The Uptake and Distribution of Radioactive Phosphorus in Chicken Eggs Containing a Rapidly Growing Mammary Tumor of a C3H Mouse*

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Tumors of rats and especially of mice have been grown in chicken eggs for a number of years. Tumors of sufficient size and uniformity as to be used in chemotherapy have been produced by the yolk sac technic, whereby the macerated tumor is injected into the yolk sac. The advantages of such a technic for screening chemicals for any tumor-inhibiting effects have already been pointed out by Taylor (1) and thus will be reviewed here only briefly.

The rapidly growing tumor and chick embryo grow within the closed system of the egg, sharing the same blood stream but without any physical contact between the two. It is thus possible to inject a substance between the egg shell and the chorio-allantoic membrane (analogous to a subcutaneous injection in mice) and, by weighing the tumor and chick embryo of both control and treated eggs, to determine whether the substance (a) has no effect, (b) inhibits tumor growth specifically, (c) inhibits embryo growth specifically, or (d) inhibits fast-growing tissues, in general.

The following experiments were undertaken to determine the manner of uptake and distribution of a substance injected between the chorio-allantoic membrane and the egg shell. For this purpose radioactive phosphorus in the form of Na₂HPO₄ was used.

METHODS

A mammary tumor of a C3H mouse was grown in chicken eggs using the yolk sac injection method (2). On the twelfth day of incubation (8 days after tumor inoculation), radioactive P³² in a 1 per cent aqueous solution of Na₂HPO₄ was injected between the chorio-allantoic membrane and the egg shell. The eggs were opened 48 and 96 hours later, and samples were taken of the various portions to be compared. These included the mouse tumor, the chick embryo skeleton, blood, allantoic fluid, yolk, and albumen. In another series the tumor and embryo brain and liver were compared as to P³² uptake.

The tissues were thoroughly macerated, and samples were dried to a constant weight which was then accurately determined. The activity of the sample was determined by use of a Geiger-Müller counter. The thickness of the samples was never enough to necessitate corrections for self-absorption. Decay corrections were made by comparison with a control sample of radioactive phosphate prepared from the same solution used for injection. The counts/minute/100 mg dry weight were used as the basis for comparison.

RESULTS

When P³² was injected between the egg shell and the chorio-allantoic membrane it was gradually taken up by the blood stream and distributed to the various parts of the egg. Table 1 shows the uptake and distribution of the P³² 48 and 96 hours after its injection.

<table>
<thead>
<tr>
<th>Material</th>
<th>Hours after P³²</th>
<th>Counts/minute/100 mg dry wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yolk</td>
<td>48</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>9</td>
</tr>
<tr>
<td>Albumen</td>
<td>48</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>35</td>
</tr>
<tr>
<td>Tumor</td>
<td>48</td>
<td>769</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>2,600</td>
</tr>
<tr>
<td>Chick embryo skeleton</td>
<td>48</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>12,900</td>
</tr>
<tr>
<td>Blood</td>
<td>48</td>
<td>11,800</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>961</td>
</tr>
<tr>
<td>Allantoic fluid</td>
<td>48</td>
<td>56,500</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>43,800</td>
</tr>
</tbody>
</table>

The uptake and distribution of the P³² 48 and 96 hours after its injection.

At 48 hours after injection most of the P³² was taken up by the blood and had passed through the system. The largest portion of it was excreted into
the allantoic fluid. The absorption by the yolk and albumen was only passive and was low enough to be unimportant. At this time there was comparatively little difference in P32 between the tumor and embryo skeleton. The skeleton continued to accumulate P32 more rapidly than the tumor and at 96 hours contained about 5 times as much. The amount in the blood dropped rapidly, due to its absorption by the tissues. There was a slight drop in the P32 found in the allantoic fluid, since the reabsorption of this liquid into the system had begun by this time.

Table 2 illustrates the results of an experiment comparing the uptake of P32 by the C3H mouse tumor, chick embryo brain, and chick embryo liver; 48 hours after injection of P32 the tumor has taken up more than either the brain or the liver.

### CONCLUSIONS

In the use of egg-grown tumors for chemotherapy it is important to know the manner in which the chemotherapeutic substances are taken up and distributed. While this process will not be identical for all such substances, a general idea may be obtained from the results of these P32 experiments.

The P32 is taken up and distributed via the blood stream, as can be seen by the increase and then decrease of the amount present. While some of the P32 is absorbed by the various tissues, the greater part of it passes into the allantoic fluid. It is thus evident that before a substance is discarded as having no tumor-inhibiting effect, it is necessary to determine whether its impotency is due to rapid excretion or physiological inactivity. If it is the former, then another method of injection designed to prevent rapid excretion (e.g., yolk, oil solvents, etc.) is necessary.

The greater accumulation of P32 by the bones of the chick embryo than by the tumor is in agreement with results obtained in mice. Such a distribution of P32 would result in destruction of the bone marrow before enough could accumulate in the tumor to destroy it.

### ACKNOWLEDGMENTS

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### REFERENCES

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