotherapy.

V. THIOSEMICARBAZONES

An inhibiting activity of the p-aminobenzaldehyde-thiosemicarbazone on the multiplication of vaccinia virus in the vitellin sac of the embryonic chicken egg was observed in 1950 by Horme and his collaborators (149). These same authors ascertain that this product administered per os or intranasally by the vaccinia virus, significantly increases the survival of the animal (150,151). Thompson and his collaborators observed later than the per os administration to the mouse of benzaldehyde thiosemicarbazone increases the survival of the animal to the intracerebral infection by a neurotrope variant of the vaccinia virus (152), on the other hand, among a whole series of thiosemicarbazones studied in this respect by these authors, the derivative most active on the intracerebral infection of the mouse by the vaccinia virus is reported to be isatin thiosemicarbazone (153) (which we abbreviate as ThIs). Bauer (154) confirms these results and shows that the subcutaneous administration of a single dose (12.5 mg/kg) of ThIs 18 hours after the intracerebral infection, suffices to decrease significantly the mortality of mice.



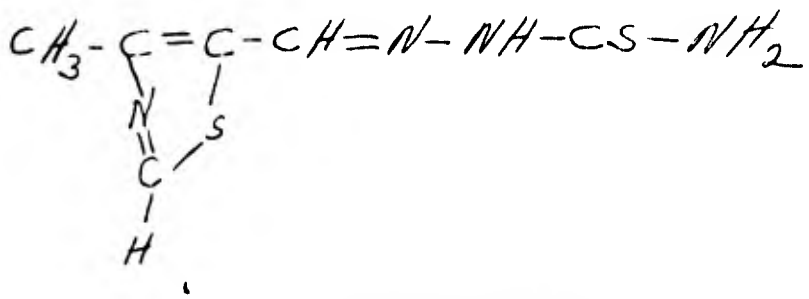
Thiosemicarbazone of benazldehyde



Thiosemicarbazone of isatin

Bauer shows that in the mice treated with ThIs the multiplication of vaccinal virus is not totally suppressed but that it reaches a plateau located at a value of 10 to 100 times less than that observed in test animals, which is enough to block the death of the animal. The animals who survive because of this treatment, are immune vis-a-vis of a re-infection by the homologous virus; precisely by reason of the fact that the multiplication of the virus in the tissues has been completely inhibited. The compound, cortisone, which by an indirect mechanism, accelerates the multiplication of vaccinia virus in the brain of the mouse, exercises an antagonistic effect vis-a-vis of the therapeutic action of ThIs (155). In tests on mice infected intracerebrally with the virus vaccine and given one dose subcutaneously, it has been verified that a prophylactic effect could be obtained if the treatment with ThIs had taken place 4 hours or more before the infection, and that a curative activity is observed if the compound had been administered no more than 3 days after infection. (280).

Correlating structure of diverse thiosemicarbazones and their therapeutic activity in the mouse infected intracerebrally by the vaccinia virus, one can express the following conclusions: a) the presence of a radical $=N-NH-CS-NH_2$ and of cyclic nucleus is essential to antiviral activity (in particular the plain oxygen semicarbazones are inactive (153); b) although isatin nucleus confers a particularly high activity it can be replaced by the phenyl, thioenyl, pyridinyl and quinolinyl nucleic) the substitution of one or both of the H's in the terminal amino group of the radical $=N-NH-CS-NH_2$ makes the activity disappear; in the case of ThIs a substitution on the N in position 1 of the indole nucleus by an ethyl group- or methyl- increases the activity, on the other hand, all substitution in position 5 of the indole nucleus (by CH_3,-OCH_3,-Cl,-NO_2,-$NaSO_3$) makes the activity disappear (155,157). Recently, Campaigne and his collaborators (156) described the activity of thiosemicarbazone of 5-methyl-4-thiazole-carboxaldehyde, administered <u>per os</u>, in the mouse infected intranasally with the vaccinia virus.



Thiosemicarbazone of
4-methyl-5-thiazole-carboxaldehyde

These authors concluded that the antiviral activity is particularly marked when the radical $=N-NH-CS-NH_2$ is separated by 2 atoms of C or of S,

the last are found on a heterocycle. This is the case of the thiosemicarbazone of isatin. Bauer and Sadler (157) studied a series of products with a structure clearly related to ThIs and have verified that the thiosemicarbazones of 1-ethylisatin have, on the infection of the mouse by a neurotrope strain of the vaccinal virus, an activity clearly superior to that of ThIs. The products were administered subcutaneously.

This discovery of significant prophylactic and therapeutic activity of certain thiosemicarbazones, in particular of ThIs, on the infection of the mouse by the vaccinia virus should logically lead to the study of the activity of these products on the infections caused by other viruses of the pox group and most particularly on smallpox. For several reasons a certain skepticism was held on clinical utility of this product to smallpox in man or in certain animal infections by the virus of the pox group (for example the contagious ecthyma of sheep): ThIs is shown incapable of modifying the dermic infection of the rabbit by the vaccinal virus (158); it does not shorten the infection of the mouse by ectromelia, a virus antigenetically related vaccinia (155,159). It is inactive on the infection of the rabbit by the virus of myxomatosis, which likewise is part of the pox group. Therefore, it has been thought for many years that the activity of ThIs is limited to vaccinia infection in the mouse; also, this product does not have the required characteristics of an effective chemotherapeutic agent, since its activity is limited to retarding the process of multiplication of the virus. The discovery in 1959 of the activity of ThIs on the intracerebral infection of the mouse by rabbit pox (160), after that of the inhibiting activity of ThIs on the multiplication of the vaccinia virus and of rabbit pox in cell cultures coming from diverse animal species (162) have renewed interest in ThIs. In 1960 Bauer and Sadler showed that the thisemicarbazone of 1-ethylisatin, administered subcutaneously at a dose of 5 mg/kg, protected the mice, aged 6 days, against the manifestations of encephalitis and consecutive death from intracerebral infection with the alastrium virus minor variola. In 1964 Bauer and his collaborators (164) showed that the thiosemicarbazone of 1-methylsatin administered subcutaneously at a dose of 10 mg/kg protected the mouse against intracerebral infection with the smallpox virus (variola major). ThIs is likewise shown active but at a higher dosage. With active dosages, both products have manifested no discernable toxicity, in spite of the fact that the experiments were on very young animals, the mice, older than 6 days, are not sensitive to intracerebral infection by the alastrium and variola viruses. ThIs, although inactive on the dermic infection of the doe rabbit by the vaccinia virus (the product does not hinder the appearance of pustules), does protect does against intravenous infection with vaccinia. Does infected

massive dose (intravenous (i.v.)) of vaccinia virus die in 5-6 days, those treated per os with 200 mg/kg of ThIs daily survive but not without manifesting marked symptoms 5 to 6 days after the infection (155).

The localized epidemics of smallpox which occurred in Great Britain in 1962 and the compaign of anti-smallpox vaccination which started in that country where vaccination is not obligatory, have furnished the opportunity for clinical tests of the activity of the thiosemicarbazone of 1-methylisatin on smallpox and on occasional generalized cowpox and vaccinia eczema. Given the small number of cases treated, the security of those which have been made the object of test treatment, which have sometimes been instituted at an already advanced stage of the disease; the uncertainty where the clinicians find themselves and which concerns the posology to be adopted, one can not again consider as conclusive the test treatment of smallpox by this product (165,166). On the other hand, many publications speaking of a significant therapeutic activity of the thiosemicarbazone of 1-methylisatin in the case of generalized cowpox of gangrenous vaccine and of vaccinal eczema have complicated the anti-smallpox vaccination (167,168,169), particularly in subjects attacked by acute or chronic leukemia (170,171). The product has been administered per os with daily doses varying for an adult between 1 and 5 g.; the opinions differ as to what the toxic effects are of this product in man (172,173).

Some recent workers have tried to elucidate the mechanisms of antiviral activity of the thiosemicarbazone of isatine and of its derivatives. The product does not inactivate in vitro the virus of the pox group, whose multiplication it inhibits; in cell cultures, it suppresses the cytopathogenic effect of the vaccinia virus (155,162), but at a concentration little less than that which is toxic for the cells (about 100 μM). At a concentration 10 times less, ThIs inhibits the production of infectious particles, whatever be the moment of the cycle of viral development in the course of which it is added to the cultures, but without hindering the formation by infected cells of certain specific antigens of virus nor the cytopathogenic effect (174). ThIs does not exercise an inhibiting effect on the adsorption of the virus on the cells, nor on the phase of eclipse, nor on the accumulation of thymidine (marked isotopically with tritium) in the cytoplasm of infected cells (174,175). In cultures of embryonic cells of mice, ThIs exercises a much more marked inhibiting effect on the production of infectious virus than on that of the hemagglutinin, which is distinct from the particle of vaccinia virus (83). In rabbit kidney cell cultures, the product inhibits the production of infectious particles by the rabbit pox virus, but it is without effect on that of diverse soluble antigens and on the manifestations of cytotoxicity associated with their production (176). In other words, ThIs causes, in the virus which are sensitive to its inhibiting action, the appearance of an incomplete cycle of multiplication, characterized by the production of certain constituents of the virus and by the absence of production of complete infectious virus. Given

