A NEOPLASM OF MONOCYTES OF MICE AND ITS RELATION TO SIMILAR NEOPLASMS OF MAN*

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PLATES 5 TO 8

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A transmissible strain of leukemia in mice with malignant cells related to histiocytes is described in this communication. The strain is derived from a mouse that had granuloma-like infiltrations in the blood-forming tissues. Upon successive passages, the granuloma-like characteristics became less noticeable and the strain was transformed into a neoplasm with malignant cells resembling histiocytes.

Terminology

Experiments described elsewhere (1) led us to conclude that monocytes, histiocytes, macrophages, clasmatocytes, polyblasts, Kupffer cells and microglia cells are synonymous terms for one cell type, which is capable of perpetuating itself by mitotic division. In this communication we shall refer to the round forms of this type of cell seen in the circulating blood, as monocytes, and to all other forms as histiocytes. Tumors of monocytes or histiocytes will be named histiocytoma (monocytoma) and the systemic disease characterized by these cells histiocytomatosis (monocytomatosis). Monocytic leukemia is a synonymous term for leukemic histiocytomatosis (monocytomatosis).

Origin of the Strain

In August, 1937, a 24 months old male mouse (Rfb 385) developed a disease associated with great enlargement of the spleen and slight enlargement of the lymph nodes. The white blood count appeared slightly elevated and the differential count was as follows: Polynuclear and young granulocytes, 60 per cent; small and medium sized lymphocytes, 15 per cent; eosinophiles, 1 per cent. The

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remaining cells, 20 per cent, were mononuclear leukocytes, most of which resembled normal monocytes, but a smaller number, 4 per cent, were unusually large mononuclear cells with a large amount of intensely basophilic cytoplasm and very large nucleus.

The mouse was killed and at autopsy the spleen measured 3.5 by 1.0 cm. in the two greatest diameters; it was firm, gray-red, with numerous nodular gray areas on the external surface and on the cut surface. Most of the lymph nodes were of normal size. The largest nodes were found in the neck and measured 7 mm. in greatest diameter. On microscopic examination there was almost complete replacement of the spleen by large, nodular, partly confluent areas, illustrated in Fig. 3. Most of the cells in these areas resembled normal monocytes with abundant pink staining cytoplasm and an oval or slightly indented nucleus. The size and shape of the nuclei varied greatly but none was hyperchromatic. Occasional giant cells were present. Mitotic figures were few. Scattered among the monocytes was a small number of lymphocytes. The compressed splenic tissue between the nodules formed by the monocytes, was composed mainly of erythroblasts and myeloid cells in various stages of development. The lymph nodes were diffusely infiltrated by mononuclear cells, similar to those in the spleen, and the architecture of the node was distorted. The section of the liver illustrated in Fig. 4 showed extensive nodular and portal infiltration by cells similar to those seen in the spleen. The bone marrow was slightly infiltrated by these cells but here cells resembling fibroblasts were numerous at the sites of infiltration (Fig. 5). A touch preparation of the spleen stained with Wright and Giemsa solution contained mononuclear cells similar to those seen in the section, but the cytoplasm of most of the mononuclear cells was more basophilic than that of normal monocytes.

Transmission Experiments

Inoculations were made with a splenic cell suspension from mouse Rfb 385 into 6 related mice. One of the injected mice died with alterations similar to those observed in the mouse with the spontaneous disease. The disease was passed by intravenous injection from this mouse to 3 of 5 related mice. In successive subpassages most inoculations into related mice were successful.

The results of the first 7 passages are summarized in Table I. 0.1 cc. of a cell suspension containing approximately 10,000 cells per c. mm. was injected into the tail vein of each mouse; it was estimated that each animal received approximately 1,000,000 cells. When the number of cells injected was decreased the number of successful injections likewise decreased and the duration of the illness was lengthened. Most mice injected with 10,000 cells and an occasional mouse injected with 100 cells died of this disease; e.g., in

