EFFECT OF INTENSE SONIC VIBRATIONS ON ELEMENTARY BODIES OF VACCINIA

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Interest in the effects of sonic energy on certain materials, including infectious agents, has increased during recent years. General reviews regarding the reaction of a number of substances to this type of energy have already been presented by Harvey (1) and by Chambers and Gaines (2). The fact that bacteria are broken up by supersonic vibrations induced us to ascertain whether elementary bodies of vaccinia are disrupted when subjected to sonic energy. We were particularly interested in the matter because disruption of the bodies would facilitate immunological studies in the virus field. The purpose of the present paper is to record the reaction of several kinds of vaccine virus preparations to intense sonic vibrations with a frequency of about 8900 cycles per second.

Methods and Materials

Sonic Energy.—A modified Peirce magnetostriction oscillator with a frequency of about 8900 cycles per second previously described and used by Chambers and Flosdorf (3) supplied the energy. The apparatus has an adequate cooling system and at no time during the experiments did the temperature of the virus preparations rise above 20°C.

Preparations of Vaccine Virus.—Elementary bodies of vaccinia were prepared according to the technic of Craigie (4) from the skin of rabbits infected with the C.L. strain of virus. Bodies obtained in this way are designated throughout the paper as prepared according to routine, whereas others, referred to as thoroughly washed, were subjected to further purification by means of additional washings. The latter preparations, although not absolutely pure, contained much less adventitious material than did those prepared in the routine manner.

Testicular vaccine virus was obtained from rabbits inoculated with the New
York City Board of Health strain. Both testes were inoculated and then removed from the animal 3 days later. After removal they were thoroughly ground; the finely divided tissue was suspended in 70 cc. of Locke's solution and centrifuged for half an hour at 2500 R.P.M. The supernatant fluid with its vaccine virus constituted the testicular virus used in our experiments.

0.25 cc. amounts of serial tenfold dilutions of the suspensions of elementary bodies and of the testicular virus emulsions were injected intradermally into rabbits in order to determine the degree of infectivity of the preparations. Each dilution was tested in two or more rabbits.

EXPERIMENTAL

Effect of Sonic Vibrations on Thoroughly Washed Elementary Bodies

Information regarding the effect that intense sonic vibrations have on thoroughly washed elementary bodies of vaccinia was obtained in the manner described in the following experiment.

Experiment 1.—Dermal pulp was obtained from 4 rabbits with 3 day vaccinal lesions. After preliminary dilution, which amounted to 30 cc. for the pulp obtained from each animal, the material was centrifuged for 2 periods of 5 minutes each in a horizontal centrifuge to remove coarse particles. The supernatant material placed in flat tubes having an inside diameter of 4 mm. and a capacity of about 4.5 cc. per tube was then spun in an angle centrifuge for an hour at 3500 R.P.M. The supernatant fluid from each tube was discarded and the sediment was resuspended in 4 cc. of a dilute citric acid-phosphate buffer solution, pH 7.2, and recentrifuged. Three such washings were carried out the first day. After resuspension following the last centrifugation in the angle machine the material was centrifuged in a horizontal machine for an hour at 2500 R.P.M. The resulting supernatant material was stored at +5°C. Once during each of the next 3 days, the elementary bodies were sedimented in the angle centrifuge, resuspended in dilute buffer solution, and then spun in a horizontal centrifuge for an hour. Finally, the elementary bodies were sedimented in the angle centrifuge and resuspended in 44 cc. of the buffer solution. Preparations of this material stained according to Morosow's technic (5) showed many discrete elementary bodies of uniform size between which little or no granular precipitate was obvious. Three portions of the material consisting of 12 cc. each were respectively exposed to sonic vibrations for 15, 45, and 90 minutes; the remaining 8 cc. were used as a control.

