STUDIES ON THE INACTIVATION OF VACCINE VIRUS AND THE ACTION OF CERTAIN SUBSTANCES UPON THE INFECTING POWER OF THE INACTIVATED VIRUS.*

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The present status of the discussion opened by the experiments of Gye and Barnard (1, 2) supporting the virus theory of cancer, can be summarized as follows. While certain investigators have been able to repeat Gye’s results and are in agreement with his interpretations (3, 4), others have failed (5–8). Murphy (9) and more recently Flu (10) and Cori (11) duplicated Gye’s work but by means of more careful control experiments have added a new fact of basic importance, namely the non-specific reactivation of the chloroformed sarcoma filtrate (specific factor) by substances other than the cultures of malignant tissues. It is obvious that Gye’s theory does not hold in the light of these later facts, although Gye himself claims to get only negative results in 150 control experiments of a similar nature.

In the opinion of Murphy also shared by Flu, the interpretation of these results is not that a virus has been rendered infective by a specific factor but that an unknown substance of tissue origin enables the agent, modified or attenuated by chloroform, to act. Flu suggests that these substances are similar to bacterial aggressins. Simon and Beck (6) explained Gye’s results on the basis of an aggregation of subinfective doses of the agent itself or of a non-specific reactivation, while Harde (12) suggests that the activator is effective only through its acidity.

In order to throw further light on this subject we have carried out

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an investigation similar to that of Gye but have substituted a typical virus in the place of the etiological agent of the chicken sarcoma. Our plan has been to determine whether the vaccine virus attenuated to such an extent that it would produce no lesion could be rendered infective by the addition of certain substances.

Methods and Materials.

Vaccine Virus.—In all of the experiments the same strain of vaccine virus has been used. Half a cc. of the vaccine emulsion mixed with an equal amount of Ringer's solution was injected into both testicles of a rabbit. Five days later when the resulting orchitis was at its height the animal was killed and the testicles removed aseptically. They were ground thoroughly with sand together with 25 cc. of glycerol and Ringer's solution. The resulting emulsion was distributed in tubes, covered with a layer of sterile vaseline and kept in the ice box.

In order to test the activity of the virus the emulsion was diluted with Ringer's solution and 0.2 cc. injected intradermally in rabbits. With a 1 to 500 dilution of the emulsion (about 1 to 5000 of the testicular material) a slight but definite

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1 We wish to acknowledge our indebtedness to Dr. T. M. Rivers for supplying the original strain of vaccine virus and for his suggestions and criticisms during the course of this investigation.
vaccine eruption appeared in 4 or 5 days. The injection of 0.2 cc. of a 1 to 10 dilution gave rise in the same time to a greatly congested circular eruption 2.5 to 3 cm. in diameter. This latter dose was the one generally used in the experiments.

Inactivation of Virus.

The same general methods have been employed as were used in Gye’s experiments. Not only chloroform but other organic solvents were tested as to their effect on the vaccine virus.

Method.—To 10 cc. of the vaccine emulsion diluted 1 to 10 and placed in a 50 cc. centrifuge tube, various amounts of one or another of the solvents were added.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Amount of acetone</th>
<th>Lesion</th>
<th>Tube</th>
<th>Amount of acetone</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>++</td>
<td>5</td>
<td>0.15</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>++</td>
<td>6</td>
<td>0.1</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>++</td>
<td>7</td>
<td>Control</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tube</th>
<th>Amount of chloroform</th>
<th>Lesion</th>
<th>Tube</th>
<th>Amounts</th>
<th>Resultant lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>-</td>
<td>4</td>
<td>alcohol</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>-</td>
<td>5</td>
<td>toluene</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>±</td>
<td>6</td>
<td>control</td>
<td></td>
</tr>
</tbody>
</table>

These included chloroform, ether, 95 per cent alcohol and toluene. The materials were thoroughly mixed by means of a pipette and were incubated at 37°C. for from 75 to 90 minutes. After this the solvents were evaporated off in a vacuum and the activity of the treated virus was tested by injection into the skin of freshly shaved rabbits.

Experiment 1.—The effect of chloroform and ether in 0.2, 0.15 and 0.1 cc. amounts on 10 cc. of vaccine emulsion was tested. The mixtures were incubated at 37°C. for 65 minutes, after which the solvents were evaporated off and 0.2 cc. of the remaining virus from each tube was injected intradermally into rabbits. The results are shown in Table I.

