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- Cover Page -

urther biochemical and electron-microscope studies

of virus infected mammalian cells

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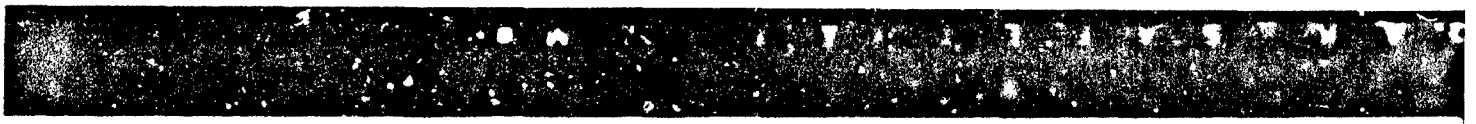

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To: European Research Office (9851 DU)
US Department of the Army
Frankfurt/Main, Germany
APO 757, US Forces

FINAL TECHNICAL REPORT

Subject:

Further biochemical and electron-microscope studies of virus infected mammalian cells

Contract number: DA-91-591-EUC-1417 01-4408-60

Covering the period March 1st 1960 thru February 28 1961

"The research reported in this document has been made possible through the support and sponsorship of the U.S. Department of Army through its European Research Office".

TABLE

SUMMARY OF THE REPORT

A number of tissue-cultured mammalian cell systems were infected with a variety of viruses to which they were susceptible.

A) A comparison was made between the cell metabolism, as judged from oxygen consumption, of normal control cells and of virus infected cells. The oxygen consumption was found in all instances to be greatly increased in cells affected with an acute virus infection, and this increase was in direct ratio to the virulence of the strain used. In chronically infected cells, no significant differences with control cell were found when the virus release was minimal or absent; in the case of sub-acute infections, where the virus production was gradually developping and enabled the cells to survive for a long time, there was only a slight increase in oxygen consumption.

B) Electron microscope studies could show no direct correlation between the cell ultrastructure lesions and the metabolic variations. In chronically infected cell-systems it was not possible to find any typical virus structures. It seems probable that in such infected cells, where no virus is produced, the infecting virus is blocked at the eclipse phase.

C) When chronically infected cells were reinfected with a virulent strain of the same virus or with another virus to which these cells were susceptible, the virus yield of the acutely infecting virus was greatly decreased or negligible. In some cases a complete protection of the cell occured when using as reinfectant a virulent strain of the same virus-type

as the one responsible for the chronic infection. Biochemical extraction of the active substance yielded a substance of proteic nature the properties of which are those of interferon.

D) This interferon was capable when introduced in a control non infected cell-system to induce a resistance to virus infection similar to the one observed in chronically infected cells. It was not possible with interferon produced on a given cultured mammalian cell-system to transfer resistance to cells from another zoological species. Whereas the viral specificity of interferon seems to be rather slack or even inexistant, the cellular specificity is high.

Implications of these results are discussed.

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FINAL GENERAL REPORT

The results obtained after one year of research and experimentation carried under the present contract can be condensed and finally reported as follow:

Object of the contract: The object of the present contract, which in fact was a continuation from a preceeding contract, was to further investigate with biochemical, histochemical and electron microscope studies, the behaviour of mammalian cells infected with different types of viruses.

The tissue culturel virus infected cells system was selected as the simplest which could yield results with the proposed techniques within the available period of time. The cells which were placed under

study were on the one hand either primary explant cells such as kidney cells (monkey, calf or pig kidney cells, hamster or mouse kidney cells) an occasionally human amnion cells or on the other hand pathologic cells maintained in continuous growth by the cell line culture system: these were either KB or HEP strains of cell lines.

The viruses which were studied under the present contract and used for infecting cells were:

a) Myxoviruses and specially the EA 102 strain of Myxovirus parainfluenzae type 3 as well as the other Myxovirus strains and in particular the IF strain of the Sendai virus.

b) Enteroviruses, namely human polioviruses type 1, 2, 3 and Echo viruses type 9 and 11.

c) Foot-and-mouth disease virus and in particular the Vallée O strain.

d) Rabies virus: in particular the Louis Pasteur strain of fixed virus, the CVS virus strains obtained from Dr Karl Habel and different street virus strains including the Farcha strain;

e) Polyoma virus as obtained from the laboratory of Dr Karl Habel, N.I.H., Bethesda, Md.

All the cells were grown either in a completely synthetic medium (Morgan, Morton and Parker 199 medium) or in a semi-synthetic (Pasteur Institute casein hydrolysate) medium. These media were supplemented with various amount of mammalian serum, either calf or cob serum, added in the necessary amount to provide growth for the cells, i.e. from 0,5 to 3 per 100 vol. in primary explanted cells and from 10 to 16 per 100 vol. in cell lines.

Metabolic studies were carried with the Warburg apparatus and with the usual standard biological and cytochemical methods.

