PRESIDENTIAL ADDRESS

Some Recent Advances in Experimental Chemotherapy

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In 1928 the writer contributed a chapter on "Precipitins and their Applications" to a book entitled The Newer Knowledge of Bacteriology and Immunology, edited by Jordan and Falk of the University of Chicago. This chapter was concerned with what is now an almost forgotten area in the one-time classical field of immunology, a subject founded by the great works of Ehrlich, Bordet and others. The last chapter in this volume of 1928 happens to be a long one on "Chemotherapy of bacterial diseases" by John A. Kolmer. Perusal of this last chapter by anyone interested will reveal that about all we had at that time in the way of disease-curing agents comprised (a) quinine for malaria, and (b) arsenicals, etc., for syphilis, and practically nothing for bacterial diseases. This situation continued for another eight or ten years following 1928, then came the sulfonamides. We are citing these old items not on account of their importance but as a background of old works rendered obsolete by new knowledge.

The present status of successful everyday chemotherapy of many of the severe infectious diseases of human beings, including use of the sulfonamides and antibiotics, attests a tremendous change which has come about in a very few years. This has been a spectacular period indeed in the conquest of human disease. There has been nothing quite like it in the previous history of human infectious disease.

However in spite of this it may be well to take a conservative look at what has come about in practical and useful chemotherapy. First, it is noticed that the wonder drugs lose a little luster as time goes on. Sometimes microorganisms get used to these drugs and on rare occasions a few bacteria have become so acclimated to a wonder drug that they need the new drug as part of their diet. This is disconcerting to say the least. Sometimes the wonder drugs cause some undesirable side reactions.

The particular area of chemotherapy upon which we wish to focus attention in this report however is not the above limitations but a segment of infectious disease against which no drug as yet has had any effect. This includes the infections caused by the so-called small viruses. This implies that some of the large viruses have been overcome by chemotherapy and this is indeed true. Other diseases, possibly of infectious nature, which have not at all been overcome by new drugs are the tumors, however we shall not be concerned here with the tremendous subject of tumor study.

The small viruses, including some types of encephalitis, poliomyelitis, and also influenza and others, comprise a hard subject to deal with. Sometimes the infected host becomes so riddled with virus, even in a matter of hours, that what ordinarily would be a good drug with leisure action would have no chance in such rapidly progressing infections. We have

been nevertheless attracted to this study mainly on account of the virgin nature of the field and the recent availability in our laboratories of large numbers of possible antibiotics made from fungi collected from soil samples from many areas over the world. A good many thousands of these materials have been subjected to so-called antiviral screening tests in which they are tried out, mainly in infected white mice in the laboratory. Out of this great number has come several antibiotics, one of which is of experimental interest in this field and some facts about this one may be presented.

In August, 1952, we published a short report on this subject in the journal, Antibiotics and Chemotherapy (1). In this report a Penicillium mold extract or filtrate designated serially as 8450 was shown to have preventive action in mice against 100 to 1,000 fatal doses of either MM or Semliki Forest virus and curative action against ten fatal doses or less of virus. These viruses are truly small viruses. Best prevention was exhibited by mice to which antibiotic 8450 had been given about twenty-four hours before infection with virus. Cures were obtained in a small way for a short time post-infection. These results appeared of interest since antibiotic and virus could be given by different routes of injection with successful results, and the action appeared to be truly in vivo, although the 8450 apparently did not act directly on the virus. Previously we had observed no similar success with many previous mold filtrates, drugs, etc. and previous publications appeared discouraging.

In the report of last year we mentioned that although multiple dose treatment of mice with 8450 was first used and found effective, the duration of treatment might be shortened to a single dose of filtrate. This single dose of filtrate was found to have a considerable carry-over effect in terms of prevention, and its effect could be observed for as long as three days. Moreover, mice which were treated with a series of doses of antibiotic 8450, then given a rest, were found again to be protectable by a new dose of 8450. Also, we noted practically no active immunity in mice once protected from MM virus with 8450 and then rechallenged with the same virus after a period of resting. We obtained no prophylactic results against influenza A and B virus, fixed rabies virus, poliomyelitis virus when given intracerebrally, meningopneumonitis virus, lymphocytic choriomeningitis virus, lymphogranuloma virus, and typhus rickettsiae.

