AN ANTIVIRAL SUBSTANCE FROM PENICILLIUM FUNICULOSUM

VII. AN Attempt to Determine Whether the Material Responsible for the Antipassive Immunity Effect Exhibited by Mice Injected with Helenine is an Interferon

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(Received for publication 18 July 1966)

In the preceding paper of this series (1) some evidence was obtained that the antipassive immunity (API) effect shown by mice injected with helenine before receiving anti-Semliki Forest virus serum of swine origin was, like the antiviral effect of helenine, the result of an interferon induced in mice by helenine. Other evidence presented in the same paper indicated that the antipassive immunity and the antiviral (AV) effect of helenine might not result from the same basic mechanism and that the API effect might have a basis other than interferon induction for its activity. The point seemed of sufficient importance to warrant further study in greater depth since, if the API effect did indeed have interferon as a basis, this represented a new and totally unsuspected action of interferon. To do this, helenine was compared with two other known interferon inducers, Newcastle disease virus (NDV) and statolon, for similarity of activities both as to their AV as well as their API effects. The results obtained will form the basis for this paper.

Materials and Methods of Test

The materials and methods of test have been extensively presented and discussed in preceding papers (1, 2) and hence will not be given in detail here except where new procedures or materials are introduced into the experimental design.

NDV.—The Hickman strain of NDV, kindly supplied by Dr. Michael W. Rytel, was used throughout. It was allantoic fluid of infected hens' eggs, had a hemagglutination titer of 256, and contained $1 \times 10^9$ plaque-forming units per 0.2 cc. It was ordinarily administered to mice intraperitoneally in 0.5 cc amounts of fluid diluted 1 to 5 with physiological saline just before use.

Statolon.—A generous supply of the antiviral substance statolon was furnished by Eli Lilly & Co., Indianapolis. It was dissolved in saline just before use to make a 0.5% solution. 0.5 cc amounts of this solution were administered intraperitoneally to the test mice.

Time of Administration.—The helenine, NDV, and statolon used in these experiments were ordinarily given intraperitoneally 12 hr before infection with Semliki Forest virus (SFV) in tests for antiviral activity. They were given by the same route, in the API tests, usually 2 hr before the administration of anti-SFV swine serum. These mice were tested for the acquisition of passive immunity to SFV 18 days after receiving the antiserum.

* Dr. Shope died October 2, 1966.
RESULTS

The findings with regard to the AV effects of the three materials under comparative test will be presented first. As shown by the results recorded in Table I, all three exerted a strong AV effect and 90% or more of the animals, regardless of whether treated with NDV, statolon, or helenine, survived a dose of virus that killed all of 56 control mice. Since Baron and Buckler first showed the interferon-inducing capacity of NOV (3), it has become the standard method for interferon induction in mice. There can, therefore, be no doubt that NDV exerted its life-saving effect through this mechanism. Also helenine (4), and earlier, statolon (5) had both been demonstrated to induce an interferon and good evidence indicates that both exert their AV effects through the medium of the interferon that they induce. It was therefore apparent that all three of the materials under test exhibited the AV properties that they are shown to possess by the data given in Table I through the induction of interferon in the mice to which they were administered.

The results gotten in comparing helenine, NOV, and statolon for their API effects were similar in that all three substances lowered the survival achieved when they were administered shortly before 0.5 cc of anti-Semliki Forest virus serum of swine origin. As shown in Table II, 90% of the animals that received antiserum alone were fully resistant to fatal infection from Semliki Forest virus 18 days later. In contrast to this, only 25% of those receiving statolon 2 hr before antiserum were resistant at 18 days, and only 23% of those that received helenine 2 hr before antiserum survived the later challenge infection. NDV given intravenously proved much more effective

### TABLE I

**Prophylactic Effectiveness of Helenine, Newcastle Disease Virus (NDV), and Statolon against Semliki Forest Virus Infection in Mice**

<table>
<thead>
<tr>
<th>Time and route of administration of helenine, NDV, and statolon</th>
<th>Infection with Semliki Forest virus subcutaneously</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survivors</td>
</tr>
<tr>
<td>Helenine intraperitoneally 12 hr before infection</td>
<td>80/56*</td>
</tr>
<tr>
<td>Statolon intraperitoneally 12 hr before infection</td>
<td>35/35</td>
</tr>
<tr>
<td>NDV intravenously 9 hr before infection</td>
<td>37/41</td>
</tr>
<tr>
<td>Saline intraperitoneally 12 hr before infection</td>
<td>28/29</td>
</tr>
<tr>
<td>Controls, untreated</td>
<td>0/14</td>
</tr>
</tbody>
</table>

* Surviving mice/No. of mice in group.
than the same material given intraperitoneally, and here the percentage of survivors respectively were 0 and 43%. This difference probably reflects the superiority of the intravenous over the intraperitoneal route in achieving interferon induction with this virus. All three substances, however, proved qualitatively to exert a pronounced API effect even though there were quantitative variations in the degree to which they interfered with the establishment of passive immunity. The conclusions to be reached in consideration of the data given in Table II is that like the AV effect discussed earlier, all three substances exercised a similar inhibitory action on the establishment of passive immunity and as in the case of the AV effect, though the materials under test differed markedly one from the other in composition, the API effect probably resulted from the same mechanism as the AV effect. The fact that the results gotten in the two experiments were comparable, although in opposite directions, suggests strongly that the mechanisms involved in the two phenomena were identical even though in the one set of experiments (AV) the end result was the saving of lives of treated animals while in the other (API) the end result was quite the opposite.