that the interruption of the production of infectious particles can be caused by ThIs, no matter which moment of the phase of exponential multiplication of the virus to which this product is added to the cultures and that this addition affects neither the formation of the principle specific antigens nor that of viral A.D.N., it is logical to suppose that the product intervenes at the moment of assemblage of diverse constituents of the virus in a particle endowed with infectiousness. Electron microscope examination by Easterbrook (174) of KB cells infected with vaccinia virus and treated with ThIs, showed, that in the presence of this product, the cells contained almost exclusively the incomplete particles, possessing a limiting membrane, but endowed with dense central masses which characterize the normal infectious vaccinia virus. These incomplete particles are of a mean diameter (300 mμ) which is greater than the normal particle (250 mμ) and reminiscent by their aspect of incomplete non-infectious particles of influenza viruses which are observed under certain experimental conditions (28). It, therefore, semms clear that ThIs is neither an inhibitor of the synthesis of viral proteins nor an inhibitor of that of viral A.D.N., but that this product intervenes at the level of the reaction by which the constituent and other possibilities (polysaccharides, lipides, coenzyme, biotine, Cu^{++}) are integrated for a complete and infectious particle. We recall on this subject that it is not possible to demonstrate that the A.D.N. of the virus of the pox group is only endowed with infectiousness. Whereas, in cell cultures an adequate concentration of ThIs can completely stop the production of infectious particles, in the mouse, infected intracerebrally with vaccinia virus and treated per os or subcutaneously with the production of infectious viruses in the brain virus growth is simply retarded and diminished. It is possible in this case also, predominance of incomplete cycles of multiplication, at least in the majority of the cells infected, and the survival of the animal is assured by the beginning of the immunological mechanisms as well as non-specifics (for example production of interferon) which do not intervene when the cycle of multiplication is normal. The antagonistic action of cortisone vis-a-vis of the therapeutic activity of ThIs lends support of the latter hypothesis, for antagonism is not observed in cell cultures (155). Successive passage of vaccinia virus in the brain of mice, treated with the dose of ThIs insufficient to protect completely has not made it possible to obtain a variant which would be resistant to the action of ThIs (280).

The relations existing between the chemical structure of thiosemicarbazones with antiviral activity and their property to interfere with the process of maturation of particles of vaccinia virus are not known. The capacity of these particles to form the chelates with metals (in particular Cu^{++}, which enters in the composition of the virus) deserves to be considered, but it does not furnish sufficient explanation, for the numerous types of molecules endowed with that property are devoid of antiviral activity. There is no relation between the activity

of certain thiosemicarbazones on mycobacterium tuberculosis and their antiviral activity(153). The disappearance of the antiviral activity of the thiosemicarbazone of isatin, when the indole ring contains a substitutant in position 5 is a fact which deserves an explanation. It is likewise of the highest interest to ascertain that a 2-alkyl substitution on the amino terminal group of the thiosemicarbazone chain makes the activity of ThIs on the vaccine virus disappear, but confers on the molecule a significant activity on the infection of the mouse by ectromelia virus (177). On the other hand, the 4,4-dibutylthiosemicarbazone of 1-methylisatin has an inhibiting effect on the multiplication of cell cultures of poliovirus type 2, but not on that of polio virus type 1 (178).

VI. UREA AND GUANIDINE

Many interesting observations have been made on the subject of the antiviral activity of urea and of guanidine and of their respective derivatives.

Weinstein and his collaborators (179) noted the activity of many urea derivatives (alloxan, alloxantine, 1-benzoyl-2-thiohydantoin, hydantoin) on the multiplication in ovo of influenza virus. In these tests, the products are mixed with the virus diluted before inoculation in the allantoic cavity; it is therefore impossible to eliminate a direct inactivating effect of these products on infectious particles. The same authors have verified a protective activity of the chlorohydrate of carbamilide on the infection of the mouse by the influenza virus. Likewise, certain derivatives of thiourea, administered subcutaneously to the mouse, increase the survival of the animal after intranasal infection by influenza virus, according to Buu-Hoi and his collaborators (180); the most active product is 4-chloro-4-fluorothiocarbanilide.

Particularly interesting are the observations of A. and M. Lwoff (181) who dealt with the action of urea on the multiplication of poliovirus. At a concentration of 0.1 to 0.3M (much less than the cytotoxic concentration) urea diminished the yield in infectious particles of poliovirus by the KB cells. This inhibiting effect is all the more marked when the temperature at which the cultures maintained is very high, for a like concentration of urea and the same temperature the inhibiting effect is more marked on a strain of poliovirus r^t equal to 38^o3 than on a strain of polio virus at r^t equal to 40^o8 (the notation r^t designates the supraoptimal temperature to which the yield in infectious particles is diminished by 90%). On the other hand, the delaying effect of heavy water on the primary phase of viral development, an effect previously observed by the same authors (182), is suppressed by the presence of urea in the medium, while the inhibiting effect of urea on the multiplication at 36^o of the strain of poliovirus

where r^t is equal at $38^{\circ}3$ is abolished when the medium contains 50% D_2O.

According to A. and M. Lwoff the development of poliovirus in cell cultures is limited to certain states of a viral macromolecule. Only one of these states is compatible with the development of the primary phase, the other with that of the secondary phase. The supra or infra-optimal temperatures displaces the equilibrium of a reaction affecting the structure of that macromolecule; heavy water which reinforces the effects of an infra-optimal temperature and diminishes a supra-optimal temperature, modifies the temperature of transition. It is, therefore, probable that these hydrogen bonds play an essential role in the change of structure of the macromolecule. The chemical agents, such as urea, which weaken the stability of hydrogen bonds, worked in the same sense as the supra-optimal temperature, but their effect is counterbalanced by the presence of D_2O; the deuterium bonds are more stable than the hydrogen bonds. The urea therefore works like a thermomimetic substance on the development of poliovirus, thus is explained the fact that it inhibits more the multiplication of a strain at a very high r^t and an antagonism exists between the action of the urea and that of heavy water.

Urethane, administered subcutaneously to the mice, infected intranasally with the influenza virus, diminishes the mortality of the animals. The total mortality of the animals, treated with the product, and of their mean survival, is as if the dose of the virus inoculated were effectively less than that used as there exists a linear relation between the dose of urethane and the apparent reduction of inoculated virus (183,184). There is reason to wonder if urethane, by its hypnotic properties, does not simply diminish the inhalation of infectious particles at the time of the intranasal inoculation of the virus.

The inhibiting activity of guanidine on the multiplication of poliovirus in cell cultures has been observed independently by Melnick, et al, (186) and Laddo, et al, (187). In the presence of 60 μg/ml of guanidine chlorhydrate, the production of poliovirus in cell cultures (H.Ep.-2 monkey kidney cells) is from 10^4 to 10^6 times weaker than that observed in the absence of the product; the cytopathogenic effect is considerably inhibited. The product only has a discernable cytotoxic effect at a concentration of 200 μg/ml. It likewise inhibits the multiplication of other viruses appertaining to the group of enterovirus (Coxsackie A and B, ECHO), but it is without effect on that of the myxovirus, of pox group viruses, of reovirus, of arboviruses. A single passage of poliovirus type 1 (Mahoney strain) in the presence of a sub-inhibiting concentration of guanidine, leads to the production of viral particles 1000 times more resistant to the inhibiting effect of the product than the original viral population: this resistance seems to

maintain itself in the course of some additional passages in the absence of guanidine. On the other hand, it is interesting to verify (188) that the weakened strain of (LSc) of poliovirus type 1 is more sensitive to the inhibiting effect of guanidine than the virulent strain of the same type (Mahoney): it is the same for the strains of type 2; yet the attenuated strain of type 3 (leon) is no more sensitive to the inhibiting effect of the product than the virulent strain, or one can say that the strain is probably less stabilized in its attenuation of virulence than the others, since after propagation in the digestive tract of man, it acquires a certain degree of neurovirulence for the monkey (189). The sensitivity vis-a-vis of guanidine would represent for the virus a "markes" of virulence.

Guanidine does not exercise an inactivating effect on the enteroviruses which are sensitive to its inhibiting action; it does not hinder the adsorption of the virus on the cells, but following its addition to the infected cells, at any moment of the cycle of viral development, it interrupts the production of new infectious particles (188). One can therefore suppose that it inhibits the process of maturity of enterovirus, by interfering either with a certain terminal reaction of the synthesis of viral A.R.N., or with the synthesis of certain proteins which precede the maturity of enterovirus. Diverse metabolic tests have not demonstrated the antagonistic effect of guanidine (187). All substitution products of guanidine tested lack antiviral activity (187,188). The macaques rhesus monkey, treated per os with guanidine in near lethal dose, have been partially protected against the intramuscular infection with poliovirus type 1; while 83% of untreated monkeys have presented at the autopsy specific histopathologic lesions of poliomelytic infection, only 50% of those treated with a daily dose of 60 mg/kg and 17% of those treated with a daily dose of 120 mg/kg (dose still close that of DL_{50}) have presented specific lesions similar to those of the controls (186). Poliovirus type 1 isolated from monkeys infected orally with this virus and treated the same way with guanidine was in cell cultures nearly 1000 times more resistant to the inhibiting effect of guanidine than the original virus (185). The rapid emergence of resistant viral particles, even in cell cultures, render general use of guanidine impossible in animal chemotherapy. Laddo and his collaborators (190) nevertheless showed that after many passages in cell cultures, in the absence of guanidine, of a variant of poliovirus 1, rendered relatively resistant to the inhibiting effect of guanidine, the characteristic of sensitivity to guanidine again becomes similar to that of the original virus. That can be explained in supposing that in the absence of guanidine, the resistant viral particles multiply less rapidly than the normal particles and end up by being eliminated following numerous passages. The same authors have moreover shown that it is possible to obtain the variants of poliovirus 1 which multiply much more rapidly in the presence of guanidine than in cultures devoid of this product.

Naturally it would be very interesting to understand the mechanism of the inhibiting action of guanidine on the development of enterovirus. Guanidine like urea, whose inhibiting activity on the development of poliovirus, described, has the property of breaking the hydrogen bond, but it is little possible that the activity explains its inhibiting effect on the multiplication of enterovirus, which is observed at a concentration 10^{-3} times weaker than that effective in the case of urea. Guanidine at a concentration 5×10^{-2}M increases the utilization of glucose and the production of lactic acid by the KB cells cultivated in vitro (191): therefore one can consider it as an inhibitor of cell respiration by disengagement of the reactions of phosphorylation oxydation. But that biochemical activity does not explain the selectivity of the inhibiting action of guanidine, which only works on the multiplication of enterovirus, when other agents capable of disengaging the reactions of oxidative phosphorylation (2,4-dinitrophenol, sodium azothydrate) have much less specific inhibiting activity on the multiplication of many types of viruses, activity only observed in doses not devoid of toxicity for the cells. On the other hand, some substituted guanidine have a more marked inhibiting effect, whereas only guanidine is capable of inhibiting the multiplication of enterovirus.