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TABLE I

Transmission Experiments

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>Material injected</th>
<th>Route</th>
<th>Mice</th>
<th>Length of life after inoculation</th>
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</thead>
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<tr>
<td>I</td>
<td>Spleen and lymph node</td>
<td>i.v. and s.c.</td>
<td>Rf</td>
<td>6</td>
</tr>
<tr>
<td>II a</td>
<td>“ “ “ “ “ “</td>
<td>Rf</td>
<td>5</td>
<td>K 21-57, D 35</td>
</tr>
<tr>
<td>II b</td>
<td>“ “ “ “ “ “</td>
<td>x-Rf</td>
<td>6</td>
<td>K 36-63, D 28</td>
</tr>
<tr>
<td>III a</td>
<td>Spleen</td>
<td>i.v.</td>
<td>x-Rf</td>
<td>5</td>
</tr>
<tr>
<td>III b</td>
<td>12 day tissue culture</td>
<td>i.v.</td>
<td>x-Rf</td>
<td>2</td>
</tr>
<tr>
<td>IV a</td>
<td>Spleen</td>
<td>Frozen and thawed spleen</td>
<td>x-Rf</td>
<td>3</td>
</tr>
<tr>
<td>IV b</td>
<td>“ “</td>
<td>i.v.</td>
<td>x-Rf</td>
<td>4</td>
</tr>
<tr>
<td>V</td>
<td>“ “</td>
<td>i.v.</td>
<td>x-S</td>
<td>2</td>
</tr>
<tr>
<td>VI</td>
<td>“ “</td>
<td>i.v.</td>
<td>x-Rf</td>
<td>8</td>
</tr>
<tr>
<td>VII</td>
<td>“ “</td>
<td>i.v. and s.c.</td>
<td>CRf</td>
<td>4</td>
</tr>
<tr>
<td>VIII</td>
<td>“ “</td>
<td>i.v.</td>
<td>CRf</td>
<td>3</td>
</tr>
<tr>
<td>IX</td>
<td>“ “</td>
<td>Rf</td>
<td>5</td>
<td>K 15, 15, D 20-23</td>
</tr>
<tr>
<td>X</td>
<td>Cell-free supernatant</td>
<td>“ “</td>
<td>x-CRf</td>
<td>11</td>
</tr>
<tr>
<td>Sediment</td>
<td>“ “</td>
<td>x-CRf</td>
<td>5</td>
<td>D 8-13</td>
</tr>
</tbody>
</table>

Abbreviations Used in the Tables.—K = killed; D = died; i.v. = intravenous; s.c. = subcutaneous. The origin of the stocks of mice named Rf, Af and S has been described (2). Stocks Ak, Af, S and C are unrelated to stock Rf in which the spontaneous disease originated. CRf is a first generation hybrid between the C stock of the Roscoe Jackson Memorial Laboratory and our Rf mice, and AkRf are similar hybrids of the corresponding stocks. x means that the mice were x-rayed from 1 to 7 days before inoculation with approximately 400 r.

one experiment the 2 mice that received 1,000,000 cells died 17 days after injection, 2 of 3 mice receiving 10,000 cells died after 27 days, and 1 of 4 mice receiving 100 cells died after 29 days.
None of the 23 unrelated mice (Table I) that were injected developed the disease even though 17 of them were irradiated with 400 r before inoculation. Mice of the first generation of hybrids between resistant and susceptible stocks were susceptible.

The material introduced into the subcutaneous tissue produced either a small, nodular growth at the site of injection or no lesion detectable in the gross. In this respect this strain resembled the transmissible chloroleukemia described by Hall and Knocke (3).

All 3 mice of the related stock Rf injected with splenic tissue that had been frozen slowly and kept during 1 hour at $-70^\circ$C. died with monocytoma. This result may be explained by the resistance of these cells to slow freezing (4).

**Attempts to Transmit the Neoplasm with Material Free from Living Cells**

1. Splenic tissue dried *in vacuo* in the frozen state failed to produce the disease in 4 irradiated mice of stock Rf. 3 months later the same mice received additional irradiation and were inoculated intravenously with live malignant mononuclear cells. Monocytoma characteristic of this strain developed in all 4 mice from 11 to 40 days after the reinjection.

2. 10 irradiated mice received intravenous injections of unfiltered cell-free splenic extract, obtained by spinning a thick splenic suspension at 3500 R.P.M. and 4 control mice received the sediment. The control mice died with monocytoma from 8 to 13 days after injection; the mice that received the cell-free extracts remained healthy.

3. Previous experiments have suggested that irradiation may be used to discriminate cells from virus in inocula containing both (5). Viruses are resistant to x-rays, whereas cells of mice present in the material are destroyed by approximately 500 to 6000 r of x-rays. X-rays do not bring about immediate destruction of the cells, but they may survive for several days and even multiply, so that viruses, should they be present within the cells, have an excellent opportunity to obtain a foothold in the new host.

In one experiment 4 mice received, with no ill effect, an intravenous injection of approximately 1,000,000 cells, exposed to 2000 r, and 4 mice a similar number of cells exposed to 4000 r. 2 control mice that received the same number of unirradiated cells died with this disease 17 days after injection; 2 of the mice that
had received approximately 10,000 cells each died after 25 and 28 days respectively; and one of 4 mice receiving approximately 100 unirradiated cells died after 29 days. In a second experiment that will not be described in detail, approximately 1,000,000 cells that were exposed to 1000 r and 2000 r respectively were injected into each of 4 mice, with no ill effect. The control injections were as effective as in the first experiment.