The following results, depicted in Chart 1, were obtained in the above experiment. The infectious titer of the untreated suspension was $10^{-8}$; the material vibrated 15 minutes titered $10^{-4}$; that treated for 45 minutes titered $10^{-8}$; while that subjected to vibration for 90
minutes was not infectious in a $10^{-2}$ dilution, lower dilutions were not tested. The suspensions subjected to sonic vibrations were definitely more opalescent than was the control. There was a slight tendency for the elementary bodies to clump in the vibrated materials, but this clumping could not have accounted for the drop in titers because the opalescence and clumping were no more marked in the material treated for 90 minutes than they were in those subjected to vibrations for 15 and 45 minutes, respectively. Densitometer (6) readings,

![](chart.png)

**Chart 1.** Graphic representation of inactivation of thoroughly washed elementary bodies by sonic vibration.

† The suspension of elementary bodies vibrated 90 minutes was inactive in a dilution of $10^{-2}$, lower dilutions were not tested.

stained preparations, and dark-field examinations gave no indications that any of the elementary bodies had become disintegrated as a result of subjection to sonic vibration; nevertheless, inactivation of the virus occurred.

**Effect of Sonic Vibrations on Elementary Bodies Prepared According to Routine and on Testicular Vaccine Virus**

Having found that thoroughly washed elementary bodies were inactivated without being broken up by sonic vibrations we decided
to test this kind of energy on bodies prepared according to routine and on testicular vaccine virus.

Experiment 2.—Dermal vaccine pulp was secured from 4 rabbits, and, after the preliminary dilution with 120 cc. of buffer solution, the coarse particles were removed from the suspension by means of centrifugation in a horizontal centrifuge. The elementary bodies were then washed 3 times in an angle centrifuge after which large particles or large clumps of elementary bodies were removed by centrifugation in a horizontal centrifuge for an hour. Four lots of 18 cc. each were treated in the following manner: one was vibrated for 2 minutes, another for 15 minutes, still another for 45 minutes, while the control was placed in the vibrating machine for 90 minutes without the oscillating current being active. The infectious titers of the 4 portions were the same, viz., $10^{-7}$.

A suspension of testicular vaccine virus as described above was divided into 4 portions, each consisting of 15 cc. The control portion was kept in the vibrating machine for 90 minutes without the current being turned on, while the other portions were vibrated for 15, 45, and 90 minutes, respectively. The titers, $10^{-5}$, of the different portions were the same.

The results of the above experiment indicate that the elementary bodies prepared according to routine and vaccine virus in testicular emulsions were not inactivated by the sonic vibrations used. The vibrated materials became somewhat more opalescent than the controls; there was a slight tendency for the elementary bodies to clump but the clumping was not sufficient to influence the titers; an alteration in the hemoglobin resulted in a change in the color of the testicular emulsion.

Protective Action of Protein on Elementary Bodies

The decided differences in the action of sonic vibration on the 3 preparations of vaccine virus used in the preceding experiments led us to consider the possibility that such amounts of protein, as are present in suspensions of elementary bodies prepared according to routine or in testicular virus emulsions, might prevent the inactivation of vaccine virus by the type of energy used. The idea was tested in the following manner.

Experiment 3.—Elementary bodies were prepared in the routine manner from dermal pulp of 5 rabbits. The bodies were further washed in the angle centrifuge on 3 successive days but were centrifuged in the horizontal machine only after the third of the rewashings. The final preparation was diluted to 120 cc. Two
portions of 55 cc. each were removed; to one, 5 cc. of dilute buffer solution were added; to the other, 5 cc. of normal rabbit serum. Each of the 2 suspensions was divided into 4 equal parts; one part from each suspension was kept as a control, while one part from each suspension was vibrated 15, 45, and 90 minutes, respectively.

The results of titrations of the controls and the vibrated elementary bodies are shown in Table I and indicate that the suspensions of thoroughly washed elementary bodies without added serum were inactivated approximately to the same extent as were those in Experiment 1, whereas the titers of the suspensions containing serum were not significantly altered. Furthermore, no evidence was obtained that the elementary bodies in the inactivated material had been broken up.