Attention in the first report was also called to two particular limitations, namely in antiviral tests in mice infected with MM or Semliki Forest virus, although we observed successful results from several combinations of different routes of injection of antibiotic 8450 and virus, we got practically no positive results in tests in which (A) 8450 was given orally, or (B) virus was given intracerebrally. The 8450 on the one hand was either destroyed or not absorbed from the gastrointestinal tract, and on the other hand intracerebrally injected virus proved too much for the agent to overcome. These particular limitations may or may not be related to the fact that antibiotic 8450 is still in a crude form and activity is confined to a substance of large particle size incapable of dialysis and possibly incapable in the crude form of rapid transport extravascularly through the tissues.

In presenting further observations on the action of 8450 against viruses we shall utilize tests against MM virus in mice and shall not refer to Semliki Forest virus infection since the activity of 8450 against these two viruses is very similar. Also, we would like to present some results of the action of 8450 in mice infected peripherally with type 2 poliomyelitis virus.

It may be mentioned at this point that work on purification of 8450 is not sufficiently advanced to merit a report at this writing. The mold which produces the agent called 8450 is identified as *Penicillium stolonif-erum,* and the preparation which we have used for the most part has been a dried filtrate with very little purification. This is rehydrated before use to original volume.

The maximum extent of effective chemoprophylaxis of 8450 against MM virus mice which we have attained is indicated as follows: Different groups of ten mice were treated chemoprophylactically by the intraperi-toneal route with a constant dose of 0.5 ml of 8450, and twenty-four hours later these mice along with controls were challenged with different decimal dilutions of MM virus administered subcutaneously. Protection against more than 1000 MLD of virus is obtained in this way.

An alternative method of assaying the titer of 8450 is illustrated as follows: In this experiment a constant dose of challenge MM virus is used, namely 10^{-6} ml amounting to about 100 LD_{50}, and diminishing doses of 8450 employed. It may be observed that dilutions of 8450 of 1:8 to 1:16 appear to span a 50 percent effective chemoprophylactic dose, and the untreated virulence control mice indicate an LD_{50} of test virus of about 10^{-8} ml. This scheme of testing is about as satisfactory as we have used for comparing titers of different batches of 8450. Since 8450 appears to have no antibacterial or antiphage spectrum, the titer of any given batch has to await the outcome of a ten day mouse test employing MM virus as just described.

Although we have administered 8450 mainly by the intraperitoneal route and MM virus mainly by the subcutaneous route, other combinations of routes of injections are of interest. Antibiotic 8450 may be injected chemoprophylactically by the intraperitoneal route, and MM virus in decimal dilutions may be given by either of two alternate routes, namely intranasally and intramuscularly. Corresponding virulence control mice are used. A considerable measure of chemoprophylactic activity of 8450 is indicated against virus given in these ways, namely intranasally or intramuscularly, just as it may be shown that virus may be overcome when it is injected under the skin.

It is premature to present experimental results dealing with the nature of 8450. It may be repeated however that it appears in its present form to be of large particle size, and it does not dialyze. Moreover it is moderately thermolabile. Comparative tests of native 8450, along with material immersed in a small test tube in boiling water for fifteen minutes, and also material which had been autoclaved at fifteen pounds pressure for one hour indicates a trace of activity in the boiled sample, however the autoclaved sample has lost about all of its activity.

It appeared of interest at this point, since most experiments hereto-

fore had dealt with treatment given ahead of infection to give some results of post infection chemotherapy. Three groups of sixty mice were injected subcutaneously with MM virus in a dilution of 10^{-7}. One group was given 8450 intraperitoneally three hours and twenty-four hours afterwards and another group was given the agent at three hours after infection only. Corresponding control mice are used and these include an additional group of mice given a 10^{-8} dilution of virus. This experiment on completion shows a definite measure of chemotherapeutic effectiveness against a dose of virus which kills most but not all of the control mice. A single dose of 8450 given three hours after infection is as good as two doses given at three hours and twenty-four hours after infection.

