Migration of Intraperitoneally Injected Thyroid Cells in the Amazon Molly, *Poecilia formosa*¹

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ABSTRACT

We have previously reported the development of an extensive invasive growth of the thyroid gland of the gynogenetic teleost, *Poecilia formosa* (the Amazon molly), following i.p. injection of UV- or γ-irradiated thyroid cells. This result was surprising by comparison with mammalian work, in which the thyroid is rarely the site for tumor metastases, but the anatomy of the circulation of fish is different from mammals, and in fish the gills and thyroid gland would be among the first tissues in which injected cells might be arrested. Techniques using a fluorescent dye, ¹²⁵I membrane label, or [³H]thymidine label were used to follow the distribution of i.p. injected cells in the Amazon molly. Fish sampled as soon as 30 min after injection had some labeled cells dispersed in the connective tissue around the ventral aorta and in the bases of the gills, and by 1 to 4 hr large numbers of cells had moved into the thyroid region. A few cells still persisted there 200 hr later.

Experiments on the distribution of heat-killed cells indicated that the initial distribution of the cells was largely governed by mechanical factors. Injected cells would appear to be disseminated in fish by mechanisms similar to those in mammals.

INTRODUCTION

The spread of malignant cells from primary tumors involves 2 mechanisms of movement, one of passive transport and the other of active mobility. Detached tumor cells penetrate blood vessels and are carried in the blood stream to distant organs where they are halted in narrow capillary beds; there they become attached to the vessel wall, subsequently move through the capillary membrane, and invade neighboring tissue. Several studies have demonstrated that radioactively labeled tumor cells are distributed in the body at considerable distances from their original site of injection (1, 3). In humans, metastases are frequently found in the liver and the lungs; the brain, muscles, spleen, and thyroid are less likely sites for the development of metastases. A major factor which influences their place of development is the state of the circulatory system of the animal. Tumor cells tend to become arrested in the first tissue with a narrow, abundant capillary bed that they encounter. It would seem that their initial distribution is governed by mechanical factors.

Active pseudopodial activity is characteristic of most embryonic tissues but apparently of only a few normal adult tissues, notably blood cells. Normal thyroid cells, injected i.v. into the tail vein of mice have, however, been found in the lungs and have persisted there for periods of up to 1 year (9). Untransformed adult cells may also have the potentiality for movement and the capacity to pass through endothelial walls and move into extravascular tissue.

In recent papers, we have reported the development of extensive invasive growth of the thyroid gland of the Amazon molly, *Poecilia formosa* (Cyprinodontiformes, Poeciliidae), following the injection of UV- or γ-irradiated thyroid cells (5, 12). The Amazon molly, a small viviparous gynogenetic teleost, is an all-female species which grows in clones, so that cell and tissue transplants between members of a clone are not rejected. Dissociated thyroid cells from donor mollies were treated *in vitro* with measured doses of UV or γ-irradiation, and then injected i.p. into isogenic recipient fish. Nine months later, the recipients had developed massive invasive overgrowth of the thyroid gland.

This result was surprising by analogy with mammalian work in which the thyroid gland is rarely the site for tumor metastases. It might, in fact, be questioned whether the i.p. injected thyroid cells would reach the thyroid, to be able to initiate the lesions which we had described. The anatomy of the circulatory system in teleost fish is different from that of mammals, however, and the thyroid gland would be among the first tissues in which the injected cells might become arrested. The thyroid gland is located around the ventral aorta, close to the afferent arteries to the gills, into which area the chances of blood-borne carriage are high.

**The Anatomy of the Thyroid Gland and Gills in Fishes.** The thyroid gland of most teleost fish is diffuse and unencapsulated, consisting of isolated thyroid follicles dispersed in loose connective tissue, extending along the course of the ventral aorta and afferent branchial arteries. Follicles commonly occur in the bases of the gill filaments. The thyroid of the Amazon molly is atypically large compared with that of other closely related poeciliid fishes, and numerous scattered follicles occur from the level of the first afferent branchial arch posteriorly to the bulbus arteriosus (11).

The gills of fish consist of a gill arch, filaments, and lamellae. The filaments are supported by cartilage at one side of which is the afferent arteriole. Blood enters the gill lamellae from the afferent arteriole and leaves by the efferent vessel, which lies directly opposite (Chart 1a). The gill lamellae, delicate leaf-like structures which are covered by thin squamous epithelium, project from both sides of the filaments. The terminal blood vessels of the lamellae are not typical blood capillaries with definite boundary walls, which would present a barrier to the passage of the cells; instead the blood vessels terminate in sinusoids, which do not always have a complete endothelial layer separating them from the gill tissue. Between the outer epithelial walls of the gill lamellae are groups of supporting "pillar" cells with flanges only partially extending around the sinusoids. Except for the pillar cells, the lamellae are open.

