Pathologic Anatomy of Experimental Leukemia Produced by Injection of Benzol Extracts from Organs of Leukemic Patients

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THE PRESENT STUDY deals with the pathology of leukemia experimentally induced in animals by the injection of organ extracts from patients who had died of leukemia.

Material was obtained from the Pathophysiology Laboratory of M. O. Rauschenbauch. His previous study\(^1\) sets forth in detail methods of transmission, peripheral blood findings, and data concerning the breed of animals used.

The mice were divided into six groups according to the disease of the donor organ injected: (1) chronic myelosis, (2) chronic lymphadenosis, (3) acute leukemia, (4) myeloma, (5) cancer of the stomach. The sixth group consisted of mice receiving organ extracts from healthy individuals who had died in street accidents. The last two groups were the controls. In all groups some of the animals died during the first two or three months of the experiment. In these animals were noted numerous foci of necrosis in the viscera, thromboses, focal hyperplasia of reticuloendothelial cells, and, occasionally, amyloidosis of spleen and liver. These changes were related to the toxic effects of the benzol extract.

Group 1 consisted of mice receiving organ extracts from four patients who had died of chronic myelosis. Of the 75 animals surviving four or more months from the start of the experiment, four showed changes in the viscera and bone marrow, consistent with the development of leukemia.

Mouse No. 1 received a benzol extract from the liver of a patient who had died of chronic myelosis. The mouse was killed four months and ten days after the start of the experiment; there was no change in blood picture. Autopsy revealed normal appearing internal organs. At the injection site in
the inguinal region a tumor the size of a pigeon’s ear was noted, which on cross section was gray with large foci of necrosis.

Microscopic examination:

Liver: aggregations about central veins and capillary lumina of large round and oval cells with large, clear nuclei and weakly basophilic cytoplasm; ring-shaped nuclei in some of the cells (myelocytes); foci of destruction of the liver cells.

Spleen: follicular hyperplasia and focal myelosis; endothelium of sinuses swollen; a few megakaryocytes.

Axillary lymph nodes: diffuse hyperplasia with complete obliteration of normal pattern. Subcapsular foci of the large myeloid cells described above. Small aggregates of the same cell were also found in lung and kidney blood vessels.

Iliac marrow: polymorphous; many lymphoid elements interspersed with groups of myeloblasts, myelocytes and undifferentiated cells of the hemocytoblast type.

Tumor: Non-necrotic areas showed atypical elongated cells of varying size, with elongated nuclei—also giant cells with deformed, hyperkeratotic nuclei and many mitotic figures. These formed clusters typical of spindle cell sarcoma. The presence of foci of myeloid metaplasia in marrow and viscera indicated a developing leukemic process.

Mouse No. 2 received organ extracts from a patient with myelosis. It was killed 4 months and 23 days after the start of the experiment. Peripheral blood showed 57,800 leukocytes, 2 per cent myeloblasts. Grossly no changes were noted in the viscera. A tumor the size of a pigeon’s ear appeared at the injection site (loin).

Microscopic examination revealed the following:

Liver: dilated capillaries, filled with myeloid elements (fig. 1) which form patchy large aggregates with an obliteration of the normal lobular pattern. Kupfer cells swollen; an occasional megakaryocyte found in the capillaries. Some invasion of Glisson’s capsule by myeloid cells also seen.

Spleen: follicles well developed. Myeloid elements predominate in the pulp.

Bone marrow: mainly cells of the myeloid series, ranging from myeloblasts to segmented leukocytes, with myelocytes predominating. A great number of megakaryocytes, many with pyknotic nuclei.

Tumor: picture consistent with spindle cell sarcoma. In summary, Mouse No. 2 developed myeloid leukemia, with myelocytes predominating.

Mouse No. 3 received organ extracts of a chronic myelogenous leukemia case. It was sacrificed 10 months and 18 days later. Gross study revealed a large amount of blood in the peritoneal cavity. The liver was found to be enlarged and a tumor was present at the injection site.

Microscopic examination showed well defined leukemic infiltration of the liver, myelosis of the spleen and lymph nodes, and myeloid metaplasia of the iliac bone marrow.