**DISCUSSION**

The hypothesis of the type just proposed that two almost opposite end results can be yielded by the same mechanism is a difficult one to prove, especially since there is no prior example of interferon exerting an API effect. However the findings make it so self-evident that if three diverse substances induce the same material to account for their AV activity, the same three sub-

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### TABLE II

**Comparison of Helenine, Newcastle Disease Virus (NDV), and Statolon for Their Capacity to Prevent the Establishment of Passive Immunity in Mice by Anti-Semliki Forest Virus Serum of Swine Origin**

<table>
<thead>
<tr>
<th>Injected intraperitoneally with 0.5 cc anti-Semliki serum (swine)</th>
<th>Inoculation with Semliki Forest virus 18 days after antiviral serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survivors</td>
</tr>
<tr>
<td>9 hr after helenine intraperitoneally</td>
<td>1/14*</td>
</tr>
<tr>
<td>2 “ “ “ NDV intravenously</td>
<td>8/34</td>
</tr>
<tr>
<td>2 “ “ statolon “</td>
<td>0/10</td>
</tr>
<tr>
<td>2 “ “ intraperitoneally</td>
<td>15/35</td>
</tr>
<tr>
<td>No preliminary treatment</td>
<td>13/51</td>
</tr>
<tr>
<td>Controls untreated</td>
<td>25/28</td>
</tr>
<tr>
<td></td>
<td>0/35</td>
</tr>
</tbody>
</table>

* Surviving mice/No. of mice in group.
stances producing a similar uniform effect probably exert their API effect by
a mechanism common to the three. Such reasoning takes into account Common
Notion 1 of Euclid which states that “things which are equal to the same thing
are also equal to one another” (6). These common notions were supposed to
be self-evident truths incapable of scientific proof. That is, they fall into the
category of axioms which no less an authority than Aristotle emphasized were
self-evident truths which it is impossible to demonstrate. He goes on to say
that if anyone should attempt to prove them, it could only be through igno-
rance (7). It would seem that the evidence presented in this paper indicates
clearly the likelihood that statolon, NDV, and helenine exert their separate
but similar AV effects through the medium of interferon induction by each.
They thus, in the terms of Common Notion 1, possess in common the capacity
to induce interferon. Since all three materials exert their AV effect through a
common mechanism, it seems obvious that when another common effect (API)
is revealed, the most likely explanation of it is that it results from the same
mechanism, in line with the second portion of Euclid’s “Notion.” Following
this type of reasoning, the conclusion to be reached from the results presented
is that the API effect, like the AV effect, results from the same mechanism in
the cases of all three of the materials studied; namely their abilities to induce
interferon in mice to which they are appropriately administered. Both effects,
therefore, result from interferon in all likelihood.

The phenomenon of interference with the establishment of foreign protein
in a host is a property that has not heretofore been attributed to interferon.
It is possible that this new activity of interferon may play a very important
role in protecting the host against undesirable sensitization to the many foreign
proteins to which it is exposed. It is probably a normal physiological mechanism
acting at very low levels of concentration in the animal body. The experiments
reported in this paper were carried out with really massive foreign protein
insults (0.5 cc swine serum per mouse), and hence required the induction of
unusually high levels of interferon to demonstrate any effectiveness at all.

The findings of two experiments in the preceding paper of this series (1)
seemed to indicate a different mechanism for the AV and API effects of hele-
nine. It was shown that, while the AV effect could be achieved only when
helenine was given for a period of 36 hr before virus administration, the API
effect could be demonstrated from 4 days before to 2 days after the antiserum
was given. Also the AV effect could be exhausted by multiple preceding injec-
tions of helenine while the API effect could not similarly be abolished. It seems
likely in the light of the present experiments that the finding of these differ-
ences did not reflect a difference in the mediation of the AV and API effects
by interferon but instead indicated a quantitative difference in the sensitivity
of the host to induced interferon in expressing the AV and the API effects.
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SUMMARY

Helenine, NDV, and statolon, all known inducers of interferon in mice, all exerted a marked antiviral effect against Semliki Forest virus. This AV effect was, so far as can be demonstrated, mediated through the induced interferon.

The same three materials also exerted a marked antipassive immunity effect. All the evidence that can be brought to bear indicates that this API effect like the AV effect is mediated through interferon known to be induced by the three materials.

If the API effect does indeed have interferon as its basis, this represents a new and totally unsuspected action of interferon.

BIBLIOGRAPHY


