The selective removal of colloidal material from the bloodstream by cells of the reticuloendothelial system makes it possible to irradiate such cells preferentially by the intravenous administration of radioactive colloids. Thus, intravenously injected colloidal Au\(^{98}\), owing to its localization in Kupffer cells, has been found to produce cirrhosis in dogs (6) and severe liver damage in rats (11). Similarly, neoplasia of reticulum cells or vascular endothelium in the liver has been observed in animals (2, 17) and in man (15) after the intravenous injection of thorotrast.

The present experiment was undertaken in an attempt to produce reticulum-cell tumors in mice, since in a previous study reticulum-cell sarcoma had been encountered in two mice surviving the intravenous administration of large doses of colloidal Au\(^{98}\) (5).

**MATERIALS AND METHODS**

Colloidal Au\(^{98}\), diluted in 0.1—0.25 ml of sterile physiologic saline solution, was injected intravenously into 27 1-month-old RF and 23 4-month-old C57BL female mice (Table 1). Varying amounts of Au\(^{98}\) were administered, in search of a dose which would produce tumors of reticuloendothelial cells; the total radioactivity of each mouse, determined by assaying each animal in a 4 \(\pi\) geometry ion chamber (9) 2 days after injection, varied from 44 to 1350 \(\mu\)c. Studies of the radioactivity distribution, carried out 5 days after injection, indicated that more than 80 per cent of the administered gold was concentrated in the liver (Table 2), in agreement with observations in animals and man (13, 21, 23). Red blood cell, total leukocyte, and differential blood counts were made on representative mice, and a limited number of animals were sacrificed for histological study during the first 2 weeks postinjection.

Thirty RF female mice 1 month of age, injected intravenously with an aliquot of the colloidal Au\(^{98}\) after it had been allowed to decay for 60 days to negligible levels of radioactivity, served as additional controls. All animals were housed in groups of approximately eight per cage in an air-conditioned room and were observed until natural death, or were killed in extremis. Purina Laboratory Chow and drinking water were supplied ad libitum. Post mortem examinations were performed on all mice, and histological studies were carried out as needed for diagnostic purposes.

**RESULTS**

**Survival.**—Of eight animals receiving more than 400 \(\mu\)c., seven died within 2 weeks from acute radiation sickness, and one after 9 months from cirrhosis. Those dying early exhibited marked lymphoid atrophy, necrosis, and aplasia of bone marrow, hemosiderosis of the spleen, gastroenteritis, purpura, and necrosis of granulosa cells and of ova in the ovarian follicles. Blood counts performed 2–5 days after injection disclosed that doses in excess of 400 \(\mu\)c. resulted in marked leukopenia and lymphopenia; whereas, after doses of less than 100 \(\mu\)c., the neutropenia was only slight and the lymphopenia moderate. The survival time and incidence of liver damage, hepatoma, and leukemia in mice receiving 150 \(\mu\)c., or smaller doses, of Au\(^{98}\) are shown in Table 2.

**Liver damage.**—On gross inspection, the liver disclosed slight enlargement and bluish-red discoloration in mice sacrificed or dying within the first 2 weeks after injection of more than 400 \(\mu\)c. On microscopic study, necrosis of isolated liver cells first became apparent 5–7 days after injection. Confluent areas of necrosis were conspicuous several days later, chiefly in the midzonal region of the lobule (Fig. 1). The early alterations noted were essentially identical to those reported by
Koletsky and Gustafson (11). In 45–88 per cent of mice dying 8 months or more after injection of 44–160 μc of colloidal Au\textsuperscript{198}, the liver was characterized by lipoidosis, patchy parenchymal atrophy, and nodular regenerative hyperplasia (Table 1). The distribution of these changes within the liver was highly irregular. The atrophy was primarily central, associated with displacement of central veins and obliteration of the normal lobular architecture (Fig. 2). Occasionally, massive, fibrous scars were found, transecting the greater part of a lobe (Fig. 3), but periobular or intralobular fibrosis was scanty or absent, in contrast to that in the human cirrhotic liver. Bizarre patterns of hepatic cords were often encountered in which the parenchymal cells were of highly varying size and shape.