Further assays of chemotherapeutic effectiveness have been done from time to time in which treatment follows infection by a longer time and results of such tests show that a moderate degree of chemotherapeutic effectiveness is obtained when 8450 is given four hours after virus, and this is also evident at six and eight hours after infection, although demonstrable activity decreases at these times.

In the first brief report on 8450 of a year ago, we stated that mice once protected by 8450 showed no substantial immunity to a rechallenge dose of MM virus. It appears that mice treated before infection show less subsequent active immunity than those treated after infection, also the size of the infecting dose of virus against which protection is obtained may be related to presence or absence of subsequent active immunity.

In the past few months we have gotten some positive results in tests of chemoprophylaxis with 8450 against type 2 poliomyelitis virus injected peripherally into mice. Previous unsuccessful results mentioned in the initial report of last year were based on experiments in which Lansing virus was given intracerebrally to mice. In a recent short report (2) on poliomyelitis mouse work we have used a special line of MEFl virus repeatedly passaged in very young hamsters. This virus has definite peripheral virulence for mice and intraperitoneal, intramuscular, or intravenous routes of injection of virus result in fatal infections. With successive passages of this MEFl strain of virus in seven day old hamsters, peripheral virulence has increased greatly. Mice of 12 grams in weight suffice best for these tests and it appears such experiments provide a basis for in vivo chemotherapy tests against type 2 poliomyelitis virus administered peripherally in small animals before resorting to monkeys. The combined results of several chemoprophylactic poliomyelitis tests of antibiotic 8450 in these mice show that the strongest effects, i.e. against about 10 MLD of virus, are produced by treatment of mice intraperitoneally with 8450 followed by MEFl virus injection intraperitoneally twenty-four hours later. If repeated intraperitoneal doses of 8450 are used, injection of 8450 is skipped on the day of intraperitoneal virus injection. Chemoprophylactic effects are also produced by injection of 8450 intravenously twenty-four hours ahead of intraperitoneal injection of virus. Other combinations of routes of treatment and virus injection show decreasing degrees of positive results. Oral treatment with 8450 did not appear effective chemoprophylactically, nor did intravenous injection of MEFl virus prove very susceptible of chemoprophylaxis.

Some poliomyelitis mouse experiments have been done in which mice once protected against MEFl virus infection by 8450 were kept for some time and were then rechallenged with virus along with new controls. Sometimes no active immunity appears to have been acquired. These results are similar to some of those obtained in mice once protected from MM virus by 8450 and then rechallenged subsequently. In both cases protection by 8450 against the first exposure to virus seems to ensue before the virus has had time to initiate an immunizing action. We have observed in mice suitably protected by 8450 that the animals soon rid themselves of the initial dose of virus. Lack of active immunity in mice once protected with 8450 is not always the case. In some instances mice once protected against MEFl virus appear after a longer rest to have acquired a little active immunity.

At this writing two of our associates, Hull and Lavelle, have reported the action of 8450 on all three types of poliomyelitis virus in tissue culture (3). Suitable pre-infection treatment of roller tube monkey testicular cultures renders these cells refractory to the usual cytopathogenic action of poliomyelitis virus.

Subsequent to our first report on this antibiotic in August, 1952, Shope in May of 1953 has published three papers (4) dealing with a *Penicillium funiculosum* agent which seems to be quite similar in activity to 8450.

Finally, more time is needed for more work on 8450 in several directions of considerable interest. Since theoretical considerations of mechanism of action do not help very much, we have refrained from an exposition of this matter.

SUMMARY

Additional results have been presented concerning extent of chemoprophylaxis obtained against MM virus infections in mice with a filtrate from *Penicillium stoloniferum* known as 8450. This action amounts to 2 or 3 logs of protection, and filtrate and virus are injected by different routes.

Chemotherapy up to about eight hours after MM virus infection is effective provided infecting dose of virus is near the 1 MLD level.

This filtrate, having a serial number of 8450, accomplishes chemoprophylaxis and chemotherapy in vivo when given to mice by routes of injection separate from virus injection.

A limited degree of chemoprophylaxis with 8450 has been obtained in mice infected peripherally with type 2 poliomyelitis virus.

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