1 This research was carried out at the Brookhaven National Laboratory under the auspices of the United States Department of Energy.

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Received May 22, 1978; accepted April 10, 1979.
Afferent arteriole surrounding the blood sinusoid (S). A ABC (A) is shown in the sinusoid. Epithelial layer (E) and the supporting pillar cells (P) with flanges (F) partially lamella (A-A. on Chart 1a) [after Grizzel and Rogers, (4)], showing the outer the efferent vessel, which is directly opposite. b, transverse section through a gill structures into which detached cells might readily move (Chart 1b).

From the disposition of the venous circulation, we expected that i.p. injected cells would be carried in the 2 portal systems, the hepatic portal and the renal portal, to the liver and kidneys and thence to the heart and gills. The circulation time in fish is ~60 sec so that injected cells would be transported rapidly from their initial site. The cells might then be arrested in any of these organs; from the gills, they could easily spread to the thyroid gland. The present study was undertaken to show that i.p. injected thyroid cells would migrate to the thyroid gland and remain there for several days.

MATERIALS AND METHODS

Studies of the dissemination of radioactively labeled tumor cells in mammals have met with various criticisms because of the use of mixed cell cultures, the release and reutilization of the label, and the rapid rate of loss of label from the cells (2). In view of these objections, we used 3 different labeling techniques to demonstrate the movement of i.p. injected cells in the Amazon molly. First, we labeled the cells with a vital dye, tetracycline. In a second series, the cell membrane was labeled with diazotized iodosulfanilic acid (125I). In our third series, cultured cells from embryonic tissue of P. formosa labeled with [3H]thymidine were injected. We sampled the fish at various times (0.5, 1, 2, 6, 24, 48, 72, 96, and 144 hr) after injection.

Collection of Thyroid Cells. Young Amazon mobbies, 2 months old and 2 cm long, from Clone 2 were used in these experiments. Donor fish were placed on ice for 5 min and then transferred to cold 95% alcohol, 2.6% sodium hypochlorite, and finally into a sterile fish Ringers solution (without calcium or magnesium). Scales were removed from the lower jaw, from the heart forwards to the tip of the jaw, and the thyroid tissue was dissected out (details are given in Ref. 12). The excised thyroid tissue was pooled and homogenized in buffer, until it consisted of a suspension of single cells with an occasional clump of 3 to 4 cells. Some suspensions were exposed to UV radiation at a dose of 20 J/sq m (see Ref. 5). The cells were centrifuged, washed, and suspended in buffer; 20-μl samples containing 2 to 5 × 10⁶ cells were injected into the abdominal cavity of recipient fish. Parallel studies made with unirradiated thyroid cells showed that there were no differences in the distribution of irradiated and nonirradiated cells with time.

Tissue was also taken from the liver and kidneys and prepared for injection in the same way.

Cell Labeling

Tetracycline Labeling. Thyroid cells were exposed for 1 hr to a solution of tetracycline (50 μg/ml; Squibb & Sons, Princeton, N. J.), in sterile fish Ringer’s solution. The cells were then washed 3 times until all traces of the tetracycline adhering to them had been removed; this was checked by examining the washings under UV. Tetracycline was not toxic at this concentration, nor did it diffuse out of cells which were held in buffer for 48 hr. In 2 separate experiments 4 recipient fish were given injections of thyroid cells, killed 4 hr later, and fixed overnight in buffered formaldehyde. A control group of 4 fish was given injections of tetracycline alone. The fish were dissected with a sharp razor blade into halves along the backbone, and the exposed organs were viewed under UV. The distribution of tetracycline-labeled cells was indicated by the distribution of fluorescence.

Iodosulfanilic Acid (125I) Labeling. High-specific-activity iodosulfanilic acid (125I) specifically labels cellular membranes (6). It does not penetrate the cell or appear to damage the membrane components or alter the surface properties of the cell. Suspensions of thyroid cells (irradiated and unirradiated), approximately 1.7 × 10⁶/ml, were exposed to freshly prepared iodosulfanilic acid (125I) (New England Nuclear, Boston, Mass.), and the mixture was left to react on ice for 60 min. The treated cells were centrifuged and washed 4 times with buffer. The fresh thyroid cells were very adhesive and tended to become attached to the sides of the reaction tube during labeling and to the centrifuge tube during the repeated washings. The cells were resuspended in a buffer at a concentration of 4 × 10⁶ cells in 20 μl for injection. Radioactivity of the sample was approximately 0.5 cpm/cell. The cells of one sample were killed by holding them at 60° for 15 min before they were injected. Dissociated liver and kidney cells were treated in the same way. A control sample of iodosulfanilic acid (without cells) was prepared which had the same amount of radioactivity, and this was injected into 4 control fish.

