A FURTHER STUDY OF GYE'S HYPOTHESIS ON
THE ETIOLOGY OF MALIGNANT TUMORS *

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According to Gye (1) the cause of all malignant new growths
of man and the lower animals is a filterable but cultivatable
microbe which, however, is unable to produce tumors unless
the resistance of the tissues of the host is first specifically
reduced by a second cellular product or "chemical stimulant"
called the "specific factor."

The latter has been so designated because it and not the
microbe is held to determine the kind and species specificity of
tumors. For example, the same microbe is believed by Gye to
cause cancer of man, sarcoma of chickens and both cancer and
sarcoma of mice and rats. But according to his hypothesis,
the microbe can produce sarcoma of the chicken only when
inoculated into Plymouth Rocks along with a "specific factor"
prepared from this tumor; if the mixture is injected into mice
or rats tumors do not result. Or if a "culture" of the sarcoma
is injected into these chickens along with a "specific factor"
extract of some other tumor like a cancer of man or mouse,
tumors are not produced. To produce a cancer in a mouse the
"culture" of microbe may be from any tumor but the "specific
factor" must be prepared of mouse cancer tissue and the mixture
must be injected into mice. In other words the very strict
species specificity of malignant growths is not due according to

*Read before the Section of Pathology and Physiology of the American Medical
Gye to a specificity on the part of the microbe but rather to the tumor extract or so-called "specific factor" which has the power of breaking down the resistance to infection by the malignant tumor microbe of the tissues of only those animals belonging to the same species from which the tumor was taken. Furthermore, while the microbe of cancer and sarcoma are held to be identical, the kind of tumor produced depends upon whether the "specific factor" was prepared of a sarcoma or carcinoma.

The hypothesis was based upon the following premises: (a) The inoculation of Plymouth Rocks with a subculture of the Rous chicken sarcoma did not produce tumors even though the "microbe" of malignant tumors was supposed to be present. (b) Inoculation with a "specific factor" prepared by treating a sand and paper filtrate of tumor emulsion in Ringer's solution with chloroform did not produce tumors if the virus of the tumor had been completely destroyed. (c) Inoculation with subculture and "specific factor" produced tumors because the "specific factor" contained a chemical agent which increased susceptibility to infection by the "microbe" in the culture.

The nature of the "specific factor" therefore, commands much interest because it is believed to control not only the infectiousness of the hypothetical microbe but likewise to determine the kind of tumor produced. And of course the theory has greatly renewed interest in the possible parasitic nature of malignant new growths. Unfortunately, however, the experiments upon which it is based lack confirmation; at least this has been true of our previous and present investigations as well as of the majority of others.

**REVIEW OF LITERATURE**

Harkins, Schamberg, Kolmer and Kast (2) in a study employing the same Rous sarcoma and the technic of Gye followed with meticulous care, showed that the "specific factor" is in reality nothing more than a suspension of attenuated living virus which may or may not produce a tumor depending upon the fowl's susceptibility or resistance. When "specific factors"
were prepared with a "few drops of chloroform" as stated by Gye they almost always produced tumors alone if the chickens were kept long enough under observation; a concentration of approximately 5 per cent chloroform was required for the production of "specific factors" surely sterile of virus and alone unable to produce the growths. When such were inoculated along with subcultures of the sarcoma, tumors were not produced. Mongrel Plymouth Rock chickens were found to be more resistant than pure bred stock and as stated above we could not confirm the evidence of growth of the virus in subcultures nor the statement that the so-called "specific factor" acts by reason of the presence of a chemical substance.

Mueller (3) has had similar results and states that tumors have never been consistently obtained in a mixture of the two substances unless one or the other alone produced tumors. The preparation of suitable chloroform filtrates (Gye's "specific factor") appeared to be the chief difficulty since individual filtrates varied considerably in their resistance to inactivation by equal quantities of the reagent. Murphy (4) has reported that anaerobic "subcultures" of chick embryo and rat placenta have proved just as effective as so-called cultures of malignant tumors in activating the chloroform treated "specific factor," thereby eliminating the assumption that a living microbe was present in the subcultures of tumor in Gye's experiments. Mackenzie and Illingworth (5) however, were unable to confirm Murphy's observations using embryo and autolyzed tissues; but they were likewise never successful in subculturing the virus of fowl sarcoma. Baker (6) also was unsuccessful with cultures of chick embryo but on two occasions was able to produce tumors in chickens by inoculating subcultures of Jensen's rat sarcoma along with chloroform prepared "specific factor" of fowl sarcoma; at least these tumors were much larger than those produced in the controls. Simon and Beck (7) were likewise unsuccessful in producing tumors by combining "embryonic tissue cultures" with chloroformed tumor extracts when the latter had been definitely inactivated and were also unsuccessful in confirming Gye's work, believing that his positive
findings were due to the fact that the treatment of tumor extracts with chloroform, in the manner advocated by him, does not uniformly lead to the complete inactivation of the contained virus. Flu (8) has also failed to confirm Gye's hypothesis in experiments employing rat sarcomas and believes that from the histo-pathological standpoint the Rous tumor is not a true sarcoma at all but a chronic infectious granuloma.