Experiment 2.—The same procedure as in Experiment 1 was carried out except
that the mixtures were incubated for 90 minutes. The results are shown in Table II.

**Experiment 3.**—The action of acetone was tested by the same method as described above, the mixtures being incubated for 90 minutes. The results are shown in Table III.

**Experiment 4.**—Additional tests were made with chloroform, 95 per cent alcohol and toluene, the mixtures being incubated for 75 minutes. The results are given in Table IV.

The above results show that the vaccine virus is very susceptible to the action of chloroform and the action seems to grade off as the dilution is reduced. Generally 0.2 cc. will inactivate 10 cc. of the emulsion so that no lesion results from its injection. When 0.15 cc. of chloroform was used, the virus was inactive in doses of 0.2 cc. Subsequent tests have shown that when as much as 1 cc. of this virus is injected a definite but small eruption appeared in from 10 to 12 days. The injection of still larger amounts, from 5 to 10 cc. gave rise to lesions almost equivalent in severity and duration to that produced by 0.2 cc. of the untreated virus. It should be noted that there is naturally some variation in the results as the amount of the virus present in the testicular emulsion must be subject to considerable variation, as well as the natural susceptibility of the animals.

Ether, 95 per cent alcohol, acetone and toluene in amounts comparable to the amounts of chloroform found to be effective seem to have little or no action on the virus.

**Reactivation of Chloroformed Virus.**

With the fact demonstrated that vaccine virus is sensitive to chloroform, the next step was to determine whether the inactive or very slightly active virus could be so influenced by the addition of other agents that it would become infective in small doses. For the secondary agents, we used those found effective in activation of the chloroformed filtrates of the chicken tumor.

**Preparation of Auxiliary Agents.**—Chicken embryos 7 to 10 days old were obtained aseptically after careful disinfection of the shell of the egg, and were placed in tubes containing 5 cc. of Hartley's broth with glucose, potassium chloride and rabbit serum. These were incubated at 37°C. under anaerobic conditions from 4 to 25 days. After removing the tubes they were shaken in order to mix the
tissue with the fluid and were then allowed to stand or were centrifuged in order to obtain a more or less clear fluid. Sterility was tested either by smears or subcultures.

The same procedure was carried out using fragments of chicken sarcoma instead of embryos.

The third substance used in the experiments was a light suspension of kieselguhr in distilled water.

**Effect of Embryo “Cultures” and Small Amounts of Chloroformed Virus.**—In the first series of experiments, carried out on ten rabbits, the action was studied of a mixture of supernatant fluid of embryo “cultures” with small amounts of chloroformed virus (0.2 cc.), namely, the same amount which untreated gives rise to a typical vaccine eruption in 4 to 5 days. The results were negative as regards a vaccinal lesion. Nevertheless, the experiments brought out some interesting facts. At the end of the first 24 hours, occasionally, but generally in 2 days, there appeared a circular zone of erythema and edema, similar in dimensions to the true vaccine eruption, but more diffuse and less indurated. This increased during the 3rd day, then ceased to spread and finally disappeared more quickly than the typical vaccine eruption. Without proper control experiments, namely injection of the embryonic fluid alone, this eruption might have been the source of considerable misunderstanding. The irritating power of the embryo culture fluid proved to be weak if the cultures were young (3 to 4 days), strong if the cultures were older. The irritation is greater if the supernatant fluid to be injected contains tiny bits of embryonic tissue in suspension.

**Effect of Chicken Sarcoma “Cultures” or Kieselguhr Together with Small Amounts of Chloroformed Vaccine Virus.**—Some experiments were carried out with small amounts of vaccine virus, such as those mentioned above, the reactivating substance being the supernatant fluid of chicken sarcoma “cultures.” These experiments were few in number, but, in general, the results justify the conclusion that these “cultures” by themselves are capable of producing in rabbits similar reactions to those produced by the supernatant fluid of embryo “cultures.”

Kieselguhr produces, when injected intracutaneously, a very wide reaction, resembling the reaction caused by the vaccine virus even
more than do those produced by embryo "cultures." This point will be discussed in greater detail further on in the paper.

Effect of Embryo "Cultures" Kieselguhr Together with Larger Amounts of Chloroformed Vaccine Virus.—After the reactions induced by the embryo "cultures" had been studied and differentiated from true vaccine eruptions, the experiments were repeated with larger doses of the chloroformed vaccine and of the "culture" fluid. We further tested the activity of larger doses of the chloroformed vaccine alone keeping in mind Flu's statement that he could obtain reactivation of the chloroformed sarcoma agent only when he got tumors from the injection of large amounts of the chloroformed filtrate alone.