[3H]Thymidine Labeling. A homogenous population of cultured embryonic cells from P. formosa (buffered minimal Eagle’s medium with 4-(2-hydroxyethyl)-1-piperazinanesulfonic acid and 10% fetal calf serum) was labeled with [methyl-3H]thymidine (0.3 μCi/ml) for 24 hr. The cells were harvested, washed, and suspended in buffer for injection; radioactivity was 0.12 cpm/cell. One sample of labeled cells was killed.
were made each day. The cells remained viable, and there was
could be reutilized after the breakdown of cells that die after
the result of cell death and reutilization of thymine. The label
thin wax sections (10 μm) taken at selected intervals along the
scintillation spectrometer. Counts were also made on single
(or an accurately weighed part) and measuring radioactivity.
formic acid hydrolysate of cells showed that virtually all (99.5%)
of the label was in thymine. Since there is negligible DNA
synthesis in confluent cultures, the persistence of label is not
the result of cell death and reutilization of thymine. The label
could be reutilized after the breakdown of cells that die after
injection into animals, but the amount of such label per cell
would not only be appreciably less than in the injected cells
but it would be in a different type of cell.

Assessment of the Distribution of Labeled Cells

Radioactivity Counts of Whole Organs. In studies with
mammals, an assessment of the distribution of labeled cells
has commonly been obtained by dissecting out the whole organ
(or an accurately weighed part) and measuring radioactivity.
Similarly, we removed the liver, spleen, ovary, gut, thyroid
gland, and kidneys from injected mollies; weighed them; and
measured their 125I radioactivity in a Beckman Gamma 8000
scintillation spectrometer. Counts were also made on single
thin wax sections (10 μm) taken at selected intervals along the
length of the body.

After our initial series of assays (see "Results"), we consid-
ered that this method did not give a reliable estimate of the
distribution of labeled cells. The Amazon mollies were small,
averaging 1.5 cm in length; further, the thyroid gland and
kidneys are diffuse structures, so that it was difficult to dissect
out comparable samples of tissue. The ovaries were immature,
very small, and difficult to weigh accurately. Because of these
disadvantages, we preferentially used autoradiography of serial
sections to follow the dispersion of cells.

 Autoradiography of Labeled Cells. Five fish were killed at
each time period, fixed in buffered formaldehyde, decalcified,
and routinely embedded in paraffin wax. Serial sections
throughout the entire fish were cut at 10 μm, and every fifth
section was mounted on slides treated with 1% gelatin; altern-
atively, the slides were immersed in celloidin before proceed-
ing. The slides were processed for autoradiography with NTB
emulsion (Kodak) and exposed for 24 hr. After developing and
fixing, the slides were routinely stained with hematoxylin and
eosin.

RESULTS

Distribution of Cells in Whole Organs

Tetracycline-treated Cells. The preliminary experiments
with tetracycline-treated cells gave a gross but clear indication
of their distribution. In all of the experimental fish from both
experiments, fluorescence was heavily concentrated around
the heart, ventral aorta, and bases of the gill lamellae; the
remainder of the body showed little fluorescence. There was
a small concentration in a loop of the gut in several fish close to
where the injection had been given. The control fish which had
been given tetracycline only had a general weak fluorescence
throughout the muscles and in the gut. Frozen serial sections
were made from the 4 experimental fish in the second assay in
an attempt to show the finer details of the distribution of the
cells, but the results were not clear. Tetracycline tended to
diffuse rather quickly out of the cells and into neighboring
tissues (despite our in vitro findings), and by 24 hr after
injection the entire fish had a low level of fluorescence.

Iodosulfanilic Acid-labeled (125I) Cells. Radioactivity counts
were made on whole organs taken from 4 fish at 1 hr and from
5 fish at 24 hr after injection. The values obtained were ex-
pressed as percentages to normalize individual organ counts,
and the distribution of activity was compared at the 2 times
(Table 1). Because of the variability in the assay (see "Materials
and Methods"), we calculated the significance of the difference
using Wilcoxon's 2-sample test, completely random design, as
modified by Mann and Whitney (8). The results showed that
the radioactivity of the thyroid gland increased significantly (at
the 5% level) during the period from 1 to 24 hr. There was a
decline in the activity of the spleen and kidney; the former
approached significance.