Electron microscope studies were carried according to the detailed technique given in the Final Report of contract No. DA-91-591-EUC-1069 (1-1285-59. The electron microscope used was either a Siemens Elmiskop, or for higher resolution the new OPL 100 KV electron microscope. When extraction of viruses were carried, there were made on DEAE cellulose columns for adsorption of the virus, elution being made at constant pH with phosphate buffer followed by a gradient of NaCl starting at 0,5 and ending at 2% concentration.

The results finally obtained are:

1) Metabolic studies: A comparison was made of the oxygen consumption of normal, acutely infected and chronically infected cells. The cell systems used were either KB cells infected with Myxovirus or hamster kidney cells infected with polyoma virus, or hamster kidney cells infected with rabies or monkey kidney cells infected with various strains of poliovirus.

In all instances we have found that the glucose consumption of acutely infected cells in aerobic phase is greatly increased in the case of virus infected cells as compared to the consumption of glucose in the normal control cells.

On the other hand the oxygen consumption of chronically infected cells whatever the cell/virus system considered, is of the order of the oxygen consumption of normal control cells. In a few instances where the virus production of sub-acutely infected cells was progressively low

pace which was compatible with delayed destruction and a long survival of cells such as is the case of hamster kidney cells infected with rabies virus, the glucose consumption was found to be lower degree than in the case of acutely infected cells, but it was still at a greater intensity level than in the truly chronically infected cells where the growth of the virus does not definitively impair the survival of the cell.

When an identical cell/virus system was used throughout the experiment but when strains of different degrees of virulence were substituted for the more or less attenuated strains, as for instance with different myxoviruses strains on KB cells or various polio virus strains of the same type (i.e. Mahoney against L sc or IP 1342) on monkey kidney cells, the rate of oxygen consumption increased in direct ratio to the virulence of the strain and was also indirectly related to the final yield of virus. Thus the rate of oxygen consumption is directly related to the cell metabolism which is increased in the proportion in which the cell is actively producing virus: in this respect the difference of oxygen consumption between normal and infected cells may be taken as an indirect measure of the quantity of virus produced and of the rate at which it is produced.

When for a given virus strain a separation was made between the highly infectious and more attenuated components using DEAE columns, the two resulting substrains induced in infected cells a variation in oxygen consumption which was in direct proportion to the degree of virulence of the substrain components. Thus the stimulating action of virus infection over the cell metabolism is not a characteristic of a given virus nor of a given strain itself but it reflects the degree of virulence of the virus com-

ponents present in the strain.

2) Electron microscope studies: An attempt was made at trying to establish a correlation between the cell ultrastructur lesions and the degrees of virulence of a given strain and/or the variation of oxygen consumption induced by the strain into the cell.

Apart from the obvious intensity of cell destruction which is of evidence linked to the virulence of the strain, and without consideration of the time factor related to strain virulence, it has not been possible to show any specific difference in the cell ultrastructure alterations as found under the electron microscope according to the degree of virulence of the strain. In the chronically infected cell/virus system where a state of equilibrium is reached between virus multiplication and cell survival, it was almost completely impossible to trace any detectable change in the cell structure. Nor were any virus tyrical structures present in the infected cells.

It thus appears that metabolic changes may take place in the cell before any morphological change can be accounted for and detected in the cell system under study. It is also probable that the inability to find typical viral particles in such infected cells reflects an extension in time of the eclipse phase of the viral infection.

3) Immunological studies: When investigating the metabolic system of chronically infected cells we were lead to study the ability to produce virus when such cells are reinfected with either a virulent strain of the same virus or another virus whether virulent or attenuated (see preceeding report). New examples of such double-infections were tried. KB cells were infected with Myxovirus parainfluenzae type 1 and la-

ter with poliovirus type 2; monkey kidney cells were infected with simian parainfluenzae myxovirus and reinfected with poliovirus strains attenuated or virulent. Calf kidney cells were infected with Sendai virus and secondarily reinfected with foot-and-mouth disease virus. Finally, hamster kidney cells were infected with polyoma virus and reinfected at a later stage with rabies virus in fixed or street virus strains.

In these experiments a comparison was made between the virus yield and the rate of cell destruction of the infected cells and of control cell infected with the second virus only. In all these instances a striking difference was found in the virus yield of control cells and the speed of destruction of these cells as compared with the amount of virus produced and the rate of cell destruction in cells chronically infected with a mild virus prior to infection with a highly virulent virus. There was a constant difference of at least 2 to 3 logs of virus produced when comparing the acutely infected control cells and the double-infected cells.

In some instances susceptible chronically infected cells were protected against a virulent strain of a different virus by their previous infection up to the point of producing only negligable amounts of the virulent virus. In such cases the amount of virus found in the supernate was of the order of 10^1 to 10^2 particles of virus or less, whereas in the control cells the supernate yielded from $10^{6.8}$ to $10^{8.1}$ p.f.u. per ml.