Experiment 1.—To 10 cc. of the vaccine virus emulsion diluted 1 to 10 with Ringer's was added 0.15 cc. of chloroform. After mixing well the tube was incubated at 37°C. for 78 minutes. The chloroform was removed in a vacuum. For the secondary substances the slightly turbid supernatant fluids from 18 day old "cultures" of embryonic tissue in Hartley's broth, and a light suspension of kieselguhr were used. The nature of the intradermal injections and the intensity of the vaccinal eruptions induced were as follows.

Rabbit 1.

1. Chloroformed vaccine virus 0.5 cc. plus supernatant fluid embryo "culture" 0.5 cc. Lesion + 10 days
2. Chloroformed vaccine virus 1 cc. plus supernatant fluid embryo "culture" 1 cc. Lesion ++ 10 days
3. Chloroformed vaccine virus 0.5 cc. plus light suspension kieselguhr 0.5 cc. Lesion ++ 10 days
4. Supernatant fluid embryo "culture" 0.5 cc. No lesion
5. Supernatant fluid embryo "culture" 1 cc. No lesion
6. Kieselguhr suspension 0.3 cc. No lesion

Rabbit 2.

1. Chloroformed vaccine virus alone 0.5 cc. No lesion
2. Chloroformed vaccine virus alone 0.5 cc. No lesion
3. Untreated vaccine virus 0.2 cc. Lesion +++ 5 days

Rabbit 3.

1. Chloroformed vaccine virus alone 5 cc. Lesion ++ 6 days

Part of the lesion produced by the mixture of kieselguhr and chloroformed vaccine virus was removed and injected into a susceptible animal (rabbit) and a positive vaccinal eruption was obtained. All rabbits of this experiment were tested
2 months later by a second inoculation of fresh vaccine virus and found to be immune, an indication that the lesions produced were true vaccinal eruptions.

Experiment 2.—This was similar to the foregoing experiment except that 24 day old “cultures” of embryonic tissue were used.

Experiment 2

**Rabbit 1.**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Maximum intensity of eruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chloroformed vaccine virus 0.5 cc. plus supernatant fluid embryonic “culture” 0.5 cc.</td>
<td>Lesion –</td>
</tr>
<tr>
<td>2. Chloroformed vaccine virus 1 cc. plus fluid embryonic “culture” 0.5 cc.</td>
<td>Lesion ++ 8 days</td>
</tr>
<tr>
<td>3. Chloroformed vaccine virus 0.5 cc. plus kieselguhr suspension 0.4 cc.</td>
<td>Lesion ++ 8 days</td>
</tr>
<tr>
<td>4. Supernatant fluid embryonic “cultures” 0.5 cc.</td>
<td>No lesion</td>
</tr>
<tr>
<td>5. Supernatant fluid embryonic “cultures” 1 cc.</td>
<td>No lesion</td>
</tr>
</tbody>
</table>

**Rabbit 2.**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Maximum intensity of eruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Untreated vaccine virus 0.2 cc.</td>
<td>Lesion ++ 5 days</td>
</tr>
<tr>
<td>2. Untreated vaccine virus 0.4 cc.</td>
<td>Lesion + + + 5 days</td>
</tr>
<tr>
<td>3. Chloroformed vaccine virus 0.5 cc.</td>
<td>No lesion</td>
</tr>
<tr>
<td>4. Chloroformed vaccine virus 1 cc.</td>
<td>No lesion</td>
</tr>
</tbody>
</table>

**Rabbit 3.**

5. Chloroformed vaccine virus 5 cc. | Lesion + 5 days |

The skin of the spot injected with 1 cc. of chloroformed vaccine virus alone, which did not show a definite eruption, was removed after 5 days and injected, after grinding, into the testicles of another rabbit. It gave rise to an orchitis. From this testicle a virus was obtained which gave rise to a typical pustular eruption in the skin of another animal. All rabbits of this experiment were injected 2 months later with fresh vaccine virus and found to be immune.