 Autoradiography

Iodosulfanilic Acid-labeled Cells (125I). Fish sampled as
soon as 0.5 hr after injection of thyroid cells had some labeled
cells dispersed in the connective tissue around the ventral
aorta, the pseudobranchs, and in the bases of the gill filaments.
Cells were also present in the kidney and liver tissue, usually
in close proximity to the large blood vessels. Some cells were
seen in other major blood vessels, i.e., the internal carotid
sinuses, the choroid gland, the hepatic vein, the hepatic portal
vein, the posterior cardinal veins, and also in the cells of the
anterior intestinal epithelium. There were no labeled cells in the
blood vessels, in the choroid gland, or in the pseudobranch of
the fish sampled 1 hr after injection. In an attempt to assess
the changes in distribution of the cells with time, the number of
labeled cells was counted on sections from comparable areas
in the thyroid, liver, and kidneys. Since the thickness of the

<table>
<thead>
<tr>
<th>Organ</th>
<th>% of radioactivity</th>
</tr>
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<tbody>
<tr>
<td>Thyroid</td>
<td>30.8</td>
</tr>
<tr>
<td>Gut</td>
<td>18.0</td>
</tr>
<tr>
<td>Liver</td>
<td>38.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.8</td>
</tr>
<tr>
<td>Ovary</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Table 1

The distribution of radioactivity in various organ 1 and 24 hr after
i.p. injection of 125I-labeled thyroid cells

There is a significant increase in the activity of the thyroid gland (at
5% level).
sections was standard (10 μm) and the frequency of sections mounted was constant (every fifth section), we selected for our comparison sections at the same distance from the tip of the snout. The fish differed in size, however; therefore, we verified as closely as possible that the sections were taken from closely comparable areas from the appearance of the organ in question and from the presence or absence of other structures. Thus, the section chosen for the thyroid area and gills was located in the posterior portion of the gill chamber at the level of the origin of the fourth gill arch. Counts were made on a selected section in the head kidney tissue immediately after termination of the heart. For the liver counts, a section was used at the point where the organ approached its maximum size between the head and trunk regions of the kidney. This method did not have quantitative precision; nevertheless, the results clearly showed the changes in distribution of the labeled cells in the body with time. The results are shown in Table 2. The concentration of labeled cells in the thyroid region reached a peak at about 4 hr (Fig. 2). Thereafter, the number of cells in the liver and kidneys declined, but large numbers of cells persisted in the thyroid gland for the next 72 hr. Few cells remained in the thyroid by the end of 144 hr; in about one-half of these cells, the grains were no longer tightly grouped together but had begun to spread. We gained the impression that these cells were starting to die and disintegrate.

Fish given injections of 125I-labeled liver or kidney cells showed a different pattern of distribution with time. Initially, at 0.5 and 1 hr, cells occurred in the thyroid area, gills, kidney, liver, and large blood vessels. Values on our standard sections averaged 50 cells for the thyroid and about 20 cells for the liver and kidneys. By 4 hr, however, the numbers of cells had decreased throughout the entire body, only around 10 to 20 remaining in the thyroid region. Twenty-four hr after the injection, hardly any cells remained.

Fish given injections of iodosulfanilic acid alone had at 0.5 hr very heavy concentrations of activity in the blood vessels of the gills and in the colloid of thyroid follicles lying close to the ventral aorta. The radioiodine was located in the colloid as a peripheral ring close to the epithelial cells (Fig. 2). There was no concentration of activity in the kidney or liver tissue. Activity, measured in a scintillation spectrometer on thin-wax sections, was high throughout the length of the body and remained high for 24 hr. Thereafter, activity declined indicating that radioiodine was being discharged from the body. As activity decreased in the body, it continued to intensify in the colloid of the thyroid follicles, particularly in those proximate to the ventral aorta. This trend continued for 48 hr, but later samples showed a fall in activity of the colloid. The distribution of radioactivity was quite distinct from that seen after the injection of labeled cells.