These experiments do not support the opinion that there is a virus in this material capable of producing monocytoma under the circumstances of our tests.

Anatomical Changes

The gross characteristics of the disease are illustrated in Figs. 1 and 2. In the advanced stage, the liver was enormously enlarged, greatly distending the abdomen. It was thickly spotted with minute round or irregular gray-white, red and yellowish gray areas. The gray areas were due to the presence of minute masses of the malignant mononuclear cells, the red areas to hemorrhage, and the yellowish gray opaque spots to necrosis. Usually all of these alterations were present, although occasionally some were inconspicuous. The spleen was moderately or greatly enlarged and was the site of similar alterations. A few lymph nodes, usually the cervical, were slightly or moderately enlarged; others were normal in size. The lungs showed small or extensive spotty areas of hemorrhage and occasionally yellowish gray tumor nodules.

The microscopic findings in the mouse with the spontaneous disease, and in the first few subpassages, have already been mentioned. In the spontaneous disease and in mice of the second subpassage, the microscopic examination of the blood-forming organs showed collections of mononuclear cells, resembling those seen in infectious granulomata of man. Most of these monocytes were not hyperchromatic (Fig. 6). The cytoplasm was abundant, pink staining, and the shape of the nucleus varied from vesicular to multilobed forms. In later passages these cells were few, whereas cells with larger hyperchromatic nuclei and relatively less but more basophilic cytoplasm were present in larger numbers. The shape of the nucleus varied greatly from vesicular to multilobed forms, shown in Figs. 12, 13 and 17. The photographs do not faithfully reproduce the lobations of the nucleus.
because the cells were so large that the nuclear shape could be ascer-
tained only by focusing at different levels on the same cell.

The infiltration in the liver was diffuse, usually with the formation
of nodular tumor masses. The malignant cells often invaded vessels
of medium size, almost completely occluding them. Mitotic figures
were present in large numbers. Cells other than variants of the
malignant monocytes were few in the tumor-like infiltrations. In
the spleen the infiltration was equally extensive and almost completely
replaced the pulp. A small amount of lymphoid tissue and of granulo-
blastic and erythrogenic foci often persisted about the follicles and
trabeculae. In mice that died at an early stage of the disease the
infiltration was limited to the splenic pulp. The infiltration in the
lymph nodes was scant or often absent. It formed nodular growths
that first appeared about the lymph sinuses. The degree of involve-
ment of the bone marrow was variable, being usually scant. In an
occasional instance the greater part of the femoral marrow in the
sections examined was replaced by neoplastic cells. In the first few
subpassages a tendency to stimulate connective tissue growth was
noted; this was less conspicuous in the course of the later passages.
When introduced into the subcutaneous tissue the malignant cells
either produced no gross alterations or a small tumor measuring
from 2 to 5 mm. in greatest diameter, and the mice died with
extensive metastases in the blood-forming tissues, similar in appear-
ance to those found in mice that had been injected intravenously.
Microscopic examination of these subcutaneous growths showed that
there was invasion of the skin and subcutaneous tissue by malignant
cells, with extensive areas of hemorrhage and necrosis.

In 2 mice a chronic disease was produced by the introduction of a tissue
culture 12 days old into the subcutaneous tissue. When killed 125 days after
the injection one mouse had a small nodular growth at the site of injection, measuring
approximately 8 mm. in greatest diameter and there was no evidence of infiltration
in the internal organs. The second mouse that died 125 days after the injection
had, in addition to a similar local growth, a generalized neoplasm with tumor
nodes composed of monocytes, many of which resembled the less hyperchromatic
type of cell seen in the spontaneous disease and in the earlier passages.

The malignant cell evidently may assume many different forms
(Figs. 3, 6, 7, 12, 13, 17). Giant cells like those seen in Hodgkin's
disease were found in large numbers in occasional mice (Fig. 13). The factors that determine the morphological characteristics of the malignant cells of the strain require further study.

Occasionally in mice in which the disease had a relatively long continued course there was slight or moderate fibrosis in the tumor-like infiltrations. In sections of these lesions stained with Foot's modification of Masson's trichrome stain there was a moderate amount of green staining amorphous material with occasional fibrils between the neoplastic cells. In sections stained with Foot's stain for reticulum a moderate number of reticular fibers were seen between the neoplastic cells.