The antiviral activity of guanidine can be compared to that of 2-(2-hydroxybenzyl)-benzimidazole (H.B.B.) which we have described above. It is a question, in both cases, of products selectively inhibiting the multiplication of enterovirus and comparison of the kinetics of their inhibiting effects. Tamm and Eggers (192) showed not only that acquisition by the enterovirus of resistance to the effect of H.B.B. is not accompanied by a lessening of sensitivity to the inhibiting action of guanidine, the reciprocal is equally true. On the other hand, in cell cultures, guanidine and H.B.B. work synergistically. In the vast group of enteroviruses one can distinguish 3 sub-groups: a) those whose multiplication is inhibited by guanidine and H.B.B.; poliovirus of three types, Coxsackie virus B, most of the ECHO viruses; b) those whose multiplication is inhibited by guanidine but not by H.B.B.: Coxsackie virus A, and the strain of H.G.P. (Salisbury) of virus isolated from catarrah; c) those whose multiplication is not inhibited by guanidine nor H.B.B.: ECHO virus 22 and 23, and ECHO virus 28 (2060-JH). These viruses, particularly ECHO virus 28 (2060-JH), have been arranged arbitrarily among the enteroviruses without valid reason, which demonstrates the different behavior, against both inhibitors.

We think that the inhibiting effect of H.B.B. and guanidine on the multiplication of enterovirus have revealed differences of great interest between the reactions of cell synthesis of A.R.N. and likewise between the mechanisms of biosynthesis of diverse enteroviruses. The elucidation of the mode of action would certainly contribute to establishment of a rationale for antiviral chemotherapy.

VII. POLYSACCHARIDES

Horsfall and McCarty (193) showed in 1947 that the capsular polysaccharide of Klebsiella pneumoniae type B, inhibits the multiplication o the pneumonia virus of the mouse in the lungs. This product is active only when it is administered intranasally, which is the natural route of infection; the inhibiting effect of the polysaccharide on the multiplication of virus in lungs is still observed when it is administered 10 hours after the infection, that is to day, during the first part of the phase of eclipse of the virus in the pulmonary tissue. Nevertheless, when the product is administered after the end of the first part of the phase of eclipse, it can again interfere with the later cycles of multiplication of the virus and hinder the appearance of pulmonary lesions and the death of the animal. A one dose treatment 4 days after the infection, at a moment when the pulmonary lesions are already apparent, can again assure the survival of the animal (194). The same polysaccharide has no effect on the multiplication of influenza virus in the lungs of the mouse (195) but it inhibits the multiplication of another myxovirus, the virus of mumps, in the allantoic cavity of the embryonic chicken egg (195): in this case, also, the product seems to work during the phase of eclipse of the virus (196).

Certain other polysaccharides have an activity comparable to that of the capsular polysaccharide of Kl. pneumoniae type B on the multiplication of pneumonia in the lungs of the animal; the capsular polysaccharide of Kl. pneumoniae types A and C, of streptococcus MG, the specific polysaccharide of blood group A, dextran (197). The polysaccharide of Diplococcus pneumoniae type 2, close antigenically to that of Kl. pneumoniae type B, however is inactive (195).

It is interesting to verify that the oxidation of polysaccharides by periodic acid, which destroys the serologic specificity and diminishes its toxicity for the mouse (194) does not affect in any way its viral activity (193).

Meier, Kradolfer and their collaborators (198,199) showed that certain polysaccharides isolated from gram-negative bacteria can partially protect the mouse against the mortality due to the infection by the encephalomyicarditis murine virus (Columbia SK) with the condition that it be administered intravenously 24 hours before the inoculation of the virus. The mechanism of that activity is again not completely clear.

A polyanionic polysaccharide, made from Penicillium stoloniferum, and which received the name Statolon, exercises a chemoprophylactic activity, in the mouse infected by an arbovirus (Semlike Forest virus) (200), by an encephalomyocarditis virus of human origin (M.M.) (200) or by poliovirus type 2 (M.E.F. strain) (201). It likewise has a chemo-

prophylactic activity on the monkey infected with poliovirus (202) and in the chicken infected with the Rous Sarcoma virus (200). In cell cultures this polysaccharide inhibits the cytopathogenic effects of many viruses etiologically associated with infectious coryza: H.G.P. virus (Salisbury) (203), the Coe strain of the Coxsackie virus A 21 (204) and of JH-2060 virus (204). The inhibition of the cytopathogenic effects of the Coe virus for aminiotic human cells cultivated in vitro, for example, is observed when statolon is added to cultures later than 4 hours after inoculation with the virus. The polysaccharide, which does not inactivate the virus, seems to protect the cell against the cytopathogenic effect of the Coe virus rather than inhibit the multiplication. The chemical analysis of the polyanionic (205) polysaccharide revealed that it is composed of glacturonic acid, galactose, galactosamine, glucose, arabinose, xylose, and rhamnose. It is absolutely devoid of toxicity for the mouse, its antiviral activity is abolished by oxidation with periodic acid.

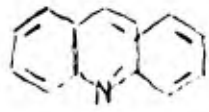
Takemoto and Liebhaber (206) recently described the interesting inhibiting activity of dextran sulfate on the cytopathogenicity plaque formation of certain enteroviruses (ECHO and Coxsackie A9), influenza virus type B and herpes virus, which were observed by adding this polysaccharide at a concentration of 100 mcg/ml with gelatin covering the cell cultures. On the other hand, dextran sulfate stimulates the plaque formation by the poliovirus type 1 (Mahoney strain) and accelerated the multiplication of this same virus in liquid media; a polycation, diethylaminoethyldextran inhibits the multiplication and the cytopathogenic effect of this virus. A weak strain of poliovirus type 1 (LSc) is considerably inhibited by dextran sulfate, but not the virulent Mahoney strain.

Mandel and Racker (207, 208) isolated from the intestinal tissue of the mouse a mucopolysaccharide which combines specifically with the particles of poliovirus muris (Theiler disease, GD VII strain) and neutralizes in this way the infectiousness for the mouse and their hemagglutinant power for the human hematites. This complex virus-polysaccharide is dissociable by simple diminution of the concentration of electrolytes of media.

A complex of the same nature is formed between the mucoproteins of the cell membranes or of certain biological liquids and of myxovirus, nevertheless it produces, in this case, no neutralization of the infectious virus, this last possess the neuraminidase enzyme which specifically decomposes the mucoproteins and disassociates the complex. But if these mucoproteins are previously treated with periodic acid they become resistant to the enzymatic action and by combining with the virus form a stable complex, in which the infectiousness of the virus is neutralized (209).

In summary, certain polysaccharides and mucopolysaccharides are proof of significant activity in diverse stages of viral infections. Certain of them work by combining specifically with viral particles, others by protecting the cells against the cytopathogenic effect of viral infection, finally, others by inhibiting certain processes of the multiplication of the virus. It would be of very great theoretical interest and would also be practical, to clarify the mechanism in particular by which protection of the cell against a cytopathogenic agent is produced.

VIII. DERIVATIVES OF ACRIDINE



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After many years, the antiviral activity of certain derivatives of acridine is understood, but it is only recently that the mechanisms of the inhibiting effects have begun to be specified.

Certain nitroacridines inhibit the multiplication of myxovirus in the chorioallantoic cavity of the embryonic chicken egg. The degree of inhibition varies according to the nature of the virus and according to the specific toxicity for the cells of the chorioallantoic membrane of the individual nitroacridine (210,211,212,213).

In quantitative research with cultures of embryonic chicken cells infected with the avian pest virus, Franklin (214) showed that the addition of proflavin, 2,8-diaminoacridine selectively inhibits the cytoplasmic production of viral hemagglutinin without affecting intranuclear A.R.N. of the soluble antigen, consequently the production of complete and infectious viral particles is blocked by the presence of proflavin. The product itself accumulates principally in the mitochondria. Ledinko (215) studied the multiplication of poliovirus (types 1 and 2) in the HeLa cell cultures in the presence of proflavin. When the product is added to the cultures after the infection, the cytopathogenic effect and the synthesis of the viral particles (counted by electron-microscope) proceeds as in the test culture, but the particles formed in the presence of proflavin are noninfectious. The addition of proflavin to the cultures already containing an important quantity of intracellular virus effects an immediate interruption of the production of new infectious particles and inhibits the liberation of particles, previously formed. These observations recall those made by DeMars (216) and by Kellenberger and Sechaud (217) on the synthesis of T_2 bacteriophages, in the presence of trypaflavin 3,6-diaminoacridine: the viral proteins and the structures constituting the head and tail of the phage are formed normally, but those structures are not associated in order to constitute the morphologically complete and infectious viral particles.

One can express the hypothesis that these acridine derivatives interfere with the intracellular synthesis of certain proteins, such as those whose formation precedes the synthesis of A.R.N., of poliovirus, the mucoproteins, of the hemagglutinin of the virus of the avian pest or of proteins eventually necessary to the integration of constituent particles of bacteriophages. Moreover, it is suitable to consider likewise the possibility of the formation of complexes between the acridines and the nucleic acids; Achaffer (218) showed that proflavin combines with the A.R.N. of poliovirus, and that the complex formed was inactivated by light, the light absorbancy of this photoinactivation corresponds to that of the absorption by proflavin. It s therefore possible that the effect of the acridine derivatives on the synthesis of certain essential proteins depends on the inactivation of A.R.N. by this process. The alteration of A.R.N. could explain the mutagenic effect of proflavin on the poliovirus, observed by Dulbecco and Vogt (219).