[3H]Thymidine-labeled Cells. The thyroid gland has a strong affinity for iodine, which is rapidly accumulated in the colloid of thyroid follicles. Although the distribution of thyroid cells labeled with 125I contrasted with that of 125I alone, we further confirmed our findings using cultured embryonic fish cells, which had been labeled with [3H]thymidine. The resulting distribution of labeled cells with time was essentially the same as we had obtained in the previous assay with 125I-membrane-labeled cells. Small numbers of cells (averaging 10 to 20) were located in the thyroid area, liver, and kidneys 30 min after injection. Their numbers reached a maximum in the thyroid at 4 hr (averaging 50 cells) but had declined by 48 hr when counts of 10 to 20 were observed. They occurred only sporadically in the 96-hr sample. A few cells were observed in the kidney at 2 hr, and groups of intact cells were noted in the opisthionephric ducts, the main excretory ducts, which extend throughout most of the kidney tissue and end in the urogenital pore. A few cells persisted in the liver for 48 hr.

Labeled cells were not observed in the muscles, ovaries, and brain in either series of experiments. In 2 fish samples, 0.5 hr after injection, there were small groups of cells lying close to the posterior intestine where the needle had penetrated. No labeled cells were seen, however, in the later samples.

The Migration of Dead Cells. We had suggested that injected cells would, in the first instance, be passively transported in the circulation to the thyroid gland and gills. In order to demonstrate this passive carriage, we gave fish injections of 125I- or [3H]thymidine-labeled cells, which had been subsequently killed by heat treatment, and followed their distribution in the body with time. The results with [3H]thymidine-labeled cells were the most striking. Cells were present in the gill arterioles after 30 min, and after 1 hr there were heavy concentrations of cells in these vessels (Fig. 3). The cells were not found in the connective tissue around the ventral aorta and did not appear to have moved from the gills. Occasional cells were noted in the blood capillaries of the kidneys and of the liver. No activity was found in the gills of fish taken 4 hr after injection. Dead cells labeled with 125I were also transported to the gills but in fewer numbers. Thyroid cells are very adhesive and readily form clumps and stick to surfaces. In the fish sampled 1 hr after injection, there were clumps of about 12 to 20 cells lodged amongst the fat or lying free in the peritoneal cavity; some cells were already located in the gill arterioles. There was sparse activity at the periphery of the colloid of the thyroid follicles, and activity was also associated with the proximal sections of the renal collecting tubules. Clusters of labeled cells persisted in the peritoneal cavity of fish sampled at 6, 24, 48, and 75 hr after injection, although the number of cells in the clusters gradually diminished as the cells disintegrated. In our last sample (75 hr), the clumps had been reduced to about 2 to 4 cells. There was some activity in the thyroid colloid throughout the entire period which did not intensify at any time. Similarly, activity was seen in the kidney tubules in all of the samples. No activity was found in other organs.

Clearly, dead cells labeled with 125I were transported in the circulation to the gill arterioles but in fewer numbers than dead labeled cultured cells. We attribute this to differences in the

Table 2

<table>
<thead>
<tr>
<th>Time after injection</th>
<th>No. of cells</th>
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<tr>
<td>Thyroid</td>
<td>Liver</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>30 min</td>
<td>70 ± 12</td>
</tr>
<tr>
<td>60 min</td>
<td>190 ± 10</td>
</tr>
<tr>
<td>4 hr</td>
<td>315 ± 24</td>
</tr>
<tr>
<td>12 hr</td>
<td>265 ± 18</td>
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<tr>
<td>24 hr</td>
<td>224 ± 15</td>
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<tr>
<td>48 hr</td>
<td>150 ± 19</td>
</tr>
<tr>
<td>72 hr</td>
<td>108 ± 8</td>
</tr>
<tr>
<td>96 hr</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>120 hr</td>
<td>27 ± 3</td>
</tr>
</tbody>
</table>

* Mean ± S.D. of 5 fish.
contact behavior of cultured cells and thyroid epithelial cells, the latter having a greater tendency to establish permanent contacts with each other. Thus, throughout the experiment, many of the thyroid cells remained in clumps in the peritoneal cavity, and as they slowly disintegrated, iodine was released into the circulation. Some of this was accumulated by the thyroid colloid, but at the same time it was excreted from the body so that the amount concentrated in the colloid was never great.

Persistence of Cells in the Thyroid Region. In order to demonstrate that injected cells would lodge and remain in the thyroid area for several days, we exposed a sample of cells to a lower level of radioactivity (0.03 μCi ³H per ml), giving a value of 0.012 cpm/cell, and extended the experiment to 200 hr. A few labeled cells persisted among the connective tissue around the ventral aorta; in our standard section through the gland area, between 2 to 5 cells were observed. Occasionally, isolated labeled cells were seen in the bases of the proximate gill arches. On the majority of liver sections, 1 or 2 labeled cells were found located amongst the hepatocytes close to branches of hepatic portal vein. There were no labeled cells seen in the kidney.

