Peters and associates (9, 11) demonstrated that the accumulation of citrate in normal tissues with fluoroacetate poisoning involves the activation of fluoroacetate and its condensation with oxalacetate to form fluorocitrate. This compound competitively inhibits aconitase and produces an accumulation of citrate. Potter and Busch (12) showed that when rats bearing transplanted tumors were given toxic doses of fluoroacetate, large but varying amounts of citric acid accumulated in most organs examined, but not in the tumors. Using cell suspensions of a mouse lymphosarcoma, Kit and Greenberg (7) found that fluoroacetate in vitro induced a slightly higher accumulation of citrate. In fortified homogenates, Dietrich and Shapiro (4) found that in only one of seven varieties of transplantable mouse tumors did fluoroacetate produce a significant increase in citrate accumulation; they therefore concluded that most mouse neoplasms cannot effectively activate fluoroacetate. From subsequent experiments, Busch et al. (1) concluded that rat tumors do possess the enzymatic capacity to activate fluoroacetate and accumulate citrate. The earlier failure of transplanted rat tumors to accumulate citrate in vivo (12) was ascribed to limiting amounts of oxygen and substrate within the transplant.

A dependent variety of the transplantable mammatropic pituitary tumor developed by Furth and his associates (6) arose and was briefly available for in vitro studies, along with the more readily available autonomous variety. Since both neoplastic and normal tissues exhibit different intrinsic abilities to activate fluoroacetate, we measured the enzymatic capacity of tumor tissue to activate fluoroacetate and to accumulate citrate. We were especially interested in possible differences between the autonomous and dependent varieties. The cellular heterogeneity of the normal adenohypophysis makes comparison of the tumors with their tissue of origin circumstantial. The subsequent transformation of the dependent variety to an autonomous state prevented further studies.

**MATERIALS AND METHODS**

The animals were maintained and the transplantations performed as previously described (6). The autonomous tumors (strain MtT F4) were transplanted into castrate male F1 hybrids of highly inbred Fischer and Wistar strains. The dependent tumors (MtT F10) were transplanted into similar hosts into which a pellet containing 1.0 mg. of diethylstilbestrol and 18 mg. cholesterol was implanted simultaneously into the subcutaneous tissue of the back of the neck. Six control rats were given injections with the same suspension of dependent tumor cells, but the stilbestrol pellets were omitted. The control animals were kept for an additional 6 months (or until death) and then autopsied. In no case was a gross tumor found in the untreated controls; this demonstrated the stilbestrol-dependent state of the tumor (6).

The kidneys and livers were obtained from male Fischer rats. Since the normal rat adenohypophysis weighs only ca. 10 mg., pituitaries from 38 male and 36 female Fischer rats were used in each of two experiments, respectively. The tumors used had originally arisen following stilbestrol treatment of this same inbred strain. The rats were stunned, and the tumors and organs were excised immediately and placed in ice-cold 0.9 per cent NaCl. Necrotic tissue was trimmed away, and homogenates containing 20 per cent of wet tissue (weight per volume) were made with 0.25 M sucrose in a chilled, smooth-glass homogenizer fitted with a motor-driven Teflon pestle. The pooled adenohypophyses were homogenized in a chilled, ground-glass hand homogenizer, 10 per cent wet weight of tissue per volume, but were
of fluoroacetate were incubated to serve as controls. The homogenates were centrifuged at 700 × g for 10 minutes at 3° C. This centrifugation sedimented nuclei, residual whole cells, and debris, and floated any small amounts of fibrous connective tissue present. The resulting homogenates were incubated in beakers in a Dubnoff incubator at 37° C. with shaking at 25 cycles per minute. Each 5-ml beaker contained 0.33 ml of freshly prepared homogenate and 0.67 ml of water containing 23 µmoles of K+, 3.3 µmoles of Mg++, 6.7 µmoles of Cl−, 3.3 µmoles of phosphate, 18 µmoles of fumarate, 0.75 µmoles of diphosphopyridine nucleotide, 0.67 µmoles of adenosinetriphosphate, and 5 µmoles of monosodium fluoroacetate, all adjusted to pH 7.4 with phenol red as an indicator. The fluoroacetate was freshly prepared each time or stored at −20° C. Duplicate vessels containing equimolar amounts of acetate instead of fluoroacetate were incubated to serve as controls.

The mixture was deproteinized after a 1-hour incubation by the addition of 1.0 ml of ice-cold 40 per cent trichloroacetic acid, and the contents of the vessels were transferred to centrifuge tubes. After centrifugation, aliquots of the supernatant fluid from each vessel and from similarly prepared non-incubated controls were analyzed for citric acid content of each homogenate was determined by a micro-Kjeldahl method.

RESULTS AND DISCUSSION

The fluoroacetate-activating ability of dependent and autonomous varieties of stilbestrol-induced, mammotrophic pituitary tumors and of the normal pituitary tissue of the same highly inbred strain from which the tumors originally arose was determined with citrate production used as a measure of this ability. Fumarate was used as substrate, since previous in vitro studies (9) had shown it to be a convenient, stable source for the generation of oxalacetate. Pyruvate was not used as co-substrate, since it was demonstrated that it actually decreased the inhibition produced by fluoroacetate, in spite of higher citrate accumulations in both the poisoned homogenates and their controls (9).