Hurst and his collaborators (220,221) showed that the per os administration to the mouse of a derivative of an antimalarial acridine, mepacrine, protects this animal against death resulting from the infection by the Eastern or Western equine encephalomyelitis, the louping ill and Rift Valley Fever. The chemotherapeutic activity of mepacrine on equine encephalomyelitis virus infection is likewise manifested in the young rat but it is not observed in the infection of guinea pigs, chicks, doe rabbits and monkeys by the same virus (222), mepacrine is, on the other hand, without effect on the louping ill infection of sheep (220). In mice, treated per os with an adequate dose of mepacrine, even 24 hours after intramuscular injection of the quine encephalomyelitis virus, the amount of virus present in the circulating blood is considerably less than that observed in the control mice, at the point that the mice treated do not manifest the immunity vis-a-vis of a future reinfection with the homologous virus. The deposits of a substance which is probably a metabolite of mepacrine are observed in the cells of the reticuloendothelial system (222). All the derivatives of acridine possess an antiviral activity analogous to that of mepacrine, giving rise to these deposits when the inactive derivatives are not deposited in the reticuloendothelial system (222). The minimal active dose of mepacrine in the female mouse is 2 times weaker than in the male (223). It is therefore clear that mepacrine and other active derivatives of acridine only exercise their antiviral activity by the intermediary of metabolites, which are formed under well determined conditions linked to the species and even the sex.

IX. ANTIBIOTICS

A certain number of fermentation products by the Actinomyetes or Penicilliums exercise interesting antiviral activity. Any one of these products have partially, an antibacterial activity which justifies the

general application of antibiotic; but is useless to insist again on the fact that the antibacterial antibiotics of current use have no therapeutic influence on virus infections, with the exception of those caused by rickettsiae and the agents of the Bedsonia group. We will only describe here the products of natural origin whose antiviral activity has been clearly demonstrated and principally those whose chemical structure has been elucidated.

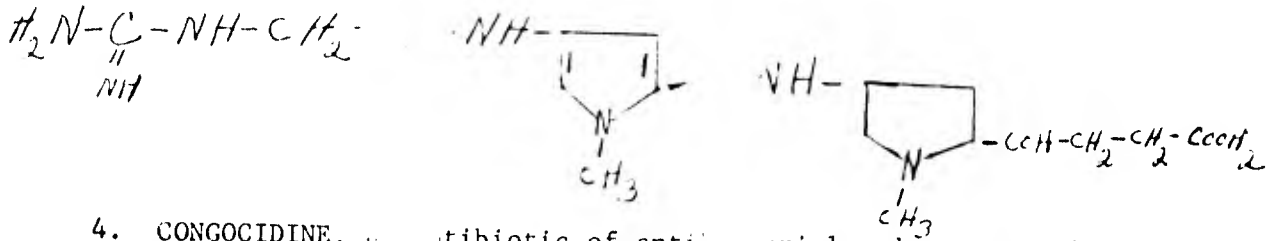
1. EHRLICHINE, an antibiotic made from a strain of Streptomyces lavendules which is a non-producer of streptothricin, inhibits the multiplication of influenza virus type B in the chorioallantoic cavity, when the addition of the product has been done at the beginning of the phase of eclipse of the virus. The same product, administered subcutaneously or i.p. to the mouse, diminishes the size and the number of the pulmonary lesions resulting from the intranasal infection by the influenza virus type B. On the other hand, ehrlichine has no inhibiting effect on the multiplication of influenza virus type A (whose phase of eclipse is shorter than that of type B virus) in ovo and in the lungs of mice. The active principle of ehrlichine is not dialysable, but resists heat and the action of trypsin; it is therefore linked possibly by a polysaccharide (224,225).

2. HELENINE, substance extracted from the cilium of Penicillium funicolosam administered i.p. to the mouse, diminishes the death rate of the animal following the infection by the encephalomyocarditis virus (Columbia SK) and the Semlike Forest virus; this activity is only observed if the treatment has been made during inoculation (226,227). Helenine administered i.p. to the macaques monkey before subcutaneous infection with poliovirus type 1, protects a considerable proportion (80%) of the animals against the paralytic phenomena and definitely retards the appearance of paralysis in those which are not saved (duration of incubation: 24 days, instead of nine days in the test monkeys) (228). On the other hand, helenine does not have a significant chemoprophylactic activity on the mouse infected with poliovirus type 2 (228). The physico-chemical characteristics of helenine are those of a ribonucleoprotein (229) which presents great interest from the point of view of its action. Furthermore, precise information concerning the characteristics of inhibition by helenine on the development of poliovirus in cell cultures is missing.

3. NETROPSINE, an antibiotic made from Streptomyces netropsis and active on a great number of gram-negative and gram-positive germs (230), administered i.p. to the mouse significantly increases the survival of the animal to the intracerebral infection with a neurotropic varriant of the vaccinic virus (231). Netropsine does not hinder the appearance of vaccinic pustules in the doe rabbit inoculated subdermally with the same virus (231). The active dose of netropsine on the vaccinia infection of the mouse (30 mg/kg per day) is close to the toxic dose. Netropsine is 5 times more toxic for the rabbit than for the mouse.

Netropsine has no vity on influen . Western equine enc lalo-myelitis or poliovirus t in mice (231).

The chemical stru e of netropsine h. een clarified by Waller and his collaborators (2 ho propose the f wing formula:



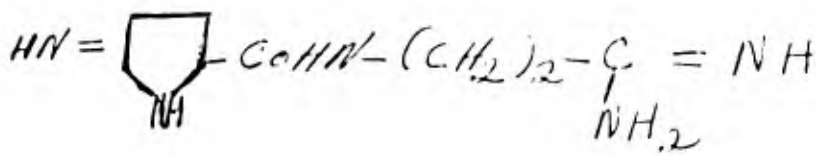
4. CONGOCIDINE, tibiotic of anti rial and trypanocide activity, made from les 2 strains of sti myces (233) has a chemical structure very lar, if not identic , to that of netropsine (234). Like the latter, ngocidine, administe ubcutaneously or i.p., protects the mouse aga intracerebral infecti th a neurotropic strain of vaccinia vir 55). In cell culture inhibits the multiplication and cytopathogenic effect of the vaccin virus and herpes, but this inhibiting effect is accompanied by a slight toxic action on the cells (83).

The inhibition by congocidine of the multiplication and the cytopathogenic effect of the vaccinia virus nevertheless does not proceed from an irreversible alteration of the cells, for if the product is eliminated from the cultures by washing, the cycle of viral multiplication is achieved normally. In the mouse, an effective protection against the intracerebral infection wich the vaccinia virus can be obtained by a single subcutaneous treatment 24 hours after infection. In certain respects, the antiviral activity of netropsine and of congocidine recall that of isatin thiosemicarbazone, but it is less specific: in effect congocidine is active on the infections of vaccinia virus (mouse and cell cultures), herpes (mouse and cell cultures), ectromelia (mouse) and myxomatose (rabbit) (83),whereas isatin thiosemicarbazone is without action on the last three infections. One will note, however, that the antiviral activity of netropsine and of congocidine is limited to the viruses with A.D.N., no inhibiting effect has been observed in cultures or in the animal vis-a-vis of diverse viruses with A.R.N. (influenza, Theiler disease, encephalomyocartis Columbia SK). The high toxicity of these antibiotics render improbable an eventual practical application of their antiviral activity.

5. NOFORMICIDINE, a substance made from Nocardia formica, inhibits the multiplication of influenza virus (types A and B) in mouse lungs and in ovo and the multiplication in ovo of the mumps and Newcastle Disease virus (235). In the mouse infected intranasally with the influenza virus, the administering of noformicine subcutaneously, i.v. or orally, even

24 hours after the infection, significantly prolongs the survival; but noformicidine is without effect on Newcastle disease infection of the chicken.

The chemical structure of the product is the following:



Noformicidine

It is interesting to verify a certain analogy of structure between noformicidine, inhibitor of myxovirus and of netropsine-congocidine, inhibitors of the pox group virus. None of the products inactivate the sensitive virus <u>in vitro</u>.

6. ACTINOMYCINE D, an antibiotic with polypetidic structure endowed with antibacterial and antitumoral activity, inhibits the multiplication of vaccinia virus in cell cultures (236). Actinomycin D acts, probably by inhibiting the synthesis of cellular A.R.N.; it is without effect on synthesis of cellular A.D.N. In the presence of actinomycin D the incorporation of uridine in the cell A.R.N. is considerably diminished, while the incorporation of thymidine in cell A.D.N. and that of leucine in the proteins is not affected. These observations furnish a new proof of the role of cell A.D.N. in the multiplication of a virus; actinomycin D is without effect on the multiplication of virus with A.R.N.. This proves that the synthesis of viral A.R.N. is independent of that of cellular A.R.N. It is interesting to note that actinomycin inhibits the multiplication of vaccinia virus and myxomatose <u>in ovo</u>, but that in tolerable doses it does not work on the infection of the mouse or the rabbit. On the other hand, this antibiotic seems capable of inactivating <u>in vitro</u> the myxomatose virus (280).

7. MITOMYCIN C, an antibiotic endowed with antitumoral activity, is a selective inhibitor of the synthesis of cellular A.D.N. (237,238). Added to the cell cultures at sufficient concentration to suppress all synthesis of A.D.N. and consequently of cellular A.R.N., it does not inhibit the multiplication of a virus with A.R.N. (Mengo), but affects that of a virus with A.D.N. (vaccinic) (239), which underlines again the important differences existing in the interdependence of synthesis of the cellular and viralnucleic acids according to how it acts on the virus with A.R.N. or on virus with A.D.N.