**TABLE I**

<table>
<thead>
<tr>
<th>Type of suspension</th>
<th>Time of vibration</th>
<th>Infective titer of elementary body suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elementary bodies suspended in buffer solution</td>
<td>No vibration</td>
<td>10^{-2} +++ 10^{-3} +++ 10^{-4} +++ 10^{-5} +++</td>
</tr>
<tr>
<td></td>
<td>Vibrated 15 min.</td>
<td>+++ + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td></td>
<td>Vibrated 45 min.</td>
<td>+++ + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td></td>
<td>Vibrated 90 min.</td>
<td>+ - - - - - - - - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td>Elementary bodies suspended in buffer solution</td>
<td>No vibration</td>
<td>10^{-2} +++ 10^{-3} +++ 10^{-4} +++ 10^{-5} +++</td>
</tr>
<tr>
<td>plus normal rabbit serum</td>
<td>Vibrated 15 min.</td>
<td>+++ + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td></td>
<td>Vibrated 45 min.</td>
<td>+++ + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td></td>
<td>Vibrated 90 min.</td>
<td>+++ + + + + + + + + + + + + + + + + + +</td>
</tr>
</tbody>
</table>

± = papule approximately 3 x 3 x 1 mm.; + = papule approximately 6 x 6 x 1 mm.; ++ = papule approximately 10 x 10 x 1.5 mm.; +++ = 15 x 15 x 2 mm. with neighboring edema; ++++ = papule approximately 20 x 20 x 2 mm. with central hemorrhage and surrounding edema.

**Effect of Hydrogen Peroxide on Elementary Bodies in Preparations Containing Different Amounts of Protein**

Results of work of Flosdorf, Chambers, and Malisoff (7) with an apparatus of the type used in our experiments indicate that the sub-
Injection of pure water to sonic vibrations at atmospheric pressure for 45 minutes results in the production of approximately 400 micro-equivalents of hydrogen peroxide per liter. Inasmuch as the inactivation of the elementary bodies in our experiments was not due to their disruption by sonic vibrations it seemed of interest to determine whether small amounts of hydrogen peroxide would accomplish it.

**Experiment 4.**—The control materials used in the preceding experiment were employed. 0.5 cc. amounts of the suspension of thoroughly washed elementary bodies without added rabbit serum and of that to which rabbit serum had been added were respectively treated with equal volumes of (a) distilled water, (b) 1:150 dilution of U.S.P. 3 per cent hydrogen peroxide, and (c) 1:15 dilution of the 3 per cent hydrogen peroxide. Thus, the final concentrations of hydrogen peroxide in tubes b and c were 0.01 and 0.1 per cent respectively. The mixtures were allowed to stand for 90 minutes at room temperature, after which they were titered for infectivity.

As in Experiment 3, the $10^{-4}$ dilution of the controls yielded a definitely positive reaction, while the $10^{-7}$ dilution gave only a questionable one. Elementary bodies in neither suspension were inactivated in significant amounts by exposure to a 0.01 per cent concentration of hydrogen peroxide. The 0.1 per cent concentration, however, reduced the titer of the suspension of bodies containing no rabbit serum to $10^{-2}$, while the titer of the suspension with added rabbit serum was slightly infectious at a dilution of $10^{-4}$, negative at $10^{-5}$, and questionable at $10^{-6}$.

**Experiment 5.**—An elementary body suspension was washed in the routine manner. Then a part of the suspension was further washed as in Experiment 3. Samples of both suspensions were treated with hydrogen peroxide as described in Experiment 4. It was found that the thoroughly washed elementary bodies were inactivated by 0.1 per cent hydrogen peroxide, while the bodies washed less thoroughly or according to routine were much more resistant to such treatment.

From the results of Experiments 4 and 5 it appears that 0.1 per cent hydrogen peroxide will inactivate a certain number of elementary bodies of vaccinia within a period of 90 minutes if they are not protected by adventitious protein.