**Alterations in the Blood Picture**

In spite of advanced invasion of medium sized vessels of the liver by malignant histiocytes, these cells were present in the circulating blood in only small numbers. In the blood smear of 10 mice that were x-rayed before inoculation the percentage of presumably malignant monocytes varied at the height of the disease from 0 to 37 with an average of 10.4 per cent and in 10 not x-rayed mice from 0 to 23 with an average of 3.5 per cent. The malignant monocytes differed from normal monocytes mainly by the large size of the nucleus and basophilia of the cytoplasm. Cytoplasmic granules were not seen, with the exception of occasional fine azurophile granules, and the cells did not give the oxydase reaction. Similar cells are usually designated as monoblasts. In fixed preparations many of these large mononuclear cells became smudged but were still recognizable by the outline of the large nucleus and by intensely basophilic cytoplasm. Touch preparations of infiltrated organs contained these cells in large numbers. The total leukocyte count was only slightly elevated. An occasional conspicuous rise of the leukocyte count was due to associated leukocytosis. Table II gives examples of the blood counts during the course of this disease. The largest number of malignant cells was found in mouse CRf 65.

**Characteristics of the Malignant Cells**

The behavior of histiocytes in tissue cultures (1) is highly characteristic. Whether derived from the brain (microglia cells) or liver
NEOPLASM OF MONOCYTES

(Kupffer cells) or from the blood (monocytes), these cells migrate into the explant and assume at first the form of the resting microglia cell of the brain; they become transformed into epithelioid cells or fat-laden round cells (the compound granular corpuscle), may form multinucleated giant cells of the foreign body type, and may be actively phagocytic. They are not transformed into any other type of blood cell or into fibroblasts.

The malignant mononuclear cell of a transmissible neoplastic

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Date of</th>
<th>Date of</th>
<th>Red cell count in millions</th>
<th>White cell count in thousands</th>
<th>Lymphocytes</th>
<th>Polymorphs</th>
<th>Monocytes</th>
<th>Malignant</th>
<th>Preserved</th>
<th>Smudged</th>
<th>Unclassified</th>
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<td>5/11</td>
<td>5/13</td>
<td>12.9</td>
<td>4.7</td>
<td>59</td>
<td>39</td>
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<td>0</td>
<td>0</td>
<td>1</td>
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<td>53</td>
<td>40</td>
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</tr>
<tr>
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<td>5/11</td>
<td>6/1</td>
<td>7.4</td>
<td>76.2</td>
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<td>30</td>
<td>4</td>
<td>25</td>
<td>12</td>
<td>9</td>
<td></td>
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<td>6/1</td>
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<tr>
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<td>5/16</td>
<td>7.4</td>
<td>2.5</td>
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<tr>
<td>CRf 34 and 35</td>
<td>—</td>
<td>—</td>
<td>*</td>
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<td>8.2</td>
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<td>42</td>
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</table>

* Average of 6 counts made at from 3 to 7 day intervals on these 2 mice.
disease of mice previously described (6) had many of the character-
istics of monocytes and formed in tissue cultures peculiar multinuclear
giant cells. In vitro the malignant monocytes described in the present
communication, behaved in a characteristic manner. Unlike fibro-
blasts and endothelial cells they did not anastomose, but like blood
cells migrated singly from the explant. The cytoplasm became
elongated with bulky bulbous projections. The microglia-like forms
with slender, long drawn out and branching processes usually seen
in cultures of normal monocytes were not observed. The shape
of the nuclei did not deviate conspicuously from that seen in sections
of the blood-forming organs of animals with this neoplasm (Figs.
8–11). In an occasional culture the cells were somewhat spindle-
shaped but did not form a reticular network. The large number of
mitotic figures, as many as six in a high power field, seen occasionally
in cultures of the buffy coat, was evidence that these cells proliferated
by mitotic division. The morphologic appearance of these cells
in tissue cultures was less variable than that of normal monocytes.

Lewis (7) compared the malignant monocytes of strain Rfb 385
with those of strain C 57 which she has recently isolated. She ob-
served that the characteristic cell of strain Rfb 385 behaved more like
a large macrophage that had become malignant, while that of mono-
cytoma C 57 was more like an epithelioid cell that had taken on can-
cerous properties. Besides the specific cell, both lesions contained
some cells of the monocyte, macrophage, and epithelioid cell type.

Phagocytosis.—One of the significant characteristics of the various
forms of monocytes is their power of phagocytosis. Phagocytosis
by the malignant cells of this strain was studied both in vivo and
in vitro.

Normal and leukemic mice were given repeated intravenous injec-
tions of India ink and the animals were killed a few hours after the
first injection. The Kupffer cells in the liver of the normal mice
were studded with carbon particles. In the normal spleen the pres-
ence of a perifollicular zone formed by phagocytes was noted in all
sections. Phagocytic mononuclear cells were less numerous in the
pulp than in this perifollicular zone. Phagocytes were scant in the
foci of myeloid metaplasia present in many normal spleens.
Carbon-filled cells were almost entirely absent in the tumor-like infiltration of the liver but were present in the normal Kupffer cells about the sinusoids of the intervening normal liver tissue. This lack of conspicuous phagocytic activity is remarkable since almost all of the medium sized and large vessels contained in their lumina many tumor cells. However numerous monocytes of both the normal and the malignant types (Figs. 10 and 11), filled with carbon particles, appeared in the blood of mice with advanced histiocytoma of this strain following intravenous injections of India ink. Parts of the cytoplasm of the large malignant cells often became detached in the preparation of blood smears and basophilic cytoplasmic fragments containing carbon particles were seen in the smears.