In summary, with the possible exception of helenine, the products of fungic origin, whose antiviral activity has been revealed, only seem, for the most part, to exercise that activity at the expense of deep

alterations in the cell metabolism. In certain cases, however, the study of their mode of action has furnished useful contributions to the knowledge of the relationship existing between the viral synthesis and the metabolic process of the above cells. We have only presented some of the examples and passed over a number of products whose chemical structure and mode of action are little known although their consideration is of general interest.

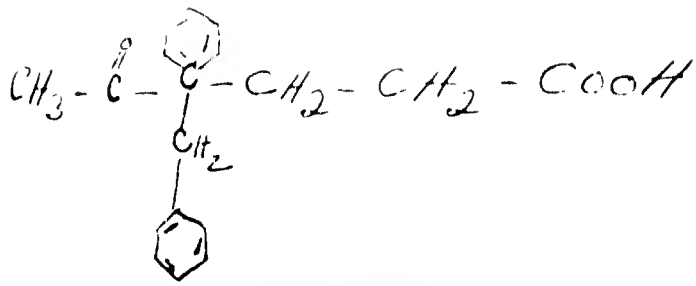
X. DIVERSE PRODUCTS

It is not our purpose to give under this method a list of all the products not belonging to the categories already considered, but only of the substances for which an antiviral activity has been described. We will therefore limit ourselves to a few examples worthy of interest.

1. CAPROCHLORONE

Lui and his collaborators (240,241) studied the activity of 4-(o-chlorobenzyl)-5-oxo-4-phenylhexanoic acid or caprochlorone on the multiplication of influenza virus, type A, in the chorioallantoic cavity membrane and in mouse lungs infected intranasally with the same virus. In ovo, caprochlorone, at a non-cytotoxic concentration, inhibits the production of hemagglutinin and the infectious virus, but only interferes with the production of soluble antigen (intranuclear A.R.N.). Administered per os to the mouse, at a rate of 3 treatments per day, the product significantly diminishes the mortality following inoculation of weak quantities of virus ($3DL_{50}$), without doubt by the partial inhibition of the multiplication of the virus in the lungs. If the treatment per os with caprochlorone is combined with the subcutaneous injection of human gammaglobulin containing a high level of antibodies against influenza virus type A, the mice are protected against death due to the inoculation of 3,000 LD_{50} of virus, while gammaglobulin alone only protects against 30 LD_{50} of virus. Although, in ovo caprochlorone exercises an inhibiting effect when it is added 20 hours after the inoculation of the virus, it is active in the mouse only if the first treatment was given at the moment of the infection; on the other hand, the treatment combining caprochlorone-gammaglobulin can start no later than 3 hours after infection to assure the survival of the animal and decrease their pulmonary lesions. This research deserved to be brought to light, it would have the advantage in certain cases of associating an antiviral chemotherapy with a specific serotherapy.

It is said that serotherapy offers only slight interest in the treatment of most infections with viruses. Yet it exercises an undeniable prophylactic effect, even if short lived, in certain cases (measles in particular).

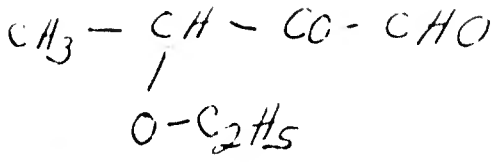


CAPROCHLORONE

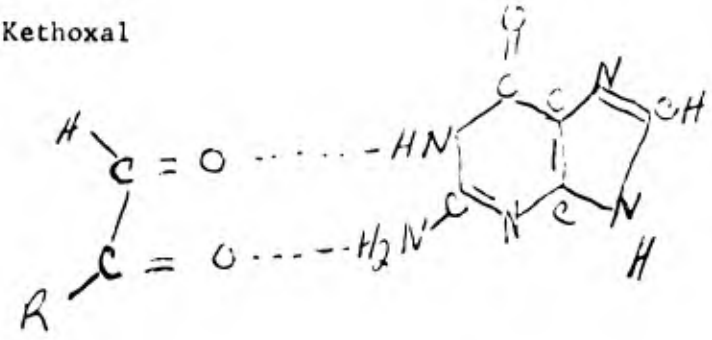
2. BIGUANIDES

The chlorhydrate of anhydro-N, N,-bis(2-hydroxyethyl)bigquanide (abbreviated as A.B.O.B.) exercises, according to Melander (242), an increase of survival and lessens the pulmonary lesions of mice infected by aerosols with influenza virus. The results published by this author nevertheless do not permit estimation of the significance, which should be attached to the activity he has observed, for mice, which were inoculated generally (according to the existing difference) 3 to 4 days after the infection, between the extent of the pulmonary lesions observed in the mice treated and that discovered in the control mice; such criteria allows a considerable margin of error. Lui and Ferlauto (243) showed that the product does not inactivate in vitro the influenza virus, and exercised only the inhibiting effect on the multiplication in ovo of the same virus with the condition that a high dose (100 mg per egg) is administered and many times during the cycle of multiplication of the virus. The same authors observed a slight favorable activity of A.B.O.B. on the survival of mice infected intranasally with a very weak dose of influenza virus.

In spite of the very restricted experimental basis on which rests the antiviral activity of A.B.O.B., this product has been made the object of numerous clinical tests on the infections caused by diverse viruses. These tests have been made possible by the fact that this product is generally well tolerated. The conclusions of these clinical tests differ according to their authors: Sjoberg (244) observed a prophylactic activity of A.B.O.B. in a fairly great number of cases of grippe caused by the virus of types A_2 and B, but Parker (245) has not noted the therapeutic effect of A.B.O.B. in the course of a study with 124 cases of acute non-bacterial respiratory diseases. Brown (246) and Hopkins (247) did not observe significant activity of A.B.O.B. in the treatment of measles. This is possible in the treatment of herpes zona and of chicken pox (both infections are caused by identical viruses) since A.B.O.B. yielded much more interesting results according to Wheatly (248), Alderman (249) and Wilkinson (250). These authors observed in diseases treated precociously, an attenuation of the extent and severity of the eruption and very rapid recovery, due to the habitual behavior of these infections. It should be noted that these clinical studies allow only a few control cases. The antiviral activity of A.B.O.B., whatever may be its mode of action or of



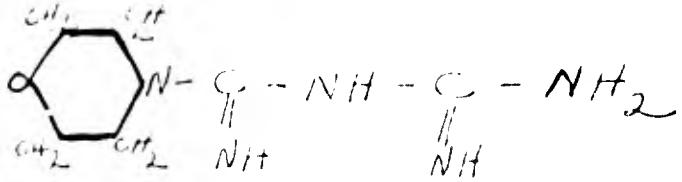
Kethoxal



Radical virucide

Guanine

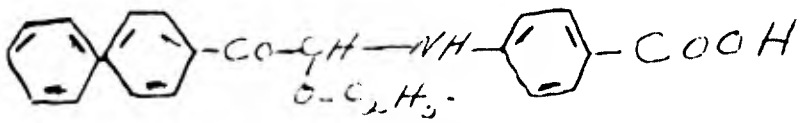
its metabolites (inhibiting effect of the viral multiplication or indirect effect on certain manifestations of infection), deserves to be specified very rigorously. This has not been done as yet.



anhydro-N_1, N_1-bis(2-hydroxyethyl)biguanide

3. KETOALDEHYDES (XENALAMINE, XENALDIAL, KETHOXAL)

Cavallini and his collaborators (251) synthesized in 1959 a series of products combining a ketoaldehyde radical, presumed antiviral, and a biphenyl radical. Amont the products were p-[2-(p-biphenylyl)-1-ethoxy-2-formylamino]benzoic acid, Xenalamine (I), and p-biphenylene-glyoxal, Xendial (II).



(I) Xenalamine

(II) Xendial

One would remark on the analogies of structure existing between these 2 products and 3-ethoxy-2-oxobutyraldehyde (kethoxal) which McLimans and his collaborators (252) in 1957 demonstrated pronounced inactivating effect on certain myxovirus in vitro. Staehelin (253) observed that the virucide activity of kethoxal and of its derivatives (general formula R-CO-CHO or R-CHOG-CHO) were linked to their capacity to react with the diamine portion of guanine, one of the purine bases constituents of A.R.N. and A.D.N.

Magrassi and his collaborators (254) studied the activity of xenalamine on influenza type A infection in the mouse subcutaneous, intranasal or oral administration of xenalamine beginning the treatment no

later than 24 hours after infection with 5 LD_{50} of virus, it permits the survival of a significant percentage of infected mice and inhibited the multiplication of the virus in their lungs.

According to the same authors, xenalamine is active at daily doses of 15 mg/kg s.c., a dose which is 100 times less than its LD_0 by the same way.

Engle and Lui (255) studied in detail the activity of xenalamine and of xendial on influenza virus type A, in vitro and in ovo. Xenalamine and xendial inactivate rapidly the infectiousness of the virus in vitro: a 90% decrease of infectious titer is observed when influenza virus is left in contact for 10 minutes at 25° with xendial at a concentration of 10 mg/ml. In the allantoic cavity the presence of 100 mg. of xenalamine or xendial inhibits the multiplication of influenza virus, all the more marked when the product is added previously in the course of the cycle of multiplication: the clearest inhibition is obtained when the product is injected at the moment of inoculation of the virus and 2,6 and 12 hours after. Under these conditions the hemagglutinant titer of intracellular and extracellular virus in the eggs containing the inhibitors is 24 hours after infection, 16 to 32 times weaker than those present in the control eggs. It seems, on the other hand, that the antiviral activity in ovo is not entirely explicable by the virucide activity which these products manifest in vitro: in effect the virucide activity is considerably diminished in the presence of serum or of allantoic fluid, where as these liquid do not interfere with the inhibiting power in ovo of the products. Lui and Engle (256) also studied the activity of xenaldial on herpes virus. Xenalamine and xendial rapidly inactivate the virus in vitro, inhibit the multiplication in ovo, partially protect the mice against mortality following intracerebral infection with the virus, applied locally in the form of ointment and considerably diminish the intensity of experimental keratosis produced by the inoculation of the herpes virus in the scarified cornea of the rabbit.