The above two experiments show that chloroformed vaccine virus in 0.5 and 1 cc. doses do not give rise to eruptions when injected alone into the skin of a rabbit, but the addition of the supernatant fluid of embryonic tissue “cultures” or a suspension of kieselguhr results in very definite and typical lesions. The same degree of eruption results from the injection of larger amounts of the chloroformed virus alone (5 to 10 cc.). Occasionally small amounts gave rise to eruptions so slight as to leave doubt as to their nature but for the fact that the virus may be recovered from such lesions in animal passage. The eruption which takes place as the result of the injection of a mixture...
of chloroformed virus and the secondary agent, comes later than that produced by the untreated virus or the larger doses of chloroformed virus.

In the next group of experiments care was taken to test the untreated vaccine virus and the chloroformed virus on different animals in order to avoid possible immunity effects. It was thought that the reaction following the pronounced eruption from the untreated virus might interfere with a weaker delayed reaction from the treated virus.

Experiment 3.—The general procedure of this experiment was the same as the foregoing. A 39 day old "culture" of 7 day old chick embryo tissue was used as the secondary factor and the vaccine virus was exposed to the action of chloroform for 70 minutes at 37°C. The results were as follows.

<table>
<thead>
<tr>
<th>Rabbit 1.</th>
<th>Maximum intensity of eruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chloroformed vaccine virus 0.5 cc. plus embryo &quot;culture&quot; fluid 0.5 cc.</td>
<td>Lesion + 8 days</td>
</tr>
<tr>
<td>2. Same as above</td>
<td>Lesion ++ 9 days</td>
</tr>
<tr>
<td>3. Chloroformed vaccine virus 0.5 cc. plus 0.5 cc. kieselguhr suspension</td>
<td>Lesion -</td>
</tr>
<tr>
<td>4. Chloroformed vaccine virus alone 0.5 cc.</td>
<td>Lesion -</td>
</tr>
<tr>
<td>5. Fluid from embryo &quot;culture&quot; 0.5 cc.</td>
<td>Lesion -</td>
</tr>
<tr>
<td>6. Kieselguhr suspension 0.5 cc.</td>
<td>Lesion -</td>
</tr>
</tbody>
</table>

Rabbit 2.

<table>
<thead>
<tr>
<th>Rabbit 2.</th>
<th>Lesion ++ 6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chloroformed vaccine virus 5 cc.</td>
<td>Lesion ++</td>
</tr>
<tr>
<td>2. Chloroformed vaccine virus 0.5 cc.</td>
<td>Lesion</td>
</tr>
<tr>
<td>3. Chloroformed vaccine virus 0.5 cc.</td>
<td>Lesion</td>
</tr>
</tbody>
</table>

Rabbit 3.

<table>
<thead>
<tr>
<th>Rabbit 3.</th>
<th>Lesion + + 5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Untreated vaccine virus 0.2 cc.</td>
<td>Lesion + +</td>
</tr>
<tr>
<td>2. Untreated vaccine virus 0.4 cc.</td>
<td>Lesion + + +</td>
</tr>
</tbody>
</table>

One of the spots of the skin of Rabbit 2 where 0.5 cc. of chloroformed vaccine virus had been injected was removed and injected, after grinding with sand, into the testicle of a normal rabbit. An orchitis developed from which a virus was obtained which regularly gave a typical vaccinal eruption in susceptible animals. All of the rabbits of this experiment were tested 2 months later by a second inoculation of fresh untreated vaccine virus and all proved to be immune.

Experiment 4.—The same general procedure was followed here as in the foregoing experiment except that 0.2 instead of 0.15 cc. of chloroform was added to the vaccine virus. The results were as follows.
Rabbit 1.

1. Chloroformed vaccine virus 0.5 cc. plus supernatant fluid embryonic "culture" 0.5 cc. Lesion + 8 days
2. Chloroformed vaccine virus 0.5 cc. plus supernatant fluid embryonic "culture" 0.5 cc. Lesion -
3. Chloroformed vaccine virus 0.5 cc. plus kieselguhr dilution 0.5 cc. Lesion -
4. Chloroformed vaccine virus alone 0.5 cc. Lesion -
5. Supernatant fluid embryonic "culture" 0.5 cc. Lesion -
6. Supernatant fluid embryonic "culture" 0.5 cc. Lesion -
7. Kieselguhr dilution alone Lesion -

Rabbit 2.

1. Chloroformed vaccine virus alone 5 cc. Lesion -
2. Chloroformed vaccine virus alone 0.5 cc. Lesion -
3. Chloroformed vaccine virus alone 0.5 cc. Lesion + 8 days

Rabbit 3.