Analogous changes developed in Mouse No. 4 who received similar organ extracts. It was sacrificed 10 months and 20 days after the start of the experiment. No changes in the peripheral blood were noted.
Subsequent transfers, both of tumor and visceral emulsions and blood from leukemic animals produced myelogenous leukemia in a number of instances along with the pathologic changes described above.

Thus, with organ extracts from patients having chronic myelogenous leukemia we were able to produce the donor disease in mouse hosts, with myelocytes predominating among the blood-forming elements.

Group 2 comprised those mice receiving organ extracts from three patients who succumbed to chronic lymphocytic leukemia.

A few of the 36 mice in this group showed aggregates of lymphocytes in the liver capillaries, as well as moderate lymphatic hyperplasia of spleen and lymph nodes. Otherwise leukemic changes were absent. A number of mice developed a tumor at the injection site and also at some distance from it. After further transfers, the development of leukemia was noted. The following are two protocols by way of example.

Mouse No. 2. First Transfer. Sacrificed one month and 20 days after injection of a tumor emulsion showing polymorphous sarcoma histologically. Peripheral blood contained 77,000 leukocytes, 3 per cent myeloblasts, and 11 per cent myelocytes. At the injection site a pea-sized mass was found. The spleen was slightly enlarged.

Microscopic examination revealed the following:

Liver: large necrotic foci. Non-necrotic areas showed obliteration of normal lobular pattern from extensive invasion of myeloid cells along Glisson's capsule and their piling up in the capillary lumina.

Spleen: diffuse myelosis of the pulp. Follicles preserved and superficial (fig. 2).
Lymph nodes: focal myelosis.

Lungs: alveolar capillaries dilated and filled with myeloid cells. The remaining viscera showed no changes.

Bone marrow: myeloid cells predominate, interspersed with a small number of red cell elements. It should be noted that in all organs, where there is a preponderance of myeloid cells, large and small myelocytes predominate, together with many segmented and hypersegmented leukocytes (fig. 3).

Tumor: spindle cell sarcoma.

Mouse No. 5. Sacrificed five months after start of the experiment. Peripheral blood showed 44,000 leukocytes, 8 per cent hemocytoblasts, and 6 per cent myeloblasts. Gross examination revealed a tumor on the back. A bean-sized mass was found over the left kidney, partially growing through the kidney parenchyma. On cross section the mass was white and dry. Spleen, liver and right kidney were enlarged.

Microscopic examination was as follows:

Liver: normal pattern completely obliterated. Large areas of amyloid replaced parenchyma. Capillaries dilated and filled with large, oval cells containing big nuclei and resembling hemocytoblasts. Some areas show aggregations of these cells, along with a smaller quantity of myeloblasts and single large myelocytes.

Spleen: follicles preserved, superficial. Pulp contained a large quantity of the hemocytoblast-type cells described above.

Right kidney: cortex contains focal aggregations of the same undifferentiated cells.
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On transfer, also a hemocytoblast-type of leukemia was obtained. Thus, benzol extracts from organs of acute leukemia patients were able to evoke the development of hemocytoblasts both in the original and transfer groups.

Group 4 comprised mice receiving liver and bone marrow extracts from a myelogenous leukemia case. One of the 19 mice in this group (Mouse No. 7), sacrificed 8 months and 23 days after injection, showed significant microscopic findings—namely, a diffuse myelosis with myelocytes predominating, plus an acute amyloidosis of the organs. The thyroid gland revealed diffuse lymphoid hyperplasia. Peripheral blood contained 24,000 leukocytes and 4 per cent myelocytes. Transfer from this animal produced myelosis.

Another, Mouse No. 1, sacrificed five months after injection, showed diffuse myelosis in the form of multiple tumor nodules (myelocytic) in the abdominal cavity and a preponderance of myelocytes in the bone marrow.

Group 5 contained animals receiving liver extract from gastric carcinoma patients. Two of the 18 mice examined microscopically showed myelosis with myeloblasts predominating. Peripheral blood contained 46,200 leukocytes, with 16 to 22 per cent immature myeloid cells.

Group 6 received liver extracts from healthy individuals who died accidentally. Some showed amyloidosis and focal necrosis related to the toxic effects of the benzol extracts. Leukemia did not develop in a single instance.