Numerous clinical tests have been conducted in Italy, to establish the therapeutic or chemotherapeutic activity of xenalamine and of xenaldial on diverse human viral infections, in particular, respiratory infections (257) infectious hepatitis (258,259) and chicken pox (260). We think that as has been the case of the clinical tests of anhydro-N_1, N_1-bis(2-hydroxyethyl)biguanide, these inquiries have not been, for the most part, conducted with rigor, and sufficient precision to carry a full conviction with respect to the effectiveness of these products in human therapeutics. It is interesting to note that the products in which the activity seem to be principally virucide in vitro, can have, under certain conditions, an inhibiting effect on the viral infection developing in vitro, and even when the products are not administered locally, we think the mechanism of activity of xenalamine and of xendial should be elucidated more clearly than it has been up to the present (261).

4. NUCLEASES

Another category of substances capable of interfering in vivo with the viral multiplication by a direct action of the infectious nucleic acids, not in inactivating them, but in specific division, is represented by the nucleases: ribonuclease and desoxyribonuclease. It is said that these enzymes are incapable of working on the whole viral particle, in which the infectious nucleic acid is protected by a proteinic capsule, but they rapidly destroy the free infectious nucleic acids and they can, therefore, at certain stages of viral development, inhibit, in this way, the multiplication of the virus.

LeClerc (262) showed that crystalized ribonuclease, isolated from beef pancreas, inhibits the multiplication of influenza virus in the chorioallantoic cavity providing it is present during the phase of eclipse of the virus. Moreover, it is not certain that the inhibiting action of nucleases is exercised always directly by interaction with the infectious nucleic acids of the virus. Tamm and Bablanian (263) showed, in effect, that the ribonuclease of beef pancreas inhibited the multiplication of vaccinia virus and herpes in cell cultures, that the nucleic acid of both contain desocyribose (A.D.N.), and it is therefore evident that the ribonuclease inhibits virus multiplication by destroying a cellular A.R.N. necessary to the synthesis of proteins or viral A.D.N.

Herriott and his collaborators (264) detected the presence in blood of healthy adult subjects of measurable amounts of ribonuclease (ARNase) and desoxyribonuclease (ADNase). ADNase is found principally localized in platelets, an inhibitor of its activity is found in the leucocytes; ARNase is found in leucocytes and an inhibitor of its activity is present in the red corpuscles. In 12 minutes at 37°, the ADNase of human blood destroys 90% of the activity of an infectious viral A.D.N.; while ARNase of blood destroys an equal proportion of infectious A.R.N. in 3 seconds at 37°. If one considers that a certain stage of viral infection, the free infectious nucleic acids are found in the tissues and in the blood, the presence of nucleases in the blood can constitute a defense mechanism against the infection as Herriott suggests (246) and it should be possible to work on the mechanism to increase the effectiveness. Dhennin and collaborators (266) report ARNase of beef pancreas retards the evolution of foot and mouth disease in the guinea pig.

It is surprising, in this regard, to ascertain that the polymer acids prepared by the action of oxydants on diverse dihydrocybenzoic acids and endowed of inhibiting activity vis-a-vis of ARNase are capable of interfering with the multiplication of influenza virus and vaccinia in cell cultures and that there exists a probable parallel between the inhibiting activity vis-a-vis of ARNase and the antiviral activity (267). On the other hand, the inhibiting action of ethylenemaleic anhydride copolymers of a molecular weight close to 120,000 on the cytopathogenic effect of ECHO virus type 9 and Semlike Forest virus, in cell cultures,

is not only linked to an inhibiting activity of this product on the nucleases, but seems due to their interaction with certain receptors of the cell surface (268).

5. IONS Co^{++}, Cu^{++}, $(NH_4)^+$

The list of diverse products, capable by multiple mechanisms of interfering with the reproduction of viruses, is certainly not exhausted by some of the examples we have given here. It is proper to insist on the fact that even the simplest products can, under certain conditions, work relatively specific in this respect. Thus it is that Co^{++} (269) and a chelate formed with this ion by ethylenediaminetetracetic acid (270) inhibit, at a noncytotoxic concentration, the multiplication of influenza virus in the allantoic cavity.

The cupric salt and the rhodium salts administered to the mouse subcutaneously in doses close to the maximum dose tolerated by the animal, exercise a significant protective activity against vaccinia virus ectromelia infection, but are without effect on the other viral infections studied (271).

Jensen and Liu (272) showed that the ammonium ion inhibits the multiplication of influenza virus in dog kidney cultures and that certain aliphatic amines also inhibit (273). No other viruses are affected by these inhibitors. The inhibiting action of the ammonium ion on the multiplication of influenza virus is exercised entirely at the beginning of the phase of eclipse of the virus (274).

The intracellular multiplication of the virus puts in play such a great number of structures that it is not astonishing to find out that the products capable of inhibiting this multiplication would be very diverse in their nature and mode of action.

XI. PRODUCTS WITH INHIBITING ACTION

A product can favorably influence a viral infection, either by inhibiting certain stages of multiplication of the virus or by working on the secondary mechanisms-whose nature is again relatively unknown- by which on the level of the cells, the tissues and the whole organism, biochemical or morphological lesions which result from the infection are manifested. From the practical point of view, the last type of activity without doubt is very interesting; it allows us to intervene therapeutically at the moment when the symptoms appear, while the inhibition of the viral multiplication can only be generally effective during the incubation phase of the disease. These products with indirect action have meanwhile been little studied for the appropriate experimental methods are difficult to employ. It is possible nevertheless, under certain conditions and with certain viruses to obtain a predominance of so called toxic effects, in which the phenomenum of cell or tissue alteration are, in a certain measure

dissociable from the viral multiplication. Such are, for example, the lesions produced in the lungs by the inoculation of the mouse with massive doses of certain myxoviruses (mumps, Newcastle disease) or by neurotoxic phenomena produced by the intracerebral inoculation in the same animal of massive doses of non-neurotrope virus (influenza).

1. XEROSINE: A few of the more significant works in this area were done by Groupe and his collaborators (276) who showed that a macromolecular substance made from Achromobacter xerosis, called xerosine by them, administered to the mouse subcutaneously, has the effect of diminishing the size of pulmonary lesions resulting from the intranasal inoculation of influenza virus and of suppressing those lesions by inoculation of the Newcastle disease virus. The last are due to a purely toxic action of the virus when it is inoculated intranasally in a massive dose and is not accompanied by a multiplication of virus in the lungs. Xerosine works at an optimum against Newcastle disease when it is injected during the period of rapid extension of these lesions, its effectiveness is not augmented by reducing the dose of the inoculated virus (277). Xerosine does not inhibit the multiplication of influenza virus in mouse lungs or in ovo, it does not inactivate influenza virus or the Newcastle disease in vitro . Injection of the mouse with a high dose of xerosine (100 mg/kg) causes pulmonary lesions comparable to those induced by intranasal inoculation of Newcastle disease virus. Xerosine, likewise decreases the death rate of mice following intracerebral inoculation of massive doses of influenza virus, but is inactive in vitro (278). Xerosine decreases the pulmonary lesions produced in the mouse by intranasal inoculation of a pneumotrope murine virus apparently of the Bedsonia group without inhibiting virus multiplication (278). Xerosine works, like hydrocortisone, on pulmonary lesions produced in the mouse by intranasal inoculation of E. coli endotoxin. But hydrocortisone, unlike xerosine, is inactive on pulmonary lesions produced by the Newcastle disease virus and on the death rate following intracerebral inoculation of influenza virus (279). It is therefore not very probable that xerosine acts simply as an ordinary anti-inflammatory product. Xerosine possess and action on rabbit myxomatosis, it inhibits the development of myxomatosis nodules (point of inoculation, mump nodules); but it has no influence on the death rate of the animals (180). Xerosine modifies in the chicken the evolution and aspect of Rous sarcoma tumors (281).

The compound 9-(p- guanylbenzylidene)fluorene works analogously to xerosine on the mouse pulmonary lesions following intranasal inoculation of influenza virus. It is active orally and subcutaneously, while xerosine is active only subcutaneously.

A glycolipid macromolecular extracted (ganglioside) from beef brain, decreases the neurotoxic effect in the mouse of a massive dose of

intracerebral inoculation of influenza virus; given that this product inhibits the reaction of hemagglutination of this virus, it is probable that it competitively with certain receptors of the cell surface on which the virus adsorbs and protects in this way the cerebral tissue of the mouse (283).

2. CHLOROPROMAZINE: exercises a marked inhibiting effect on pulmonary lesions caused in the mouse by intranasal inoculation of the Newcastle disease (284). This same product partially protects the mouse against convulsions, generally fatal, caused by the intracerebral injection of a massive dose of influenza virus; phenobarbital exercises, in this experimental system, a protective action, clearly less marked than that of chloropromazine (83). Chloropromazine likewise protects the mouse against the toxic effect of intravenous inoculation of a massive dose of psittacosis virus (83). In rabbits the pyrogenic effect of intravenous inoculation of influenza virus is suppressed by the subcutaneous administration of chloropromazine (83).