**Tumors of the liver.**—Foci of hyperplasia of liver cells were noted alternating with foci of degeneration (Fig. 9). In the hyperplastic regions, which were of varying size, the cells were enlarged and exhibited large, hyperchromatic nuclei and abundant, usually slightly basophilic cytoplasm. These cells formed nodular masses, which in some instances were large enough to be grossly visible; when the nodules exceeded 4 mm. in diameter, they were arbitrarily called hepatomas (Fig. 10). Such tumors occurred in 10–28 per cent of livers damaged by radiogold (Table 1) and developed long after administration and decay of the isotope (Chart 1). Although there was anaplasia and local invasion in two cases, extrahepatic metastases were not encountered.

### Table 1

<table>
<thead>
<tr>
<th>Dose (μc.)</th>
<th>STRAIN</th>
<th>No. of MICE</th>
<th>Time (mo.)</th>
<th>Mean Survival (Per cent)</th>
<th>Liver Damage (Per cent)</th>
<th>Hepatoma (Per cent)</th>
<th>Leukemia (Per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44-91</td>
<td>RF</td>
<td>7</td>
<td>20</td>
<td>3</td>
<td>45</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>85-160</td>
<td>RF</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>80</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>0 (decayed Au)</td>
<td>RF</td>
<td>30</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0 (noninjected)</td>
<td>RF</td>
<td>29</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>150</td>
<td>C57BL</td>
<td>23</td>
<td>22</td>
<td>19</td>
<td>85</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>0 (noninjected)</td>
<td>C57BL</td>
<td>27</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Includes all hematologic types.

### Table 2

<table>
<thead>
<tr>
<th>Total Body Radioactivity (μc.)</th>
<th>Liver</th>
<th>Spleen</th>
<th>Intestine</th>
<th>Kidney</th>
<th>Lung</th>
<th>Mouse no.</th>
</tr>
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<tbody>
<tr>
<td>1850</td>
<td>81.4</td>
<td>4.2</td>
<td>4.2</td>
<td>0.6</td>
<td>0.5</td>
<td>C57BL 1</td>
</tr>
<tr>
<td>615</td>
<td>81.5</td>
<td>3.1</td>
<td>3.2</td>
<td>0.2</td>
<td>0.04</td>
<td>C57BL 2</td>
</tr>
<tr>
<td>440</td>
<td>83.5</td>
<td>1.2</td>
<td>2.2</td>
<td>0.2</td>
<td>0.03</td>
<td>C57BL 3</td>
</tr>
<tr>
<td>34</td>
<td>85.0</td>
<td>1.9</td>
<td>2.5</td>
<td>0.2</td>
<td>0.0</td>
<td>RF 2</td>
</tr>
<tr>
<td>54</td>
<td>80.0</td>
<td>1.9</td>
<td>1.5</td>
<td>0.5</td>
<td>0.01</td>
<td>RF 3</td>
</tr>
</tbody>
</table>

* The radioactivity of the whole body and of the separate organs was determined in a 4 σ geometry ion chamber (see [9]).

and separated by broad, irregular sinusoids (Fig. 4). In addition, many liver cells were enlarged, particularly those in the portal regions, giving rise to giant cells with acidophilic cytoplasm and large, atypical nuclei (Figs. 2, 5). These nuclei frequently contained spherical eosinophilic inclusions (Fig. 5). Cell gigantism occasionally appeared to involve an entire lobule.

Lipoidosis (degenerative fatty infiltration) occurred in the form of many small, in part confluent, intracytoplasmic droplets of neutral fat (Figs. 6, 7). Fat-laden cells were most numerous in the midzonal region, but their frequency and distribution were irregular from one lobule to another. Heterotopic ossification in the liver with formation of bone marrow (Fig. 8) was observed in five of 31 animals studied histologically.

Proliferation of bile ducts was observed in three mice (Fig. 11); however, fully developed cholangiomas were not found. Proliferation of Kupffer cells was observed in but few animals, and neoplasia of these elements was not noted; however, the site of origin of the reticulum-cell sarcomas is uncertain.