DISCUSSION

These experiments have shown that UV-irradiated and normal thyroid cells injected into the abdominal cavity of the Amazon molly rapidly reach the thyroid gland and remain there, so as to be able to initiate the lesions which we have previously described (5, 12).

The establishment of metastases in distant organs far from a primary tumor is thought to depend upon several factors. Detached cells, carried by the blood stream, become arrested in the narrow capillary bed of the first organ that they encounter. They must survive the turbulence of the blood stream and resist destruction by the host’s defense mechanisms. They then may attach firmly to the wall of the capillary and subsequently gain entry to extravascular tissues (2). Untreated adult thyroid cells from mice, injected i.v., are disseminated in the blood stream to the lungs, where they pass through the vascular epithelium to become lodged in the interstitial tissue. Whether the cells moved actively through the endothelium or whether the endothelium developed temporary small openings, whether the cells moved actively through the endothelium or whether the endothelium developed temporary small openings, was unknown (9). We see no difficulties in explaining the movement of injected cells in the Amazon molly in a similar way. Cells might readily enter the venous circulation (veins offer little resistance to the entry of cells) and be transported to the liver, kidney, and gills where they could be arrested in the microcirculation, as we have found with dead cells which were injected i.p. and rapidly accumulated in the arterioles of the gills. The open structure of the gill lamellae provides a particularly facile passage for cells into extravascular tissue. Cells might then easily reach the thyroid gland since it does not have an encapsulating membrane as in mammals, which is an effective barrier to invasion. Further, the connective tissue, among which the thyroid follicles are dispersed, has a loose texture with abundant spaces in which invading cells might become lodged.

The ability of nonhomologous cells to establish themselves and grow in other tissues depends upon their capacity to resist destruction by the host’s defense mechanisms and their ability to adhere firmly. The Amazon molly grows in clones, and injected cells taken from members of the same clone are not injected by the recipient. Further, the follicular cells of the thyroid gland are epithelial cells which readily establish permanent contact with each other after collision. Indeed, Fidler (2) suggests that any contact between epithelial cells has a high probability of becoming a lasting one.

The initial dispersion of liver, kidney, and embryonic cells in the thyroid gland shortly after injection again indicates that this was accomplished by passive carriage. However, while thyroid cells persisted in the area of the gland, other types of cells remained for only short periods. Permanent contacts may not be established with nonhomologous cells which then die and are eliminated. A ‘‘soil’’ hypothesis was suggested many years ago (7), attributing different tissues with distinct metabolic properties which might encourage or inhibit the growth of invading cells. There is some evidence that local ‘‘soil’’ factors are important in determining whether invading cells will persist and flourish (10).

Our experiments essentially were qualitative ones, and we have no data on the number of cells which might be required to produce or initiate a thyroid lesion or upon the length of time that they must remain viable in situ to be able to do so. In a quantitative analysis of the fate of tumor emboli injected i.v. into mice, Fidler (1) has shown that only 400 cells of a total of 200,000 injected survived. He suggested that very few tumor cells were required to initiate a metastasis and that even a single cell might suffice. In our experiments, a few intact cultured cells labeled with low levels of ³H-thymidine remained in the thyroid gland for 200 hr after injection. This is well in excess of the doubling time of embryonic fish cells in our cultures, which generally reach confluency 96 hr after seeding and probably also considerably more than the cell cycle time of adult thyroid cells.

ACKNOWLEDGMENTS

We are grateful to R. W. Hart for providing the initial cultures of fish cells and to E. Grist for maintaining the cultures for us. Photomicrographs were taken by W. Marin, Jr.

REFERENCES

Migration of i.p. injected Cells in the Amazon Molly

Fig. 1. a, low-power photograph of a radioautogram of a transverse section through the head of an Amazon molly showing 125I-labeled injected thyroid cells in the connective tissue of the thyroid region. × 11. b, high-power photomicrograph from a, showing the thyroid region with the labeled cells. × 150.

Fig. 2. High-power photomicrograph of a radioautogram through the thyroid region of an Amazon molly given an injection of 125I alone. The activity is located at the periphery of the colloid. × 150.

Fig. 3. A radioautogram showing that heat-killed [3H]thymidine-labeled cells accumulate in the arterioles of the gills. × 250.
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