1. Untreated vaccine virus 0.2 cc. Lesion ++
2. Untreated vaccine virus 0.4 cc. Lesion +++

All the animals of this experiment were tested with fresh vaccine virus 2 months later and found immune.

These last two experiments indicate that the immunity possibly developing as the result of the stronger reaction to untreated vaccine did not influence the results.

The last experiment cannot be considered as entirely a satisfactory result for 0.5 cc. of chloroform vaccine alone caused a slight lesion in the control rabbit but gave a negative reaction in the test animal. The indications are that the greater the amount of chloroform used the less is the possibility of reactivating the virus. In order to get some idea of the limits the following experiments have been carried out. As the general procedure was the same and only the amount of chloroform added to the vaccine virus was varied the details of the experiment will not be gone into.

Experiment 5.—In this test 0.4 cc. of chloroform was used. Injection of as much as 2 cc. failed to produce a lesion and attempts to reactivate with "culture" fluid and kieselguhr failed to give results. The two animals developed no immunity as the result of this injection, as shown by the fact that they had an eruption 2 months later when inoculated with untreated virus.
Experiment 6.—When 0.25 cc. of chloroform was used all the injections were negative and the two animals showed no immunity on subsequent injection with fresh virus.

Experiment 7.—In this test 0.2 cc. of chloroform was used. Injection of 4 cc. of the vaccine after treatment failed to elicit a response in one rabbit and there was no reactivation by the auxiliary agent in another and no immunity developed as the result of the injection.

Experiment 8.—The above experiment with 0.2 cc. of chloroform was repeated. This time there was a slight positive eruption following the injection of chloroformed vaccine virus plus the fluid from embryonic tissue “cultures.” There was also a positive result from the injection of 4.5 cc. of the treated virus alone and slight positiveness with 1 cc. and 0.5 cc. but the lesion from the latter developed some 3 or 4 days later than the lesions from the reactivated virus. All of these animals proved immune on subsequent inoculation with fresh virus.

Experiment 9.—With 0.15 cc. of chloroform the mixture of 0.5 cc. each of the treated vaccine and the supernatant fluid of the embryonic tissue “culture” gave typical eruptions. A large amount of this virus, 4.5 cc., also gave a positive result alone but smaller amounts, 0.5 to 1 cc., resulted in very slight responses. All the animals were immune on second inoculation.

Experiment 10.—Another experiment using 0.15 cc. of chloroform was carried out. In the tests the chloroformed vaccine alone even in the smaller doses gave typical eruptions. Very little difference could be made out between these and the eruptions caused by the virus plus the auxiliary fluids. All the animals were immune on second inoculation with untreated virus.

The results seem comparable to those obtained by Flu in his study of the effect of chloroform on the agent of the chicken tumor. Where no lesions result from the injection of large amounts of the treated vaccine virus it proved impossible to reactivate the smaller doses with the fluid from embryo tissue “cultures” and no immunity is developed by the animal. Our experiments show further the great variability in the eruption produced by the same dose of vaccine virus especially when one at the lower effective limit is employed. Not only do individuals differ in susceptibility but areas in the same animal differ in their response to the same dose. An extreme example of this is to be seen in the case of Rabbit 2 of Experiment 4, in which 5 cc. of the chloroformed virus failed to produce a lesion while a typical reaction occurred in another skin area receiving only 0.5 cc. While it is conceivable that errors in technique, local injury from shaving, leakage of the fluid from the puncture wound or some such factor might explain these inconsistencies, yet as far as our observations go they exist.
Localizing Effect of Auxiliary Agents.

An occasional animal of the preliminary experiments having pronounced lesions from pure vaccine showed a typical vaccinal eruption in spots injected only with fluid from an embryonic tissue "culture." This suggested a test of the secondary fluids as localizing agents when the virus was given intravenously. It will be recalled that Calmette and Guerin (13) found that the pulling out of the hair was sufficient to localize the vaccine lesion.

Experiment.—Embryonic tissue "cultures," chicken sarcoma "cultures" and light suspensions of kieselguhr were prepared in the manner already described. As a fourth substance a 10 per cent solution of peptone was injected into the skin in 0.5 to 1 cc. amounts. The supernatant fluid from the "cultures" and the kieselguhr were used in the same amounts. Five cc. of a 10 per cent suspension of vaccine virus was injected into the ear vein of the rabbits. The animals had been shaved carefully so as to avoid injury to the skin, prior to the intradermal injection of the fluids. The resultant eruptions were tested by removing the skin area, grinding and injecting into the skin of a fresh animal, and only these eruptions proving to have active virus by this test were included as positives.