**Leukemia.**—The frequency of leukemias encountered in radiogold-injected animals was not significantly different from that noted in the controls (Table 1); one case of myeloid leukemia occurred in an RF mouse dying 9 months after receiving 95 μc., and one thymic lymphoma in an RF mouse dying 10 months after receiving 158 μc. of Au\textsuperscript{198}; the latter exhibited, in addition, severe irradiation injury of the liver. Reticulum-cell sarcoma was noted in only one of the 27 radiogold-injected RF mice and in two controls of the same
strain; it did not occur in the C57BL mice. The other leukemias were lymphomas, usually generalized, with no gross thymic involvement.

Ovarian tumors.—Although necrosis of granulosa cells and ova was evident within 2 weeks after a dose of Au\(^{198}\) in excess of 400 \(\mu\)c. and ovarian atrophy was observed late in life in all radiogold-injected mice, tumors of the ovary were noted in only one mouse of each strain. Both were lymphomas and occurred 20 and 25 months after administration of 138 and 158 \(\mu\)c., respectively.

Other neoplasms.—Pulmonary adenomas occurred in only one of the C57BL mice, a control which died at 20 months of age. In the RF strain, adenomas of the lung developed in six of 27 mice (22 per cent) receiving radioactive gold, seven of 30 mice (23 per cent) given nonradioactive (decayed) gold, and two of 29 noninjected controls (7 per cent). These adenomas developed late in life, the earliest being detected at 16 months of age.

DISCUSSION

The literature on radiation-induced hepatic damage has been reviewed by Friedman (4) and by Koletsky and Gustafson (11). With massive, local irradiation, the sequence of pathologic changes in the liver is analogous, except for distribution, to that following diffuse hepatic irradiation by incorporated radioactive substances. Direct cellular radiation damage has been postulated as the primary mechanism in the pathogenesis of these alterations (11). That dietary or metabolic factors may, however, potentiate irradiation injury of the liver is suggested by the experiments of White et al. (25), who observed the development of cirrhosis in irradiated rats fed a protein-deficient diet. The lesions noted in the mice of the present study, as in rats (11) and dogs (6) given radiogold, differ from the atrophic cirrhosis of man in lacking periportal fibrosis. This may be attributable to differences in the sites and modes of cellular damage; in the experiments with radiogold the hepato cellular necrosis occurred rapidly and was maximal in the midzonal region of the lobule; whereas, in human atrophic cirrhosis or in experimental dietary cirrhosis, the necrosis develops gradually and is most marked at the periphery, presumably because of the relative vascular insufficiency in this location (7, 19).

The development of hepatomas following administration of Au\(^{198}\) is consistent with the known carcinogenic properties of ionizing radiation. The dose administered to the liver by 40–160 \(\mu\)c. of radiogold injected intravenously was calculated to be 850–3400 rep, on the basis of the data of Marinelli et al. (16). Hepatomas have also been noted in rats given radiogold intravenously (Koletsky, personal communication), and hepatoma-like lesions have been produced in mice by the injection of radioactive colloidal chromic phosphate (10) and in mice and rats by the administration of plutonium (14). Lorenz (5) reported slight elevation of the incidence of hepatomas in mice exposed to chronic whole-body gamma irradiation, and hepatic tumor formation has been observed in guinea pigs (3, 18) and man (22) after local radium irradiation and in a rabbit following exposure to neutrons (12). The pathogenesis of the radiation-induced hepatomas is not clear (1, 5).

It was anticipated that radioactive colloidal gold might produce neoplasia of reticulum cells by virtue of its concentration in the reticuloendothelial system. Reticulum-cell sarcoma was observed in a rat after administration of thorotrast (17) and in two mice surviving the intravenous injection of large amounts of colloidal Au\(^{198}\) (5); however, since this disease may occur spontaneously in aging rodents, these few observations do not prove that the neoplasm was induced by irradiation. Cytologic changes in the Kupffer cells interpreted as “precancerous hemangioendothelioma” were produced in rats (2), and an endothelial sarcoma of the liver was produced in a woman (15), by the intravenous injection of thorotrast. The failure to induce such tumors with Au\(^{198}\) may be attributable to lower dosage or to the duration of radioactivity in the liver, Au\(^{198}\) decaying fast and thorotrast acting over a longer period.