Mice with this neoplasm were given intravenous injections of India ink and tissue cultures prepared from the spleen, liver and the buffy coat of blood of these animals. The presence of carbon was noted in numerous malignant cells (Fig. 8), though in smaller amount than in the normal histiocytes, present in the same preparations, normal and malignant histiocytes being distinguished by the characteristics already described. The basophilia of the malignant histiocytes was shown by the purple hue of the cytoplasm when stained with hematoxylin, whereas the cytoplasm of the normal histiocytes was stained very faintly in the same sections. Carbon particles were seldom seen in granulocytes.

When blood smears of leukemic mice that had been injected with carbon were treated with benzidine for the oxydase reaction, few cells that reacted contained carbon. None of the characteristic malignant cells gave the oxydase reaction but many contained the black carbon particles. Fig. 8 shows phagocytic malignant cells in a 3 day old culture of the buffy coat of a mouse with histiocytoma that had been injected with India ink shortly before death. Figs. 10 and 11 show these cells in blood smears.

The phagocytic mononuclear cells in cultures of the spleen and liver of normal mice that had been injected with India ink had the same appearance as the phagocytic mononuclear cells of the blood. Fibroblast-like cells of the spleen were not phagocytic.

The studies support the conclusion arrived at in previous work done in association with Dunning and with Hall (1): histiocytes are an independent cell type and are the cells often described as
reticulo-endothelial cells. This view is in harmony with that of Clark and Clark (8) and Lewis (7) and Lewis (9) based on studies of living tissues. The malignant cell of the strain here described seems to be related to the histiocyte.

Similarity of Neoplasms of Histiocytes of Man and Mouse

The extensive literature on neoplasms of monocytes or histiocytes has been reviewed in numerous recent articles (10-13, 18, 21). The cases described by different investigators differ greatly. Downey distinguishes between monocytic leukemia with and with no myeloid reaction and believes that most of the cases published as monocytic leukemias are probably myeloid leukemias with many monocyte-like myeloid cells. The aleukemic forms of histiocytomatosis have been described, as it has already been stated, under the misleading terms, reticulum cell sarcoma and reticulosis (11, 13, 18).

Whereas normal cells of the same cell type, e.g., monocytes of different individuals, resemble each other very closely, malignant cells of the same cell type exhibit in different individuals conspicuous differences. The first transmissible neoplasm of mice with cells resembling monocytes (strain S 2) was observed by the author in association with Dr. W. A. Barnes, and its cells are characterized by a large nucleus which has a tendency to assume bizarre shapes and forms in vitro multinuclear giant cells. It invades the bloodstream readily. The second transmissible neoplasm of cells like monocytes described here (strain Rfb 385) is characterized by somewhat larger malignant cells which have a tendency to form mononuclear giant cells. They produce tumor nodules and do not appear in the circulating blood in conspicuous numbers. Several neoplasms composed of histiocytes with and with no blood involvement have been observed in mice that have received intrasplenic injections of benzpyrene but transmission of these neoplasms has not been attempted (22).

The human neoplasms of histiocytes collected in the Lymphatic Registry differ widely one from another. The malignant cells of one of the two cases of monocytic leukemia (No. 540) of this collection resemble those of our strain

1 We acknowledge the opportunity given us by Colonel Ash of the Army Medical Museum, Washington, D. C., to study the material of the Lymphatic Registry.
S 2 and tend to form giant cells with lobed nuclei. The numerous mitotic figures among these cells and their invasion of fatty tissue in absence of other cells strongly support the view that monocytic leukemia of man is the result of unrestricted multiplication of monocytes. No. 680 of this collection diagnosed by two competent pathologists as reticular cell sarcoma and by another as Hodgkin's sarcoma, is an example of histiocytic neoplasm with no blood involvement. This neoplasm is characterized by monocyte-like cells whose nuclei are often indented or lobed or convoluted; these cells form multinuclear giant cells resembling those of strain S 2. No. 637 of the Lymphatic Registry is characterized by similar cells.

Downey (12) has described several cases of monocytic leukemia and his colored figures (Nos. 17 to 25) illustrating these malignant human monocytes resemble the malignant monocytes of strain Rf 385 so closely that these cells from men and mice can barely be distinguished.