In a more recent paper Gye failed to confirm Murphy's work and states that not once has it been possible with extracts of embryonic and adult tissues to replace the agent destroyed by antiseptic in the fowl sarcoma extract and believes that it is necessary to inject cultures of some kind of malignant tumor to furnish the "microbe" along with supposedly virus-free "specific factor." But in these more recent experiments the latter were prepared by treating 10 cc. amounts of clear, cell-free, sand filtrates with 0.2 cc. of chloroform (2 per cent) at 37° C. for three hours and also with 1 in 5,000 acriflavin at 37° C. overnight, which likewise introduced a complication since simple incubation for 18 hours frequently robbed filtrates of their powers to introduce tumor formation.

In this paper Gye confirms the observation of Rous and Murphy that extracts of fowl sarcoma are always robbed of their activity by exposure to 55° C. for 15 to 18 minutes. The former thought that this destroyed the virus which would appear to us to be the correct interpretation since the virus bodies of other diseases are known to be likewise susceptible to heat. But Gye believes that heat destroys the "specific factor" or chemical agent present in tumor tissue rather than the virus and states that the inoculation of Plymouth Rocks with heat treated filtrate (presumably containing the microbe but robbed of "specific factor") fails to produce tumors; that inoculation with acriflavin treated filtrate (presumably containing the "factor" but not the microbe) also fails but that the injection of a mixture of the two results in tumor production. We have repeated this newer work and regret being unable once again to confirm his observations, the technic and results of our experiments being briefly summarized as follows.
EFFECT OF ACRIFLAVIN AND HEAT UPON TUMOR FILTRATES

Germicides vary in their rate of action and are influenced to some extent by the amount of microbes to be destroyed as well as the presence of cellular and serum constituents in filtrates of tumor tissue. In our experience at least 5 per cent chloroform in an exposure of 3 hours at 38° C. has been required to completely destroy all virus in filtrates of the chicken sarcoma prepared by Gye's method and 1 : 5000 acriflavin has required an exposure of six hours at 38° C. as shown in Table I.

### Table I

<table>
<thead>
<tr>
<th>Fowl</th>
<th>Incubation at 38° C.</th>
<th>Amount Inoculated</th>
<th>Period of Observation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>1 hour</td>
<td>1 cc.</td>
<td>23 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>33</td>
<td>1 hour</td>
<td>1 cc.</td>
<td>27 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>20</td>
<td>2 hours</td>
<td>1 cc.</td>
<td>20 days</td>
<td>Small tumor</td>
</tr>
<tr>
<td>21</td>
<td>2 hours</td>
<td>1 cc.</td>
<td>32 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>14</td>
<td>4 1/2 hours</td>
<td>1 cc.</td>
<td>34 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>15</td>
<td>4 1/2 hours</td>
<td>1 cc.</td>
<td>26 days</td>
<td>Small tumor</td>
</tr>
<tr>
<td>24</td>
<td>6 hours</td>
<td>1 cc.</td>
<td>23 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>25</td>
<td>6 hours</td>
<td>1 cc.</td>
<td>27 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>28</td>
<td>18 hours</td>
<td>1 cc.</td>
<td>60 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>29</td>
<td>18 hours</td>
<td>1 cc.</td>
<td>18 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>16</td>
<td>23 hours</td>
<td>1 cc.</td>
<td>82 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>17</td>
<td>23 hours</td>
<td>1 cc.</td>
<td>22 days</td>
<td>No tumor</td>
</tr>
</tbody>
</table>

Inoculations were made into the pectoral muscles of fowls in 1 cc. amounts. It will be noted that the longer periods of incubation (6 to 23 hours) inactivated the suspension, while the shorter exposures (1 to 4 1/2 hours) were inconsistent. For example filtrates exposed for 1 hour and inoculated into fowls 32 and 33 did not produce tumors in 23 and 27 days, while fowl 20 inoculated with a like amount exposed for 2 hours developed a small tumor as likewise fowl 15 injected with a filtrate exposed for 4 1/2 hours. The duplicates of these two fowls, 21 and 14, remained free of tumor development.