**DISCUSSION**

The results of our experiments clearly indicate that adequately washed elementary bodies, obtained from rabbit dermal pulp, are inactivated by sonic vibrations with a frequency of about 8900 cycles per second. Small amounts of normal rabbit serum added to such preparations prevent inactivation. Furthermore, inadequately
washed elementary bodies and suspensions of testicular vaccine virus are not inactivated by the amount of energy employed.

Our findings agree with those previously reported regarding the action of supersonic vibrations on vaccine virus. Hopwood (8) found that calf lymph vaccine virus retained its activity in the presence of vibrations. In fact, it appeared to have had an increased infectious titer after treatment. This he attributed to a breaking up of particles of tissue which resulted in a greater dispersion of virus. Yaoi and Nakahara (9) noted that crude calf lymph virus was not inactivated by supersonic energy, whereas virus purified by adsorption and elution was inactivated. The Japanese workers suggested that impurities in the crude virus preparations prevented the inactivation of the agent by the vibrations. The results of our work clearly indicate that adventitious substances, particularly protein, in the preparations of vaccine virus protect the agent from inactivation by sonic vibrations. Of interest in this connection is the observation of Beckwith and Weaver (10) that protein is responsible for a diminished lethal effect of supersonic energy on bacteria in milk.

There appears to be no uniformity in the reaction of different viruses to sonic energy. Stanley (11) found that partially purified tobacco mosaic virus was less affected than was the virus in crude preparations of infectious plant juice. According to Scherp and Chambers (12), partially purified poliomyelitis virus and influenza virus in emulsions of infected mouse lung tissue were unaffected by intense sonic vibrations.

The disruption of bacteria (13) by high frequency sound waves led us to hope that the elementary bodies of vaccinia might also be broken up in such a manner. If it were possible to obtain their disintegration in this way, the solution of many immunological problems in this field would be facilitated. However, in spite of the fact that thoroughly washed elementary bodies were inactivated by vibration, no evidence was obtained that an appreciable number of them were broken up.

The mechanism by which sonic vibrations induce death and disruption of animal cells and bacteria is not fully understood (2). Moreover, it is not unlikely that the inactivation of vaccine virus is accomplished in a manner different from that operative in the de-
struction of cells and bacteria. At least no obvious disintegration of
the elementary bodies was observed. Flosdorf, Chambers, and
Malisoff (7) have found that approximately 400 microequivalents of
hydrogen peroxide per liter were formed in distilled water subjected
to sonic energy for 45 minutes in an apparatus of the type used in
our experiments. This amount of hydrogen peroxide is less than 1
per cent of that necessary to inactivate the elementary bodies used
in Experiments 4 and 5. Thus, while it seems justifiable to assume
that hydrogen peroxide was not responsible for the loss of activity
of the vibrated virus, one cannot avoid the implication that an oxidiz-
ing reaction played an important rôle in this inactivation, because it
has been observed (7) that the oxidizing capacity of water during
vibration, as determined by the oxidation of sodium bisulfite placed
in the treated water, was in certain instances as great as 20 milli-
equivalents per liter per hour. This oxidizing capacity is of the same
order as that of the hydrogen peroxide solutions (60 milliequivalents
per liter) effective in inactivating the elementary bodies. In spite
of this apparent correlation, no conclusions are warranted. However,
it can be pointed out that the oxidizing ability of aqueous prepara-
tions during sonic vibration must be taken into consideration in
explanations of the inactivation of elementary bodies of vaccinia by
sonic energy.

SUMMARY

Thoroughly washed elementary bodies of vaccinia were inactivated
by sonic vibrations with a frequency of about 8900 cycles per second;
the inactivation was not accompanied by a disruption of the bodies.
Adventitious substances, notably protein, prevented or hindered the
inactivation. There is some evidence that oxidation might have
played a rôle in the inactivation.

BIBLIOGRAPHY

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