Autopsies have been performed in recent years in this department on two human cases of this type of neoplasm and Figs. 14 to 16 illustrate the malignant cells in them.

Neoplastic cells like those shown in Fig. 16 infiltrated the skin of a woman 36 years old causing swelling and redness in focal areas. The blood picture was not significantly altered. The spleen and lymph nodes were not enlarged. The postmortem diagnosis of reticulo-endothelial (reticulothelial) sarcoma with involvement of skin, lymph nodes, tonsils (Fig. 16), spleen (weight, 680 gm.), bone marrow, mesenteric, omental and retroperitoneal tissues, pleurae, trachea, bronchi and gastro-intestinal tract. The cells infiltrating these structures had a large vesicular and slightly indented or lobed hyperchromatic nucleus. A nucleolus was present in only a few cells. The cytoplasm was moderately abundant and the relation of nucleus and cytoplasm varied greatly. Occasional cells had two nuclei. Eosinophiles were present in small numbers and slight fibrosis occurred at the site of occasional infiltrations. In a section stained with silver nitrate there was a variable and usually moderate amount of reticulum among the tumor cells. The observation that there is in a few places little if any reticulum between large groups of cells suggests the possibility that much of the reticulum is derived from preexisting reticulum.

The cells in Figs. 14 and 15 illustrate the malignant cells in a neoplasm of a man 37 years old diagnosed as round cell sarcoma with involvement of the spleen, of the stomach with extensive necrosis and ulceration, of the intestinal tract with hemorrhage into the lumen. The malignant cells in this case (Figs. 14 and 15) resemble the neoplastic histiocytes of mice of strain Rf 385 (Fig. 17). Both are large round cells with large hyperchromatic vesicular and indented or lobed nuclei and a moderate amount of slightly basophilic cytoplasm. The disease in both instances is characterized by an unrestricted growth of these cells.

2 Made by Dr. Lawrence Smith.
3 Autopsy performed by Dr. J. W. Hall.
DISCUSSION

Relation of Histiocytes to Other Cells.—It is beyond the scope of this work to review the different opinions on this highly controversial subject; this has been done recently by Forkner (10), Jaffé (11), Downey (12), Hadfield and Garrod (13) and others. It seems desirable, however, to summarize the author's opinion based on a critical study of the literature and on experiments of his own.

The existence in the adult organism of an ancestral cell of the mesenchyme which gives rise to the well recognized cells of the bone marrow and blood is now almost universally accepted. These cells are believed to form the framework of the blood-forming organs, and according to Maximow, the main exponent of this view, produce monocytes. This supposition, based on observations of fixed and stained preparations, has to be reconsidered in the light of recent studies of cells in the living state. Clark and Clark (8 a) observed that histiocytes develop in the tissue of the tail of transparent axolotl larvae from an undifferentiated strand of primitive mesoderm ventral to the notochord at the same time as do other varieties of early connective tissue cells. They have observed furthermore the increase of histiocytes by mitotic division but have not seen them change into any other form of blood or tissue cell. The source of monocytes in later life, they suggest, must be looked for in some specialized region.

The opinion that monocytes arise from lymphocytes has been discussed elsewhere (1 b), and in accordance with the observations of Clark and Clark and Lewis and Lewis the conclusion has been reached that lymphocytes do not form monocytes.

Although there is ample evidence indicating that histiocytes are derived from histiocytes and pure cultures of them have been grown in vitro by numerous workers, their derivation from other cells during postembryonal life and their ability to form fibroblasts is doubtful.

In sections of mice that had been injected with India ink the phagocytic cells containing carbon particles are scattered about the lymphoid follicles and in the pulp and appear to line the blood sinuses of the liver and spleen; but it is questionable if histiocytes are identical with the lining cells of these blood sinuses.

Our studies made in association with Dunning and those of other investigators quoted indicate that Kupffer cells are not identical with the endothelium of the sinusoids of the liver. The studies of Eliot (14) are of unusual interest in this connection. She injected into the veins of rabbits monocytes that had phagocytosed carmine particles and found that these cells became established about the sinusoids of the liver and were indistinguishable from normal Kupffer cells. These studies have been confirmed by de Haan and Hoekstra (15). The experiments of Rous and Beard (16), on the other hand, indicate that monocytes, histiocytes and Kupffer cells, although genetically related, differ morphologically to no slight
extent as also in physical properties, e.g. stickiness, and in ability to survive outside the body.

MacCallum (cf. 8 b) and several other workers found that endothelium possesses phagocytic ability and Clark and Clark (8 b) observed in the transparent tails of tadpoles phagocytosis of carmine and carbon particles by connective tissue and endothelial cells. Direct observations made upon living tissue by Clark and Clark (8 c) indicate that endothelial cells do not form monocytes and the contradictory reports of several workers, reviewed by Clark and Clark, are not convincing.