The inoculations of filtrates heated for 18 minutes at 55 to 56° C. never produced tumors in observations of 18 to 82 days. This we understand has been the experience of Gye, and checks in a general ways with the effect of heat on other viruses.
Filtrates were heated by placing 3 cc. in thin-walled glass ampoules, and completely submerging in a water bath at the above temperature.

RESULTS OF INOCULATION WITH COMBINATIONS OF ACRIFLAVIN AND HEAT TREATED FILTRATES

According to Gye the inoculation of fowls with a mixture of acriflavine and heated filtrates of Rous sarcoma results in tumor production, each alone being without effect, because he believes that the former contains a chemical stimulant or "specific factor" and the latter the living microbe of malignant tumors.

TABLE II

Result of Inoculation of Combinations of Acriflavine Treated (1:5000) and Heated Rous Sarcoma Extracts

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>1 hour</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>23 days</td>
<td>Tumor 2</td>
</tr>
<tr>
<td>33</td>
<td>1 hour</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>27 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>20</td>
<td>2 hours</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>20 days</td>
<td>Small tumor</td>
</tr>
<tr>
<td>21</td>
<td>2 hours</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>32 days</td>
<td>No tumor</td>
</tr>
<tr>
<td>15</td>
<td>4 hours</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>26 days</td>
<td>Tumor 4</td>
</tr>
<tr>
<td>24</td>
<td>6 hours</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>23 days</td>
<td>No tumor</td>
</tr>
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<td>25</td>
<td>6 hours</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>27 days</td>
<td>No tumor</td>
</tr>
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<td>28</td>
<td>18 hours</td>
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<td>18 minutes</td>
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<td>82 days</td>
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<td>17</td>
<td>23 hours</td>
<td>0.5 cc.</td>
<td>18 minutes</td>
<td>0.5 cc.</td>
<td>22 days</td>
<td>No tumor</td>
</tr>
</tbody>
</table>

1 Heated filtrates alone in dose of 1 cc. never produced tumors in observation of 18 to 82 days.
2 Acriflavine treated extract alone in 1 cc. dose did not produce a tumor in 23 days (death of fowl). The only instance of confirmation of Gye's experiments.
3 Acriflavine treated extract alone in 1 cc. dose also produced a small tumor in 19 days.
4 Acriflavine treated extract alone in 1 cc. dose also produced a small tumor in 26 days.

The results of our experiments are summarized in Table II. In these fowls multiple inoculations were made in order to eliminate the individual susceptibility or resistance of the fowl to the virus. As a result the periods of observation were
shortened because of death of the chickens from metastasis of the tumor developing at the point where the untreated filtrate (living virus) control was inoculated.

In none of the fowls inoculated with extracts exposed to acriflavine for 6, 18 and 23 hours and combined with heated filtrates were tumors produced. Gye's deductions probably would be that either the tumor from which the filtrate was prepared was lacking in the "chemical activating agent" ("specific factor") or that the length of exposure destroyed this hypothetical chemical activating agent. In the fowls inoculated with extracts treated with acriflavine for shorter periods and combined with heated filtrates tumor development was more frequent. In fowls 20 and 15 an apparent confirmation of Gye's experiments was obtained but on autopsy a small tumor was found at the site where the acriflavine treated extract alone was inoculated. This of course nullified the development of the tumors produced by the combination of acriflavine treated extracts and heated filtrates, the virus being alive. If we had been the least convinced of the existence of a "specific factor" by our previous work (2) we would have drawn the conclusion when considering fowl 15 especially, that the amount of virus present in the acriflavine treated extract when combined with the heated filtrate was activated in as much as the tumor produced by the combination was palpable in 22 days while the inoculation of the acriflavine treated extract alone in the same period was not, being found only on autopsy as a small tumor in 26 days. On the other hand the development of tumors in the two locations in fowl 20 were identical and would not support such a theory.