Previous studies indicated that fluoroacetate was well activated by kidney and poorly activated by liver (12); therefore, these tissues were examined under identical conditions to establish the validity of the in vitro system employed (Table 1). The values found for citrate accumulation in the kidney homogenates were lower but of the same order of magnitude as those found with slices of rat kidney (1) and were much higher than those found with homogenates of guinea pig kidney (9). This was not unexpected, since in the former experiments pyruvate was used as co-substrate and in the latter experiments the concentration of fumarate was approximately one third that used here.

**Table 1**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. EXPERIMENTS</th>
<th>CITRIC ACID PRODUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
<td>Fluoroacetate</td>
</tr>
<tr>
<td>Kidney</td>
<td>10</td>
<td>(5 × 10⁻⁹ M)</td>
</tr>
<tr>
<td>Liver</td>
<td>10</td>
<td>(5 × 10⁻⁹ M)</td>
</tr>
</tbody>
</table>

Analysis of variance (13) shows that fluoroacetate increases the citrate accumulation of these two tissues significantly (P < .01) as compared with the sodium acetate controls. There is likewise a significant difference (P < .01) between these two tissues in their response to this treatment.

Values are given as the mean ± standard error of the mean. The individual values for each experiment were obtained by subtracting the endogenous citrate content of the homogenate (non-incubated control) from the citrate content of the vessels containing sodium acetate and fluoroacetate after incubation.

The ∆ Citrate Production was obtained by subtracting the mean citrate production of the sodium acetate-treated homogenates from the mean citrate production of the fluoroacetate-treated homogenates.

The tumors, both dependent and autonomous, possessed the capacity to activate fluoroacetate as measured by the accumulation of citrate (Table 2). Inspection of the results would suggest that the tumors were apparently better able to activate the fluoroacetate than was the normal anterior pituitary tissue. However, the pituitary consists of a heterogeneous cell population. It seems unlikely that the metabolic differences could be due solely to differences in proportions of cell types, but this cannot be ruled out. It can be stated with certainty, however, that both the dependent and autonomous varieties of this tumor, the monomorphic origin of which is well established,1 these tumors appear to be monomorphic in the sense that they are derived from the “alpha-cell” or “acidophil” and are functionally mammo-somatotropic (4, 3, 6). Further evidence of their monomorphic character is the finding that the same electron-dense particles characteristic of the “alpha-cell” of the normal rat adenohypophysis are present in the cells comprising the dependent and autonomous tumors (5).

1 These tumors appear to be monomorphic in the sense that they are derived from the “alpha-cell” or “acidophil” and are functionally mammo-somatotropic (4, 3, 6). Further evidence of their monomorphic character is the finding that the same electron-dense particles characteristic of the “alpha-cell” of the normal rat adenohypophysis are present in the cells comprising the dependent and autonomous tumors (5).
have the enzymatic capacity to activate fluoroacetate and accumulate citrate.

In recent experiments, Reynolds\(^2\) found that increasing autonomy, as measured by decreasing responsiveness of thyrotropic mouse pituitary tumors to thyroid hormone, was associated with increasing glycolysis as measured by lactate production. In the light of current theories about dependent and autonomous tumors (8) and the biochemical characteristics of neoplastic tissue (8), it is of interest that there was no relationship between the dependency state of this tumor and its capacity to activate fluoroacetate and accumulate citrate.

### SUMMARY

1. The observation that transplantable tumors in the rat may possess the enzymatic capacity to activate fluoroacetate and accumulate citrate has been confirmed and extended with dependent and autonomous varieties of functional pituitary tumors.

2. The dependent and autonomous varieties of the tumor examined did not differ in this capacity.

### ACKNOWLEDGMENTS

All tumor-bearing and pedigree-control rats were received through the generosity of Drs. Jacob Furth and Kelly Clifton. The transplantations and autopsies were performed in their laboratory. We are very much obliged to them and to our associate, Dr. Dwain Hagerman, for their helpful suggestions.

### REFERENCES


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**TABLE 2**

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>NO. EXPERIMENT</th>
<th>CITRIC ACID PRODUCTION*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sodium acetate</td>
</tr>
<tr>
<td>Normal pituitary</td>
<td>4</td>
<td>(5 X 10^-5)</td>
</tr>
<tr>
<td>Dependent tumor MtTF10</td>
<td>4</td>
<td>7 ± 6</td>
</tr>
<tr>
<td>Autonomous tumor MtTF4</td>
<td>6</td>
<td>65 ± 20</td>
</tr>
</tbody>
</table>

* The data are expressed as in Table 1.

Analysis of variance shows that fluoroacetate increases the citrate accumulation of the tumors and the normal pituitary tissue significantly (P < .01) as compared with the sodium acetate controls. The differences between these tissues in their response to this treatment is barely significant at P < .05.
Activation of Fluoroacetate by Neoplastic and Normal Pituitary Tissue

Gerald E. Gaull and Claude A. Villee

*Cancer Res* 1959;19:726-728.

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