In sections a sharp differentiation of endothelial cells from phagocytic mononuclear cells such as Kupffer cells is not possible. In tissue cultures, on the other hand, the two types of cells, histiocytes and fibroblast-like (endothelial cells and fibroblasts), can usually be distinguished. Differentiation of endothelial cells and fibroblasts in tissue cultures, we believe, is uncertain. We have noted in the cultures of the biffy coat of the blood of animals that received intravenous injections of India ink all forms of phagocytic cells that were present in the cultures of the spleen of the same animals. Phagocytosis by fibroblast-like cells was inconspicuous. Histiocytes and fibroblast-like cells have been grown separately in pure cultures by several investigators; a few of them have stated on insufficient evidence that there is occasionally transformation of one cell type into the other (cf. 17). Direct observation of cells in the transparent chamber of the rabbit's ear by Clark and his associates and numerous studies on the behavior of histiocytes in tissue cultures indicate that endothelial cells are not related to histiocytes and suggest that histiocytes are not able to form a sinusoid or an anastomosing network of tissue. It seems more likely that fibroblasts and endothelial cells form the permanent framework of the blood-forming organs and about these cells are large numbers of motile histiocytes. Both lymphocytes and monocytes seem to be independent self-perpetuating strains of cells (cf. Lewis, 8 b).

Neoplasms of Histiocytes.—If monocytes are capable of perpetuating themselves by mitotic division it is possible that they can undergo malignant transformation and undoubted cases of such neoplasms are on record (cf. 12). These may occur in the form of solitary tumors (histiocytoma) or as a systemic disease (histiocytomatosis) involving mainly the blood-forming organs with or without leukemia. Most human neoplasms of histiocytes have been described under the term reticulum cell sarcoma or reticulosis, leukemic and aleukemic (cf. Krumphaar, 18) but since the relation of histiocytes to reticulum fibers and to the reticular fibroblast-like cells of the blood-forming organs is obscure this terminology is not desirable.

The origin of the reticulum of sarcoma has not been definitely established (cf. Arey, 19). The reticulum of sarcoma may be remnant of preexisting reticulum
or it may be newly formed by either the stroma cells or the neoplastic cells themselves. Schwann, Flemming and others derive reticulum from the ectoplasm of cells; Henle, Koelliker and others from a semifluid amorphous substance secreted by the cell. Some more recent workers assume that an enzyme of the fibroblasts passing into the surrounding fluid jelly is responsible for fibril formation but others attribute it to a coagulation process in the intercellular substance and ascribe no specific rôle to the fibroblasts (cf. Arey, 19). There is not enough evidence that quantity or appearance of reticulum characterizes a distinct type of neoplasm that could be named reticulum cell sarcoma. Our studies do not support the opinion that the reticular fibroblast-like cells of lymphoid tumors are neoplastic elements of the growth (20). They indicate that lymphoid, myeloid and monocytic leukemias are the result of unrestricted proliferation of lymphocytes, immature myeloid cells and monocytes, respectively.

For the reasons given it seems desirable to abandon the term reticulum cell sarcoma and name the solitary growth of histiocytes histiocytoma or monocytoma, and the systemic disease histiocytomatosis or monocytomatosis. Either may be leukemic or aleukemic. The transmissible neoplasm here described is sometimes aleukemic and sometimes leukemic but with small numbers of malignant cells in the peripheral blood. Monocytic leukemia may be substituted for the cumbersome term leukemic histiocytomatosis.

The Relation of This Transmissible Histiocytoma of Mice to Hodgkin's Disease.—The available evidence is not sufficient to prove that Hodgkin's disease is neoplastic in nature although its characteristics strongly suggest this view. A disease exactly like it has not been found in animals, and the experience gained in the study of transmissible neoplasms of animals including those produced by viruses does not elucidate the morphological characteristics of Hodgkin's disease.

Nevertheless, there are certain similarities between Hodgkin's disease and the neoplasm of mice described here.

Both may have granuloma-like characters though they are readily distinguishable. In the mouse with the spontaneous disease and in a few instances of the transmitted disease there was in the blood-forming organs a focal and diffuse overgrowth by large mononuclear cells like histiocytes among and about which were lymphoid cells in variable numbers. Among the mononuclear cells were occasional giant cells; plasma cells and eosinophiles were seen in a few places. There were areas of necrosis and an increase of fibrous connective tissue in some of the foci of infiltrations. In the course of successive passages the pleomorphic characteristics of these alterations disappeared almost completely and the trans-
missile strain changed into a disease unquestionably neoplastic. It is well known that the lesions in Hodgkin's disease are sometimes pluricellular and granuloma-like, sometimes monocellular characterized by an overgrowth of large mononuclear cells with giant cells named after Sternberg and Reed. Similar giant cells occur in the mouse neoplasm.