Furthermore, in Figure 1 it will be noted that fowl 32 which was inoculated with an extract treated with acriflavine for one hour at 38° C. combined with heated filtrate developed a tumor. Where the acriflavine treated extract alone was inoculated no tumor developed. This is apparently a confirmation of Gye's experiments, but as shown in Table I the effect of 1 : 5000 acriflavine upon Rous sarcoma virus for short periods is very uncertain since exposures up to and including 4$\frac{1}{2}$ hours
in our experience were sometimes unable to completely destroy the virus. Figure 2 for example, is a duplicate experiment and showed no tumor production following the injection of acriflavin and heated filtrates into the left leg of fowl 33 and

FOWL 32. Died 23 days.

"Specific Factor" = Rous sarcoma extract incub. 1 hr. 38° C.
with acriflavine (1 : 5000),
Heated filtrate = Rous sarcoma extract heated 55–56° C. 18 minutes.

**Fig. 1**

<table>
<thead>
<tr>
<th>Right Breast.</th>
<th>Left Breast.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cc. acriflavine</td>
<td>No TUMOR.</td>
</tr>
<tr>
<td>&quot;Spec. fact.&quot;</td>
<td>No TUMOR = 1 cc. heated filtrate.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Right Leg.</th>
<th>Left Leg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cc. untreated filtrate = TUMOR.</td>
<td>SMALL TUMOR =</td>
</tr>
<tr>
<td></td>
<td>0.5 cc. acriflavine,</td>
</tr>
<tr>
<td></td>
<td>&quot;Spec. fact.&quot;</td>
</tr>
<tr>
<td></td>
<td>0.5 cc. heated filtrate.</td>
</tr>
</tbody>
</table>

indeed in every additional experiment where the acriflavine treated filtrates alone produced no tumors negative results followed inoculations with a combination of heated and acriflavine treated filtrates.

Furthermore in the single experiment (Fig. 1) where the combination resulted in tumor production the question may be raised, as previously done by Mueller, whether inoculation of the chicken with untreated filtrate in the right leg as the positive control could result in a transfer of the virus to the other sites of inoculation? That the virus of the Rous sarcoma is contained in the blood has been apparently shown by Lewis and Andervont (10) who were able to reproduce the Rous fowl sarcoma by inoculation of plasma alone and washed white blood cells alone from the blood of fowls with Rous sarcomata.* But in our

*The reports of Lewis and Andervont were not seen by us until just before this paper was presented at the Amer. Med. Assoc. meeting, May 1927. However, since that time, we have succeeded in confirming their experiments. Tumors readily de-
A FURTHER STUDY OF GYE’S HYPOTHESIS

experience there was no evidence at all to indicate that the virus growing in a tumor of the right leg for example was transferred to sites of inoculation in the opposite leg and both sides of the breast with heated filtrate alone, acriflavin treated

FOWL 33. Died 27 days.

“Specific factor” = Rous sarcoma extract incub. 1 hr. 38º C.
with acriflavine (1 : 5000).
Heated filtrate = Rous sarcoma extract heated 55-56º C. 18 minutes.

<table>
<thead>
<tr>
<th></th>
<th>Right Breast.</th>
<th>Left Breast.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cc. acriflavine</td>
<td>= No tumor.</td>
<td>No tumor = 1 cc. heated filtrate.</td>
</tr>
<tr>
<td>“Spec. fact.”</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Right Leg.</th>
<th>Left Leg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cc. untreated filtrate = TUMOR.</td>
<td>No tumor =</td>
</tr>
<tr>
<td></td>
<td>0.5 cc. acriflavine, “Spec. fact.”</td>
</tr>
<tr>
<td></td>
<td>0.5 cc. heated filtrate.</td>
</tr>
</tbody>
</table>

Fig. 2

filtrate alone or with a combination of these. In the single instance (fowl 32) where the combination yielded a tumor we believe that a small amount of living but greatly attenuated virus was present in the acriflavin treated filtrate even though it alone failed to produce a tumor in the short period of only 23 days when the animal died as a result of the tumor developing in the right leg where untreated filtrate had been injected.