Thus, of 162 mice over six months of age, surviving not less than 4 to 4½ months after injection of benzol-extracted organs of leukemic patients, eight or 4.9 per cent developed leukemia.

In addition to the original study, a series of 250 mice receiving injections
of tumor emulsion, visceral emulsion and blood from leukemic animals was studied. Among these was noted the development of myelogenous leukemia and hemocytoblastosis along with pathologic changes analogous to those described above. Leukemia reached its peak after three of four transfers in which myeloid metaplasia appeared in a greater number of animals (approximately 80 per cent) and with noticeably greater rapidity. If in the original mice leukemia developed four months or more after start of the experiment, on transfer this delay decreased to 20-30 days, and even to 15 days in isolated instances.

The histologic findings of our mouse leukemia experiments may be summarized as follows: diffuse myeloid hyperplasia of the bone marrow, myelosis of spleen and lymph nodes, and myeloid invasion of the liver parenchyma.

It is noteworthy that the changes which occur in experimental leukemia produced by known synthetic carcinogens (Yudina, Engelbret-Holm) are morphologically identical to those brought about by benzol extracts of organs from leukemic patients. These changes have already been described in part in our study of leukemia induced by the synthetic carcinogen 9,10 dimethyl 1,10 benzatratstes (Nemenova and Khokhlova).

Thus, along with diffuse hyperplasia and myelosis of the bone marrow, involvement of most of the liver was also noted. In the latter, the growth of leukemic cells occurred mainly in Glisson's capsule and, to a lesser degree, along the course of the capillaries. In the lymph nodes, notwithstanding the intensity of the leukemic process, the myelosis was usually focal, with a preservation of the parenchymal structure. In the spleen there was diffuse myelosis through which one could discern a decrease of normally structured follicles. It also seemed to be a characteristic of experimental leukemia that in the marrow as well as in the viscera one always found among the primordial myeloid cells a larger or smaller amount of mature elements—segmented leukocytes. And finally, in a significant number of mice, leukemic changes in organs were not expressed macroscopically.

The sometimes noteworthy enlargement of liver and spleen was due mainly to amyloidosis, as shown by histologic examination. In some of these mice leukemia was absent altogether. It was possible to discern in the viscera and bone marrow three types of leukemia—hemocytoblastic, myeloblastic and myelocytic—the last being the most frequently found. Leukemic changes in the organs were almost always accompanied by a leukemic blood picture, and only rarely was there aleukemic variation.

A salient fact is that, regardless of the donor disease (chronic myelogenous, chronic lymphocytic, and acute leukemias), the mice developed myeloid leukemia. It should be noted that in one case, in which organs from a myelosis patient were used, the mouse developed changes consistent with diffuse myelosis.

In our study we did not see any difference in the changes which developed as a result of using different organs for our extracts. The highest percentage of induced leukemia (5.3 per cent) together with intense extramedullary hematopoiesis and markedly positive transfer results was obtained by the use of liver and spleen from chronic myelogenous leukemia cases. With chronic
lymphocytic leukemia organs we induced tumors only in the original hosts, and myelosis only in the subsequent transfers.

With acute leukemic organs, it is interesting to note that both in the original host and transfers, there was always a preponderance of immature forms—myeloblasts and hemocytoblasts—among the blood-forming cells.

In some of the mice, leukemia accompanied the development of tumors which usually arose at the injection site, but sometimes at a distance from it. In the original hosts, the tumor had the appearance of spindle cell sarcoma, while on subsequent transfer it assumed the form of polymorpho-cellular sarcoma (fig. 4).

In a small number of mice there appeared so-called distant tumors (Shabal) including mammary cancer, bronchogenic cancer, lymphosarcoma of the diaphragm, and peritoneal sarcoma. At times lymphoid hyperplasia of the thyroid occurred, but without the criteria of malignancy.

The changes which developed after injection of benzol-extracted organs can be divided into three main groups. Characteristic of the first group is the presence of leukemia. In the second group, leukemia was concomitant with the appearance of a tumor, usually at the injection site and occasionally at some distance from it. In the third and largest group, tumor development was observed, but leukemia was absent. Some of the mice studied showed no changes.