Occasionally a tumor definable by one pathologist as a histiocytoma has been designated Hodgkin's disease by another, e.g. No. 680 of the Lymphatic Registry already cited. Moreover, Doan and Weisman (21) have pointed out that cases that are clinically monocytic leukemia may possess the anatomical characteristics of Hodgkin's disease.

The mononuclear cells of Hodgkin's disease are not known to be phagocytic. Phagocytic ability of the malignant mononuclear cells of this strain Rfb 385 could be demonstrated only by special tests. It is noteworthy that India ink injected intravenously into mice with this neoplasm was taken up almost exclusively by normal histiocytes.

The mononuclear cells of Hodgkin's disease do not appear in the blood but the malignant mononuclear cells of this neoplastic disease of mice are in many instances present in the blood in small numbers during the terminal stage of the disease.

The transmissible disease here described is characterized by proliferation of mononuclear cells which resemble the monocytes of the blood. Proliferation of these malignant monocytes is occasionally associated with stimulation of other cells when the disease is granuloma-like and resembles Hodgkin's disease but is not identical with it; more often the growth of these cells is unaccompanied by those of other cells, the malignant cells appear in the blood in small numbers, and the disease closely resembles monocytic leukemia.

**SUMMARY AND CONCLUSIONS**

A transmissible neoplasm of mice characterized by malignant cells resembling histiocytes (monocytes) is described. The morphology of these cells and the microscopic appearance of the lesions are similar to those of human neoplasms formed by histiocytes.

The malignant histiocytes form tumor-like masses in the liver and spleen and infiltrate these and other tissues. They are present in small numbers in the blood of many mice when the disease is far advanced. The malignant cells have scant phagocytic ability. The fixed cells of the host (endothelial cells and fibroblasts) have no significant part in the production of the lesions of the disease.

Transmission is readily accomplished when material containing
the malignant histiocytes is used for inoculations, but fails in their absence. Attempts to demonstrate a cell-free transmitting agent have been unsuccessful.

**BIBLIOGRAPHY**

EXPLANATION OF PLATES

The sections were stained with hematoxylin and eosin. The blood smears were stained with Wright and Giemsa solution. The magnifications given are approximate.

PLATE 5

Fig. 1. Transmitted monocytomatosis with greatly enlarged liver and spleen. These organs are spotted with areas of hemorrhage.

Fig. 2. Transmitted monocytomatosis with numerous partly confluent grayish tumor nodules in the liver.

Fig. 3. Granuloma-like infiltrations in the spleen compressing the pulp in the mouse with spontaneous monocytomatosis (Rfb 385). × 250.
PLATE 6

Fig. 4. Portal infiltration in the liver of a mouse with the spontaneous monocytomatosis (Rfb 385); the cells resemble those that infiltrate the spleen. × 75.

Fig. 5. Focal infiltration of the bone marrow with mononuclear cells and early fibrosis in the same mouse. × 150.

Fig. 6. Infiltration by large mononuclear cells with abundant, slightly hyperchromatic cytoplasm in the lymph node of a mouse of the second passage. × 350.

Fig. 7. Infiltration similar to that shown by Fig. 6, but with more hyperchromatic mononuclear cells, some of which are phagocytic, in a lymph node of a mouse of the third passage. × 400.
Plate 7

Fig. 8. 3 days old tissue culture of the buffy coat of blood of a mouse with monocytomatosis, that received intravenous injections of India ink. The black granules in the cytoplasm of the cells are phagocytosed particles of India ink. \( \times 400 \).

Fig. 9. Touch preparation of the liver showing numerous malignant mononuclear cells. \( \times 1000 \).

Figs. 10 and 11. Malignant mononuclear cells in a blood smear of a mouse that received intravenous injection of India ink. The black granules in the cytoplasm are those of India ink. \( \times 1000 \).

Fig. 12. High magnification of the malignant cells in a tumor-like infiltration in the liver (fifth passage). There is a giant cell in the field. \( \times 800 \).

Fig. 13. Microscopic section of a tumor-like nodule in the liver of a mouse with transmitted leukemia (third passage), showing numerous giant cells. \( \times 400 \).
(Furth: Neoplasm of monocytes)
PLATE 8

FIGS. 14 and 15. The malignant cells of human round cell sarcoma or histiocytoma infiltrating the jejunum. Fig. 14, × 800; Fig. 15, × 1000.

FIG. 16. The malignant cells of human histiocytoma infiltrating the tonsil. × 800.

FIG. 17. The malignant mononuclear cells of transmitted mouse histiocytoma (second passage) infiltrating the liver. × 800.