In the experiment proper eight animals were injected in five or six different spots with the fluids prepared as described above, followed by the intravenous injection of the vaccine virus. In addition seven rabbits were used to test the presence of active virus in the resultant lesions. The results are given in Table V.

<table>
<thead>
<tr>
<th></th>
<th>Spots injected</th>
<th>Positive localization</th>
<th>Doubtful localization</th>
<th>Negative localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic &quot;cultures&quot;</td>
<td>29</td>
<td>25</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Chicken sarcoma &quot;cultures&quot;</td>
<td>9</td>
<td>6</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Peptone</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Kieselguhr</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

The above results suggest that the action of the secondary fluid is not on the virus but upon the cells rendering them more susceptible to its action. While the number of tests with kieselguhr is small the
INACTIVATION OF VACCINE VIRUS

failure of the virus to localize in a single instance in the area of reaction produced by this agent is significant. It is of interest in this connection to note that the agent of the chicken tumor on intravenous injection shows less tendency to localize in kieselguhr reactions than in the reactions produced by the several other substances (14).

DISCUSSION.

It was the purpose of these experiments to parallel the work of Gye, but with vaccine virus instead of the chicken tumor agent. In planning of the tests, we took into consideration the critical work of Murphy and of Flu as well as the various results obtained by several other investigators who have attempted to repeat Gye’s experiments. While the vaccine virus has certain advantages in a study of this kind, the chicken tumor agent has a special one in that natural resistance against it is so feeble that a tumor once started rarely fails to progress. In spite of the variability in the reactions reported in this paper, sufficient data of a positive nature are provided to justify certain conclusions.

Flu states that an auxiliary substance will render a small amount of the chloroformed chicken tumor filtrate infective only when a large dose of the chloroformed filtrate alone is capable of inducing a tumor. The evidence brought out by the present experiments indicates that the same is true of the vaccine virus. When the amount of chloroform used was large enough to render even great amounts of the virus innocuous no reactivation of the small dose proved possible. It is of interest to note that about the same amount of chloroform is required to inactivate the virus and the tumor agent. It would seem from these results that chloroform in amounts which still allow reactivation of the virus does not destroy all of the agent. One may suppose either that the number of infective elements is greatly reduced in number or that the infectivity of all of the elements is lowered by the chloroform treatment.

The secondary substances or activators would seem to exert their effect by rendering the animal’s cells more susceptible to the infecting power of the virus. This is indicated by the result of the experiments in which it was demonstrated that the fluids most effective in reac-
tivating the chloroformed virus will induce a reaction in the skin that is favorable to localization of virus injected intravenously. The fact that some substances are more active than others in this respect regardless of the amount of injury induced, opens up an interesting question as to the type of injury or reaction which determines the localization of an infective agent. This point would bear further investigation not only as concerns the vaccine virus but the chicken tumor agent as well.

The analogy between these results and those obtained by Gye with the chicken tumor agent would tend to uphold the conclusions of Murphy and of Flu that the chloroform treatment attenuates the agent but does not destroy it; and the secondary factor contained in the "cultures" is non-specific in its action, merely rendering the cells of the inoculated animal more susceptible to the enfeebled agent.

Summary and Conclusions.

Vaccine virus, obtained from testicular inoculation shows a high susceptibility to chloroform as compared with ether, toluene, 95 per cent alcohol and acetone.

Vaccine virus, after treatment with an amount of chloroform sufficient to render it incapable or only barely capable of originating an eruption in the rabbit's skin, produces a characteristic eruption when injected with the supernatant fluid of embryonic tissue or sarcoma tissue "cultures" or kieselguhr, substances all of which are markedly irritative to the rabbit's skin.

Reactivation of the chloroformed vaccine virus is not possible when chloroform has been added to it in such quantity that the injection of large amounts of the treated virus fails to cause an eruption. Whenever reactivation has been accomplished it has been possible to get a vaccine eruption of greater or less intensity by the injection of large amounts of the chloroformed vaccine alone.

Embryo and chicken sarcoma "culture" fluids when injected intradermally make the skin susceptible to the localization of the virus introduced intravenously.

The bearing of these experiments on the interpretation of Gye's theory of cancer causation is discussed.
INACTIVATION OF VACCINE VIRUS

BIBLIOGRAPHY.