In analyzing the results we had to work out clear morphologic criteria of true experimental leukemia in order to distinguish it from so-called leukemoid reactions. The latter show changes mainly in the peripheral blood (leukocytes, young forms); a swelling of the reticuloendothelial cells; and a hyperplasia of blood-forming elements in the marrow without a preponderance of white cell elements.
A diagnosis of true experimental leukemia was made only in those cases showing diffuse involvement of the hematopoietic system along with myeloid tissue growth and leukemic infiltration of necrotized viscera. A leukemic blood picture usually accompanied these changes. True experimental leukemia seems to be a fatal disease in mice at the time that leukemoid changes are observed in the peripheral blood.

In addition to morphologic proof, positive transfer results are also required. But only through pathologic technics is it possible to establish precisely the diagnosis of experimental leukemia and to distinguish it from leukemoid conditions.

Thus, in our experiment with benzol extracts, in some cases we succeeded in evoking leukemia, while in others we produced malignant neoplasm, a fact which to us betokens a common pathogenetic basis of the processes.

The induction of experimental leukemia with organs of both acute and chronic leukemic donors indicates the common nature of these diseases. Likewise, the similar result obtained through our method of organ extracts and synthetic carcinogens leads us to regard them as similar carcinogenic mechanisms.

**Conclusions**

1. By injecting mice with benzol extracts of organs from deceased leukemic patients, we were able to produce both intra- and extra-medullary myeloid hyperplasia, characteristic of leukemia. In most instances a leukemic blood picture accompanied these pathologic changes. The positive transfer results obtained seemed to confirm the presence of true leukemia.

2. Only myeloid leukemia was evoked both in the original mice and the transfers—hemocytoblastic, myeloblastic and myelocytic being the types observed. The last type occurred most frequently.

3. When chronic leukemic donor organs were used, the mice usually developed a myelocytic type of leukemia; with acute leukemia extracts, hemocytoblasts were always found.

4. The development of experimental leukemia from both chronic and acute leukemic donors indicates the identical nature of these processes.

5. In some mice we observed the development of a malignant tumor, usually at the injection site, and sometimes at a distance from it.

6. Results show that the injection of one and the same benzol extract can bring about leukemia alone, leukemia plus tumor, or tumor alone.

7. Leukemias produced by our benzol extract method and by use of chemically pure carcinogens are morphologically identical.

8. Since both methods can induce malignant tumor formation together with the leukemia, this points to a close pathogenetic relationship of these diseases.

**Summario in Interlingua**

1. Le injection de extractos in benzol obtenite ab organos de decedite patientes leucemic produceva in muses hyperplasia myeloide intra e extra le medulla. In le majoritate del casos, un tableau leucemic del sanguine accompaniava iste characteristicamente leucemic alterationes pathologic. Le
obtention de resultatos positive de transferimento pareva confirmar le presentia de ver leucemia.

2. Solmente leucemia myeloide esseva provocate in le muses original e in le subjectos del transferimento. Le typos observate esseva hemocytoblastic, myeloblastic, e myelocytic. Le typo myelocytic occurreva le plus frequente-mente.

3. Quando le organos usate veniva ab donatores con leucemia chronic, le muses disveloppava generalmente un typo myelocytic de leucemia. Quando le donatores habeva leucemia acute, etiam hemocytoblastos esseva trovate in le muses.

4. Le disveloppamento de leucemia experimental per extractos ab donatores tanto con leucemia chronic como etiam con leucemia acute indica le natura identic del duo processos.

5. In certe muses nos observava le disveloppamento de un tumor maligne, usualmente al sito del injection sed a vices etiam a un certe distantia ab illo.

6. Le resultatos indica que le injection del mesme extracto benzolic es capace a evocar leucemia sol, leucemia con tumor, e tumor sol.

7. Leucemias producite per nostre methodo a extracto benzolic e leucemias producite per chimicamente pur carcinogenos es identic in lor caracteristicas morphologic.

8. Le facto que ambe methodos pote inducer maligne formationes tumoric insimul con leucemia indica un intime relation pathogenetic inter iste conditiones.
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