EXPERIMENTS WITH BERKEFELD FILTRATES

After the publication of Gye’s original paper the question was often asked why Berkefeld filtrates were not used instead of developed following inoculation of blood plasma and washed leucocytes of a fowl having a Rous sarcoma of twenty-one days’ development. When heated for 18 minutes at 55-56º C. no tumor developed. Our own experiments were confirmed and such tumors are transmissible. These results make highly possible the suggestion of Mueller that virus from a tumor in one part of the body may be transferred to other sites of inoculation.
sand and paper pulp filtrates in order to eliminate the possibility of the injections of cells or cellular material. In our previous communication we did not go into this phase because we were concerned in attempting to confirm only what Gye had published, and at that time had no desire to modify the technique as described by him. Since then we have worked with Berkefeld filtrates of actively growing tumors by preparing the tumor extract as he described it but using acriflavine as the germicide and incubating for various periods at 38° C. in a water bath

![Figure 3](image)

in an incubator. With such filtrates it is well known that the virus is in smaller quantities and that inoculation of greater amounts is necessary to produce tumors; therefore all inoculations were in 2 cc. quantities or double that of the sand-paper filtrates. Even in this dose three fowls failed to develop tumors over a period of 61 days although curiously enough a duplicate of one of these developed a tumor in 19 days evidently constituting another example of variation in resistance of pure bred Plymouth Rocks to the virus.

Figure 3 showing the results obtained in fowl 23 is a type of the experiment carried out. In no instance did tumors develop
when the acriflavine treated or the heated extracts alone were inoculated; neither did the inoculation of a combination of these extracts produce tumors, thereby confirming the results of similar experiments employing the sand-paper filtrates.

**SUBSTITUTION EXPERIMENTS**

Gye has stated that if "cultures" of rat, mouse or human tumors are injected into fowls alone with chloroform treated filtrates of fowl sarcoma (so-called "specific factor") tumors result; if injected into rats or mice tumors do not result. Therefore he argues the "cultures" contain the "microbe" rendered infectious for fowls only by the "specific factor." But could it not be that the "cultures" were sterile and that the tumors were the result of surviving fowl sarcoma virus in the chloroform treated filtrates or "specific factors" as stated by Schamberg, Kast and ourselves? As previously stated Murphy reported that "cultures" of embryonic tissues did as well as "cultures" of tumors in such experiments but several other investigators including Gye have failed to confirm his observations. We have not used "cultures" of embryonic or adult tissues as substitutes for "cultures" of tumors in such experiments but all of our substitution experiments employing "cultures" of mouse sarcoma 37/S have shown that the only time tumors were produced was when the chloroform or acriflavin treated filtrates contained living virus; in other words we have been totally unable to confirm Gye's hypothesis.

Our investigations made in mice and chickens were attempts to replace the virus of the Rous sarcoma with the mouse sarcoma 37/S and with cultures of this tumor. A number of "specific factors" were prepared of the 37/S and attempts to replace the Rous sarcoma extract with it were made using subcultures of the Rous sarcoma, the technic of cultivation being exactly as described by Gye.

In so-called primary cultures of the mouse sarcoma 37/S incubated anaerobically 3 days at 38° C. it was interesting to note that tumor development in mice was quite rapid, the tumor cells being apparently unimpaired by incubation. This result
conforms with those of Gye and others. When such "primary cultures" of 37/S were combined with a chloroform treated (5 per cent) Rous sarcoma extract and inoculated into mice a mouse sarcoma resulted. This result was expected inasmuch as the primary culture alone was capable of producing tumors. But when such combinations were inoculated into fowls, no tumor was produced.

Further attempts were made to activate the Rous sarcoma extract treated with chloroform (5 per cent) also with acriflavine (1 : 5000) with suspensions of mouse tumor 37/S in Ringer's solution. Inoculations were made in fowls but no tumors developed. Certainly the opportunity was here presented for the mouse sarcoma 37/S to supply the activating agent, if such existed.

Subcultures of 37/S were of course unable to produce the sarcoma in mice. Inoculation with subculture and 5 per cent chloroform treated extracts of this tumor also failed since the amount of chloroform used was sufficient for completely destroying all of the virus and certainly none was present in the subcultures. Of course, mice inoculated with subcultures of 37/S and chloroform treated extracts of fowl sarcoma failed to develop tumors because even if some virus escaped destruction in the latter it is unable to produce tumors in mice. Likewise Plymouth Rocks were inoculated with subcultures of fowl sarcoma combined with chloroform treated filtrates of mouse sarcoma 37/S; tumors did not develop because the subcultures were harmless and the filtrates likewise because even if they contained the virus of mouse sarcoma it is unable to produce tumors in chickens.