It was possible to show that this resistance of cells to virus infection could be attributed to the presence of interferon in the chronically infected cells. Following the method previously described, it was possible to extract this interferon substance, and by introducing it in a con-

trol cell system of the same type of cells, to induce in non-chronically infected cells a degree of resistance strictly comparable, and in some cases superior, to the degree of resistance acquired by chronically infected cells.

Thus it is demonstrable that the resistance of virus immune cells is connected with the production and the presence in these cells of interferon which can be extracted, and from which it is possible to transfer the cell immunity of an infected cell system to another cell system of the same cell-tissue.

It was possible, using monkey kidney cells to transfer the poliovirus resistance acquired through a chronically infected system to other monkey kidney cells. These cells became not only resistant to poliovirus infection but also to Echo virus and Sendai virus infection with strains normally grown on a monkey kidney cell system.

On the other hand, when interferon extracted from monkey kidney cells was introduced in a calf kidney cell system, these cells were not protected against Myxovirus Sendai or foot-and-mouth disease virus infection.

Results with rabies virus were less clearcut because of the long duration of cell survival of rabies infected cells: but the rabies virus yield of hamster kidney cells protected with hamster kidney myxovirus induced interferon was definitely lower ($10^{-1,5}$ against $10^{3,2}$) than in non-protected cells.

IMPLICATIONS OF RESULTS AND DISCUSSION

In short, the experiments summarized above have shown the following points:

1) Virus multiplication in infected cells results in an increase of oxygen consumption which is in direct ratio to the virulence of the infecting virus strain.

2) In chronically infected cells where little or no virus is produced, no morphological changes can be traced with the electron microscope and the virus seems to be blocked at the eclipse phase.

3) Chronically infected cells or cells infected with strains of a low virulence yielding only small quantity of the infecting virus are to a certain degree partially protected or completely protected against reinfection with either virulent strains of the same virus or with other viruses.

4) This protection is due to the production and presence of an interferon which can be extracted as a relatively pure preparation. Interferon can be used to transfer immunological resistance to other cells of the same tissular type and of the same zoological species, but not to cells of the same tissular type belonging to a different zoological species.

5) The use of DEAE columns enables to separate by the method of NaCl gradients in phosphate buffer a number of virus fractions where the virulent components of the strain are separated from the non virulent or attenuated ones. The mutants thus obtained are stable. It is possible, using the attenuated components of the virulent strain, to induce resistance against the complete strain or against its virulent component into susceptible cell-systems.

From a practical standpoint, the methods of production and extraction of interferon should be improved before we can fully appreciate the spectrum of activity of interferon against viruses. There seem to be little viral specificity in the mode of action of interferon whereas the cellular specificity is obviously great.

It can be provisionnally concluded from these experiments that in the case of the human species there lies a more immediate prospect in the possibility of promoting resistance to virus infection through interferon formation induced by the use of attenuated or non virulent virus strains than with using an animal produced interferon to transfer immunity to man. In animals the problem is much simplified by the possibility of using an homotypic interferon as well as by the acceptance of a calculated risk of minor viral infections through the use of attenuated virus strains.

SUMMARY OF PERSONNEL UTILIZED DURING THE REPORTING PERIOD

The experiments described were carried by the contractant with the aid of Madame Christiane Bonissol, licenciée ès-sciences and of Dr Giovanna Cedlin, M.D., assistant; two technicians Mademoiselle Annick Poullain et Mademoiselle F. Fournier were paid on the contract. Technical assistance was given in biochemistry by Monsieur Louis Delsal, biochemist, chef of laboratory and in the electron microscope field by Mlle. C. Croissant, assistant physicist, which were not paid on the contract. A technician photograph, Monsieur Olivier Fay was used and paid on the contract during part of the period covered. All worked on a full time basis.

Secretarial and other administrative work required 2/12 working time give . by Mademoiselle D. Morice, secretary.

No property was acquired during the contract period at the contract direct or indirect expense.

Reactives, chemicals, photographic plates and other consumable items needed in investigation were bought at direct contract expenses. Monkeys bought in Africa and transported by air freight were used throughout for the preparation of cell systems and for the titration of the different viruses used in the experiments.

Before concluding the contractor wishes to congratulate all the workers on the contract, and those who have participated with technical or partial help to the contractants. All of them have shown an outstanding team spirit without which the results obtained could not have been achieved in such a short time.

The results so far obtained bear such implications that it is to be expected that further developments will come, which could not have been gained without the help given by the present contract.

It is the agreeable duty of the contractor to gratefully acknowledge such help given by the European Office of the US Army.

The present report terminates the contract N° DA-91-591-EUC-1417 01-4448-60 and puts an end to a three years period of fruitful work carried with the assistance of the European Office of the U.S. Army. The continuation of the work thus initiated will require further developments which can be deduced from the present results.

Although the contractor is fully aware that it is not in

the intentions of the European Office of the U.S. Army to continue its support, he wishes as a final conclusion to express the hope that the work still in progress will not be interrupted and that it will continue along on the impulse which the U.S. Army contract has provided.

Paris, 22 March 1961

The Contractor:

Dr P. Lépine