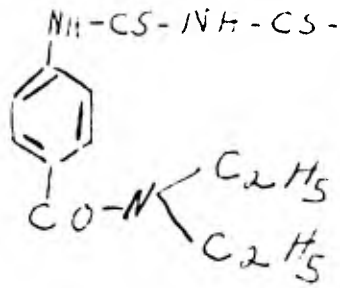
3. Certain ANTIHISTAMINICS are likewise capable of protecting the tissues against the toxic reactions of viruses. Pyrilamine,maleate, decreases the edema and the necrosis of the corneala endothelium caused by the injection of the virus of Newcastle disease in the anterior chamber of the rabbit eye; lysergic acid diethylamide antagonist of serotonin, exercises a comparable effect on hepatitis virus necrosis on the liver surface [MHV_3 strain]; lysergic acid diethylamide protects in vitro mouse hepatic cells against the cytopathogenic effect of hepatitis, without inhibiting the virus multiplication (286). The prevention of the cytopathogenic effect in cell cultures and of disseminated necrosis of hepatic parenchyma is attributed to a protective action of the antihistamines on the mitochondria (287).

4. The injection of bacterial ENDOTOXINS can transitorially increase the resistance of the mouse to diverse viral infections. The reason is probably in the reticuloendothelial system structure. For example, we cite the recent work of Nemes and Hillman (288) who showed that the lipopolysaccharide fraction of E. coli endotoxin (Lipide A of Westphal), administered to the mouse intracerebrally, partially protects this animal against the neutrotoxicity of influenza and herpes virus. Administered subcutaneously this product likewise increases the resistance of the mouse to the intranasal infection of the influenza virus; i.p. it partially protects against the infection of encephalomyocarditis virus (Columbia SK). It is without action on infections of poliomyelitis virus (Lansing strain) and of Coxsackie virus B3.

Hook and Wagner (289) showed that the intracerebral infection of the mouse with weak doses of endotoxins of Gram-negative bacilli make the animal partially resistant to the neurotoxic effect of influenza virus; and Gledhill (290) showed that certain bacterial endotoxins increase the resistance of the mouse to infection with ectromelia virus. Finkelstein (291)

increased the survival chicken embryos inoculated *in ovo* with the virus of Newcastle disease by injection in the allantoic cavity of endotoxins extracted from diverse strains of *E. coli*.

5. Girard and his collaborators (292) described the compound, 1-*p*-diethylcarbamoxylphenyl(biuret (G81), which works on the infection of the mouse of poliovirus type 2 (Lansing strain). The mice, treated daily with a dose of 500 mg/kg *per os* with this product have a total death rate less than that of the control mice. It is said that in treated mice with poliovirus (Lansing strain), the death rate is generally 50 to 70% at the most, it only reaches this maximum at the end of about 50 days after the intracerebral inoculation of the virus. Girard and his collaborators obtained the best results (total death rate 25% instead of 58% in test animals), when they treated mice 12 days after inoculation with the virus.



1-(*p*-diethylcarbamoxylphenyl)-biuret

Paradiethylbenzamiodithibiuret (G812)

The diethylbenzamido group can be replaced by N-butylbenzamido and morphalinobenzamido without appreciably changing the activity (293). Viallier and his collaborators (294) studied the mode of action of G812 and noted in the guinea pigs treated with this product the appearance of polyneuritic symptoms, with ascending progressive paralysis while G812 is well tolerated by the mice, rats and man. G812 does not inactivate the poliovirus *in vitro* and does not inhibit its multiplication in cell cultures. It is therefore probable that G812 works on the level of the central nervous system, possibly by protecting the neuron motors against destruction by poliovirus, an hypothesis supported by its neurotropism evidenced in the guinea pig. It is noted that G812 exercises no influence on the infection of the mouse by poliovirus muirs (Theiler disease) or by the encephalomyocarditis virus (Columbia SK) (183). G812 has been clinically tested on subjects attacked with poliomyelitis, in particular in cases having a severe respiratory symptoms (295,296). The paralysis has rarely progressed, and the death rate has been less than that usually observed. G812 worked in the majority of the cases of infections caused by poliovirus type 1. Nevertheless, the clinical studies of G812 should be better controlled before therapeutic activity of this product can be established.

6. CORTICOSTROIDES

The influence of conditions of nutrition and hormonal factors are part of the study of indirect action which can influence the evolution of animal viral infections. This complex subject has been reviewed by Hurst and Hull (3) and we will not deal with it here. Nevertheless, it is suitable to recall the aggravating action which certain cortiscosteroids can exercise on viral infections, in particular, on those caused by virus with A.D.N. (herpes, smallpox) without forgetting that corticosteroids can play an indirect, but favorable, therapeutic role in other viral infections (viral pneumopathies, infectious hepatitis). The temperature of the animal (297), the oxygen tension in the surrounding air likewise have their importance in the evolution of virus infections.

It is conceivable that corticosteroids working indirectly on certain mechanisms directing the development of the pathogenisis of viral infections, rather than on the multiplication of the virus, could play a role in the therapeutics of these infections in the future. The difficulties of research in this area are principally of a methodologic and experimental order.

XII THE INTERFERON

The discovery of the interferon by Isaacs and Lindenmann in 1957 (298) is the result of research to clarification of the mechanism of interference of the multiplication of one virus by another in cells culture or animals. This research first showed that the action of influenza virus, rendered noninfectious by heating to 56° or by ultraviolet radiation, on the cells of the chorioallantoic membrane causes the cells to produce a nondialysable thermolabile substance inactivated by trypsin, capable of inhibiting the multiplication of infectious particles of influenza virus in cell cultures of the same origin. The identification of the substance, called interferon explain this phenomenon.

Production of interferon by cells infected in vitro or in ovo has been observed with myxovirus (299,300), enterovirus (301,302), arbovirus (303,304,305), vaccinia (306), measles (307), mouse polyoma (308), etc. Production of interferon is not limited to the case of heat or U.V. rays inactivated virus, but it is also observed with more or less intensity with intact virus when, for one of many reasons, the virus does not multiply rapidly, delaying the destruction of the cells. This is the case, in particular of so called "chromic" infections of certain lines of cells by this virus (309) or in the course of abortive animal viral infections (310).

Interferon has certain characteristics: a) the interferon produced by the cells at the time of their infection by one virus can inhibit the multiplication of a second virus having no reaction with the first, but

only in the cells arising from the same animal species as from which it has been produced. In other words, the action of the interferon has no specific quantity of virus on which it is exercised, but with some exceptions (311), it is specific to the species of animal in which it has been produced; b) interferon can be produced at the time of interaction of cells with a virus inactivated at 56° or by U.V. irradiation of short duration, but the presence of intact nucleic acid, not denatured, in the same virus is necessary. A virus inactivated at 60° or by formaldehyde, or again an incomplete influenza virus (devoid of A.R.N.) does not cause the production of interferon by the cells. Very probably, this is the introduction into the cell of an extraneous nucleic acid, that carrier of the infectiousness which unleashes the production of interferon; c) the inhibition of the multiplication of viruses by interferon exercises on the level of synthesis of the nucleic acids: infectious A.R.N. isolated from poliovirus is incapable of infecting the cells protected by interferon (312) and in chicken embryo cells treated by interferon, the synthesis of the A.R.N. of poliovirus and of Western equine encephalitis virus does not operate at all (313); d) interferon is a protein, of a relatively low molecular weight (63,000) whose activity is destroyed at an alkaline pH, but resistant at a pH equal to 2. The protein interferon has been purified and concentrated by Burke (314).

From the point of view of antiviral chemotherapy 2 essential questions are posed: 1) what role does the production <u>in situ</u> of interferon play in the mechanism by which it produces in man a spontaneous cure for virus infections? 2) Can the administration laboratory prepared interferon to animals or man favorably influence the evolution of a virus infection? The first point has been clarified by Isaacs and Hitchcock (315) who studied the multiplication of influenza virus and the production of interferon in the lungs of the mouse. When the mice are infected with a massive dose (600 LD_{50}) of influenza virus, the rate of virus growth in the lungs increases rapidly and remains high up to the time of the death of the mice; interferon appears in the lungs 2 days after infection, but its concentration decreases rapidly beginning on the 5th day. When the mice are infected with 60 LD_{50} of virus, the rate of virus growth in the lungs reaches a maximum 3 days after infection then decreases considerably; the rate of interferon production in the lungs following a curve similar to that of the rate of virus growth; a measured quantity of specific seric antibodies only appear 11 days after the infection and the animals die toward the 12th day. Finally when the mice are infected with 6 or 20 LD_{50} of virus, the rate of virus growth reaches a maximum 3 days after infection, then decreases rapidly; the rate of interferon production in the lungs is maintained at a high level up to the 5th day, and most of the animals survive. The antibodies are only discernable in their serum 14 days after infection. It is therefore clear that the decrease in rate of virus growth in the lungs is produced at the

moment when the interferon attains its maximum concentration, but the appearance of antibodies and one to conclude that the interferon has played a role with the specific immunological response of the animal in the mechanism by which the virus multiplication has been retarded or completely arrested 3 days after infection. In the same vein, Freidman and his collaborators (316) showed that at the time of the cutaneous infection of the guinea pig with the vaccinia virus, the evolution of the local infection and of its cure proceed the same way in the animal whose immunologic reactions have been suppressed (by radiation with X-rays or by administration of amethopterin) which in the animals in which the production of antibodies is not disturbed, the presence of interferon in the skin of the animal has been revealed in both cases, it is therefore probable that the substance has played an important role in the mechanism of cure.

The prophylactic or therapeutic action of laboratory prepared interferon has already been the object of much research. Hitchcock and Isaacs (317) administered mouse lung cell culture and mouse lung extract interferon i.p. to mice, who 24 hours later, were infected the same way with Bunyamwera virus. In the mice treated with interferon prepared in cell cultures, the death rate following the Bunyamwera infection has been from 22 to 60% in the control mice; in the tests effected with interferon extracted from the lungs of mice, the death rate has been 37% in the treated mice and 76% in the test mice. Thus in both cases, the administration of interferon before the infection has exercised a significant protective activity.