The slightly reduced longevity of the radiogold-injected RF mice can probably be accounted for...
by hepatic injury; however, even in the absence of detectable organ damage or neoplasia, relatively low doses of radiation are known to significantly diminish survival in mice (24).

The elevated incidence of pulmonary tumors in the RF mice injected with radiogold is consistent with earlier observations on the effects of irradiation in this strain (24); however, a similar increase occurred in animals injected with nonradioactive (decayed) colloidal gold. Since the amount of gold injected per mouse was small (<0.1 mg.) and, as a colloid, most of it became localized in the reticuloendothelial system, a rise in the incidence of pulmonary tumors is puzzling. In view of the known carcinogenic activity of some other metals (8), it is conceivable that the metallic gold may have exerted a tumorigenic action. The extraordinary sensitivity of the lining cells of the alveoli to lung tumor induction is well known. Much of the intravenously injected gold must have passed through the pulmonary capillaries, and Rogers (20) has shown that even transient contact of pulmonary lining cells to nitrogen mustard is carcinogenic to these cells.

SUMMARY

1. Colloidal radioactive Au\textsuperscript{198} was injected intravenously into mice in large doses in an attempt to produce neoplasms of the reticuloendothelial cells. The Au\textsuperscript{198} became rapidly concentrated in these cells, over 80 per cent being retained in the liver.

2. Of eight mice receiving 475–1350 µc., seven died within 2 weeks with leukopenia, necrosis of the bone marrow, and hepatic degeneration, and one mouse surviving 9 months died with cirrhosis.

3. A dose of 44–160 µc. of Au\textsuperscript{198}, estimated to deliver 850–3400 r. to the liver, caused no early deaths but produced chronic liver damage in 45–83 per cent and hepatomas in 10–26 per cent of mice injected.

4. In five of 31 livers examined microscopically, foci of ossification with marrow formation were noted.

5. The incidence of leukemia was not significantly altered, and neoplasms of reticuloendothelial cells were not produced by the colloidal Au\textsuperscript{198}.

6. An increase in the incidence of pulmonary adenomas occurred in mice receiving both radioactive and nonradioactive colloidal gold.

ACKNOWLEDGMENTS

The authors are grateful to Mr. W. D. Gude, Mrs. E. S. Ledford, and Mrs. F. F. Wolff for technical assistance.

ADDENDUM

Since the preparation of this manuscript, Guimaraes et al. have reported the occurrence of hepatomas and lung tumors in mice surviving 13–21 months after the intravenous injection of 0.1–0.5 ml. of thorotrast (Brit. J. Cancer, 9:258–67, 1955).

REFERENCES


FIG. 6.—Advanced hepatic lipoidosis, with a lobular distribution of fat-laden (foamy) liver cells. H & E ×80.

FIG. 7.—Patchy, irregular distribution of fat-filled foamy liver cells, with some necrosis and marked nuclear abnormalities. H & E ×100.

FIG. 8.—Heterotopic bone in the liver, with bone marrow. H & E ×80.

FIG. 9.—Alternating areas of hyperplasia and atrophy of liver cells, with slight interstitial inflammation. H & E ×100.

FIG. 10.—Nodular liver containing several spheroid hepatomas; the lobe at the lower left exhibits cystic degeneration, with concavity of its inferior surface. ×2.

FIG. 11.—A spheroid hepatoma, with compression of the surrounding non-neoplastic hepatic parenchyma (upper right). H & E ×20.

FIG. 12.—Margin of a hepatoma (lower left) exhibiting infiltration into surrounding atrophic liver (upper right). H & E ×90.

FIG. 13.—Focal cholangiomatoid hyperplasia of bile ducts in an area of cystic degeneration of the liver. H & E ×100.


Liver Damage and Hepatomas in Mice Produced by Radioactive Colloidal Gold

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