Other substitution experiments were made on fowls and mice using heated extracts of the Rous sarcoma and viable mouse tumor 37/S suspension, and also viable Rous sarcoma extract and heated mouse tumor 37/S filtrates but always with similar results—no tumors developing.
SUMMARY

We believe therefore that these results confirm the previous report by Harkins, Schamberg, Kolmer and Kast in which was demonstrated the difficulty or rather the impossibility of preparing non-infectious so-called "specific factor" unless a concentration of chloroform greater than that stated by Gye was used. In attempts to confirm Gye's work by others this same difficulty was experienced. The many influences that control the action of the chloroform on the virus makes the result uncertain, unless a concentration of 5 per cent or higher is used.

In our experience we have never obtained a result that could be interpreted in the least that a "specific factor" existed and Gye's experiments and hypothesis have centered about this point. He claims that all tumors provide a virus which is universal and alone unable to produce a tumor; a second substance is necessary—a "specific factor" which is obtained from tumor extracts and which enables the virus to infect.

Almost two years have elapsed since his first announcement and to date no clear cut confirmation has been reported. MacKenzie and Illingworth and also Baker report partial confirmation but their experiments were based on such evidence as to detract from their value. For example when a fluid is inoculated in which it is known that the living virus of the Rous sarcoma exists with no attempt to destroy it other than removal by centrifugalization (a very uncertain method especially with viruses) what degree of assurance is there that the resulting tumor was not produced by inoculated virus? Furthermore in methods of dilution for removal or reduction of virus the individual resistance of the fowl may be expected to influence the results. Such methods are pure chance and in our opinion the introduction of live virus either in small numbers of a virulent form or numerous attenuated forms, offers fair opportunity for tumor development and wrongly interpreted results.

Rous and Murphy and their collaborators have shown that the virus of the fowl sarcoma is susceptible to a temperature of 55° C. for 15 minutes and is robbed of its activity to produce
tumors. Gye confirms this temperature but does not agree that the virus has been destroyed but that a substance exists in the tumor which is sensitive to heat and is variable in amount and quality. In his opinion heat destroys the “specific factor” and not the virus in tumor extracts and after testing several germicides reports briefly on the use of acriflavine as an agent “to abolish the activity of tumor extracts.” Our investigations reveal that extracts of the Rous sarcoma treated with acriflavine 1:5000 for variable periods of time show activity up to and including 4½ hours incubation at 38° C. Incubation for greater periods of time up to 23 hours were innocuous when inoculated into fowls. Like chloroform treated extracts those treated for shorter periods of time produced tumors in some fowls and not in others. Our interpretation is that the virus was reduced in numbers or so attenuated that it was infectious for one fowl and not for another, the individual resistance or susceptibility of the fowl controlling the production of the tumor; Gye, however, interprets differently believing that the destruction of “specific factor” and not of virus is the matter involved.

But if “specific factor” is such a variable and labile substance how can the ease with which tumors are produced with dried tumor be answered? We held the original dried tumor received from Dr. Rous on October 3, 1925, in a refrigerator at 6–8° C. for 278 days. The vial was dated July 1, 1925. It was opened twice during this interval and resealed (without vacuum) and still produced active tumors at the end of 278 days in our possession and 358 days from time of drying.

Our substitution experiments with the Rous sarcoma extracts and mouse sarcoma 37/S have likewise failed to confirm the experiments of Gye and no results have been obtained that would cause us to change our previous conclusion that the “specific factor” is in reality nothing more than a suspension of attenuated living virus which may or may not produce a tumor depending upon the fowl’s susceptibility or resistance.

**CONCLUSIONS**

1. The infectivity of the Rous fowl sarcoma is usually completely destroyed by treating sand-paper and Berkefeld filtrates
of 5 per cent tumor suspension with 5 per cent chloroform for 3 hours or 1:5000 acriflavine for 6 hours at 38° C. Likewise by heating at 55–56° C. for 18 minutes.

2. Inoculation of Plymouth Rock chickens with subcultures of fowl sarcoma and 5 per cent chloroform treated filtrates have produced no tumors.

3. Inoculation with subcultures and 1:5000 acriflavine treated filtrates produced no tumors.

4. Inoculation with chloroform and heat treated filtrates produced no tumors. Figs. 1 and 2.

5. Inoculation with acriflavine and heat treated filtrates have with one exception produced no tumors.

6. Substitution experiments employing mouse sarcoma 37/S yielded negative findings.

7. The results are interpreted as failing to confirm Gye's hypothesis on the etiology of malignant new growths.

REFERENCES