Isaacs and Westwood (318) on the other hand, studied the action of interferon prepared from cell cultures of rabbit kidney (by inoculation with influenza virus inactivated by U. V. rays) on the intradermic infection of the rabbit with vaccinia virus. The injection of interferon in the skin of the rabbit, 24 hours before the inoculation at the same point of a considerable dose of vaccinia virus, completely inhibited the appearance of vaccinal lesions; against the weak dose of virus, the inhibition of specific lesions can be obtained even when interferon has been injected at the same times as the virus. Some interferon prepared from cell cultures of embryonic chicken exercised an inhibiting effect much less marked on the appearance of vaccinia lesions on the skin of the rabbit than in rabbit kidney cell cultures, which illustrates the specificity of interferon insofar as the animal species in which it is produced and tested.

Cantell and Tommila (319) showed that instillation of interferon prepared from rabbit kidney cell cultures (for inoculation of mumps virus irradiated with U. V. rays), on the cornea of the rabbit infected with vaccinia virus, inhibits the appearance of keratosis which was regularly observed in the eyes of non-treated animals. On the other hand, the

local treatment with interferon has not had any effect on the lesions produced on the surface of the cornea by inoculation with herpes virus.

Atanasiu and Chany (320) studied the action of interferon, extracted from tumoral cell cultures of hamsters, infected with myxovirus parainfluenza type 3, on the protection of tumors in the hamsters inoculated at birth with the polyoma virus. A significant protective effect of interferon has been observed in animals receiving the interferon subcutaneously the day before infection, tumors appear between 30 and 200 days after the inoculation with polyoma in 35% of the cases, while in the hamsters not protected by interferon, the tumors are apparent most precociously (in 7 to 125 days after the infection) and in 89% of the cases.

Recent clinical tests showed that interferon prepared from macaques rhesus kidney cell cultures inoculated with influenza virus, have a highly significant inhibiting effect on the local infections by the vaccinia virus in man.

Isaacs and his collaborators (321) studied the appearance of vaccinia lesion in 38 volunteers, in which the vaccinia virus had been inoculated on 2 points of the skin: in the first, interferon had been inoculated 24 hours before the injection of the virus, in the second a test material (coming from the monkey kidney cell cultures not inoculated with influenza virus and therefore not containing interferon) had been inoculated likewise 24 hours before infection. The typical vaccinia lesions (primary reactions) have been observed in 37 of the 39 points of the skin not treated with interferon, on the other hand, on the points of the skin having received the interferon, the vaccinia lesions are apparent in 14 of the 38 cases.

On the other side, Jones and his collaborators (322) studied the therapeutic activity of repeated local instillations of interferon (each half hour during the day) in 5 cases of vaccinal keratosis presenting the ulcerated lesions of the cornea. In the 5 cases, the granular opacification of the epithelium has disappeared 24 hours after the start of the treatment.

It is necessary to underline that in these test cases, interferon manifested a truely therapeutic activity since it was administered locally many days after the viral infection which in all the cases studied, was an accident of the antivariolic vaccination. One can conclude that interferon is capable not only of protecting the cells against viral infection, but also of arresting the extension of that infection already established. In this respect, interferon acts like 5-iodo-2-desoxyuridine, whose antiviral activity is exercised on a different mechanism. Neither in the cell culture tests nor in man or animal, has interferon manifested the slightest toxicity.

CONCLUSIONS

We have shown in the course of this study how the problems posed by the chemotherapy of viral infections are difficult to solve due to the particular nature of these infections which resides essentially in a cell contamination by a foreign genetic material. Up to just recently, one could consider, that chemotherapy represents an insoluble problem, that of selectively inhibiting the reactions which develope within the cell and intimately tied to its own metabolism yet without disturbing the normal cell function (323). In the meantime, in the course of later years, it has been shown that under certain conditions, a similar selectivity of action is not impossible: we have given the examples. The cell infected by a viral nucleic acid is capable of producing a protein (interferon) devoid of toxicity, which specifically interferes with the synthesis of viral nucleic acids. The compounds, 2-(α-hydroxybenzyl)-o-benzimidazole and guanidine are likewise capable of selectively inhibiting intracellular production of infectious nucleic acids of certain enteroviruses. The other products, without doubt less selective, can nevertheless exploit the quantitative differences existing between the biosynthesis of the nucleic acid of virus and those of nucleic acid of cells; the most striking example is furnished by the desoxyribosides of halogenated pyrimidines. The other products, such as the thiosemicarbazone of isatin can at a concentration devoid of toxicity for the cells, inhibit certain essential stages of maturation of pox group viruses.

For practical application, it is at present evident that chemotherapy and of chemoprophylaxy are not as successful as prevention of virus infection by specific vaccines. However, vaccination is not always practical and research on effective chemical agents is needed. The clinical tests effected with 2'-5-iododesoxyuridine in the case of vaccinia keratosis and herpes, with the thiosemicarbazones in the case of variolus and generalized or gangrenous smallpox, with interferon in the case of vaccinia keratosis, have given encouraging results although often limited,principally to local infections. It is hoped that eventual clinical tests of active products will be conducted with the same scientific rigor as those which have been mentioned, and that the important problem of antiviral chemotherapy is not obscured by premature and poorly controlled clinical applications. Finally, the vast number of products with indirect activity, all the more interesting since their action can be exercised at the moment when certain symptoms of the viral infection are already apparent, deserve to be explained with renewed intensity.

Right now the work accomplished in an area where recently there was skepticism, has not only contributed significantly to the knowledge of numerous mechanisms of viral infections and by virtue of this, represented an important phase in the research of virology and cell physiology, but it allows the foreseeing of a justified optimum in the future of antiviral chemotherapy. Possibly the results obtained in this area will open new horizons for the treatment of tumoral afflictions.

The diversity of products which should be studied and the methods necessary to present their activity, seem to give meaning to a dialogue of protagonists of another drama, also as old as the world:

-"This is an idea," said Cottard "but this will not serve anyone. The pest is very strong."

-"We will know," said Tarrou, "by the shade of the patient, when we have been tested."

(Albert Camus, La Peste)

ADDENDUM

During the time which passed between the sending of the manuscript of this article (February 1963), and that of the correction of the proofs (September 1963), two journals dealing with antiviral chemotherapy have been published. The first (P.W. Sadler: Chemotherapy of Viral Diseases Pharmacol. Rev. 15, 407-447, June 1963) is a remarkable account of the question in which one finds in particular a detailed description of methods used for the research and evaluations of products with antiviral activity and the relationship existing between chemical structure and activity (principally in the area of benzimidazole derivatives and that of thiosemicarbazones). A interesting article (H.E. Kaufman: Chemotherapy of Virus Disease, Chemotherapia 7, 1-16, 1963) is dedicated, for the most part, to the activity of halogenated analogues of thymidine on the development of herpes and gives the results of the most recent studies in this area of clinical and experimental studies. On the other hand, the actual state of the matter of interferon is summarized in another article recently published (R. R. Wagner, Bacteriol. Rev. 27, 72-86, 1963).

It is therefore convenient to mention some of the important discoveries made during the last months in the area of antiviral chemotherapy. Underwood (Proc. Soc. Exp. Biol. Med. (N.Y.) 111.660.1962) showed that a pyrimidinic nucleoside, 1-β-D-arabinofuranosyl-cytosine, has on experimental herpetic keratosis of the rabbit a therapeutic activity comparable to that of 2'-5-iododesoxyuridine (I.D.U.R.) and works synergically the latter. On the other hand this nucleoside does not seem to cause incorporation in cell of viral A.D.N.

Eggers and Tamm (3rd International Congress of Chemotherapy, Stuttgart, July 1963) clarified the mode of action of 2(α]hydroxybenzyl)-benzimidazole (H.B.B.) and of guanidine on the multiplication of enteroviruses: these two products inhibit the synthesis in cells infected with a specific A.R.N. - polymerase whose appearance precedes that of the infectious virus. Inversely, the synthesis of this essential enzyme is only done in the presence of H.B.B. or of guanidine in the case of developing enterovirus dependents.

Hamilton and collaborators (Nature 198, 538, 1963) showed that actinomycin D (or: C_1) inhibits the process of synthesis of A.R.N.

(cell or viral) which depend directly on cell A.D.N. by the fact of the formation of a complex between the latter (in particular its desoxyguanosine part) and the chromophore group of the antibiotic. The viruses with A.R.N. whose multiplication is not inhibited by actinomycine D, not dependent on cell A.D.N. for their synthesis: the viruses with A.D.N. on the other hand, depend on cell A.R.N. whose synthesis is conditioned by the A.D.N. of the cell.

About interferon: Rotem and collaborators (Nature 197,564,1963) showed the inhibiting action on the viral multiplication in certain types of nucleic acids, extracts from heterologue cells (e.g. of A.R.N. of embryonic chicken cells on the multiplication of vaccinia virus on embryonic cells of mice and vice versa). The antiviral action of heterologue nucleic acids is probably due to the formation of interferon by the cells thus treated, which leads us to think that the production of interferon is the general result of a reaction of the cells against the heterologue nucleic acids.

Michael and Naseman (3rd International Congress of Chemotherapy, Stuttgart, July 1963) described the antiviral activity exercised in cell cultures and in animals on diverse virus with A.R.N. (influenza, Mengo) and with A.D.N. (vaccinia, cowpox, herpes) of certain diamidinodiphenylsulfones in the clinical tests yielding encouraging results.

Carver and Naficy described the interesting inhibiting effect exercised on the multiplication of group A arbovirus by a protein made from a Corynebacterium (Proc. Soc. Exp. Biol. Med., N.Y., 111, 356, 1962). Li and collaborators described the presence of macromolecular antiviral substances, active in cell cultures and in the animal (poliovirus and influenza) in oyster extracts (Trans. N. Y. Acad. Sc. 24, 504, 1962).

From all evidence, the area of antiviral chemotherapy is actually in full expansion in many directions, and this article will soon be obsolete.