The suitability of pressure as a means of inducing genic or chromosomal changes in the sperm cannot be decided until experiments are done using higher pressures. However, pressure higher than 544 atms. is not available at present in our laboratory.

Possibly genic or chromosomal changes were not to be expected in the range of pressure which was used, despite the relatively long duration of the compression period. However, certain viruses do begin to undergo denaturation at a somewhat higher pressure, namely, 1000 atms., applied for 45 minutes (Basset, Wollman, Machelboeuf and Bardach[9]). On the other hand, a number of proteins retain their antigenic specificity at pressures up to 4000 atms. (Basset, Macheboeuf and Perez[4]) and a few do not appear to be denatured below 10,000 atms. (Basset, Lisbonne and Macheboeuf[10]).

Conclusion. Hydrostatic pressure of 544 atms. applied to frog sperm, either continuously, or in the alternating periods of compression and decompression, for a period of 3 hours, in no way alters the motility or the fertilizing power of the sperm. More than 5000 embryos resulting from such sperm and normal eggs, developed normally in all respects.

11124

Induction of Lymphomatosis in Mice Following Painting with 9:10 dimethyl-1:2 benzanthracene.

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There is sufficient evidence to indicate the existence of a direct relationship between carcinogenic agents and certain types of tumors in mice, *e. g.*, carcinoma of the lung, sarcoma, epithelioma. Given the hereditary predisposition to tumor formation, the application of certain carcinogens will hasten the appearance and in some instances

9 Basset, J., Wollman, E., Macheboeuf, M. A., and Bardach, M., *C. r. Acad. Sci.*, 1933, **196**, 1138.

10 Basset, J., Lisbonne, M., and Macheboeuf, M. A., *C. r. Acad. Sci.*, 1933, **196**, 1540.

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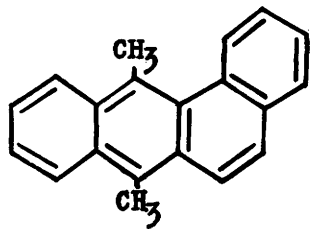
increase the incidence of the tumor. The relationship between carcinogens and the leukemoid conditions has not been so clear-cut. A leukemic condition in mice has been reported following the subcutaneous injection of 1:2:5:6 dibenzanthracene-9:10 *endo-αβ* succinate.[1] Leukemia has been reported in stock mice following painting with 1:2:5:6 dibenzanthracene combined with theelin,[2] and in derivatives of the Bagg albino strain following tar painting.[3] An atypical leukemia has been reported in mice of the S strain following intrasplenic injections of crystalline 3:4 benzpyrene.[4] Recently, a condition of general lymphomatosis has appeared in the dilute brown (dba) strain after the cutaneous application of methylcholanthrene in benzene.[5]

During the course of an experiment testing the carcinogenicity of 9:10 dimethyl-1:2 benzanthracene[6] on different inbred strains of mice

9:10-dimethyl-1:2-benzanthracene.



there appeared in one of the strains used, the dilute brown (dba) strain, individuals with a general lymphomatosis at a strikingly early age. It is the purpose of this report to record these findings.

The dilute brown strain is a high mammary tumor strain with a tumor incidence approximating 85% in the breeding females. Subline 212 is distinct from the other sublines in having a relatively high incidence of lymphoblastomas in old animals. The incidence data are as yet incomplete for this subline.[7] Ten mice of subline 212 were used. On August 1, 1939, when these animals were 4 weeks old, paintings were begun. A 0.3% solution of 9:10 dimethyl-1:2 benzanthracene in thiopene-free benzene was applied to the skin with a

1 Burrows, H., and Cook, J. W., *Am. J. Cancer*, 1936, **30**, 75.

2 Perry, I. H., and Ginzton, L. L., *Am. J. Cancer*, 1937, **29**, 680.

3 Brues, Austin M., and Marble, Beula B., *Am. J. Cancer*, 1939, **37**, 45.

4 Barnes, W. A., and Furth, J., *Am. J. Cancer*, 1937, **30**, 75.

5 Morton, John J., and Mider, G. Burroughs, *Science*, 1938, **87**, 327.

6 Furnished through the courtesy of Dr. W. E. Bachmann of the University of Michigan. See Bachmann, W. E., Kennaway, E. L., and Kenneway, N. M., *Yale J. Biol. and Med.*, 1938, **11**, 97.

7 Personal communication, Dr. G. W. Woolley of this laboratory.

No. 4 camel's hair brush. Paintings were made mid-dorsally from the interscapular to the sacral region with 2 strokes of the brush. These were continued twice weekly at the same site.

Sixty-five days after the first painting ♀$D_2$5 showed extreme bilateral lymphadenopathy of the cervical, axillary and inguinal lymph nodes and had a "swell belly". The animal appeared moribund and was killed. At necropsy there was also lymphadenopathy of the tracheo-bronchial and abdominal-mesenteric nodes. There was enlargement of the liver and spleen. The liver was pale and the spleen grayish in color. There existed a general connective tissue edema. Hydrothorax and ascites were evident. Microscopic examination of the lymph nodes showed an infiltration of lymphoid cells. The lymph node architecture was completely lost and in most cases there was infiltration through the node capsule into surrounding tissue. The nodes were grayish and soft and in most cases were larger than 1 cm in diameter. In some a brownish pigment deposit was found. Liver sinusoids were filled with lymphoid cells and dense areas were formed around the portal vessels. Large areas of the splenic architecture were completely obliterated and pulp tissue was replaced by lymphoblasts.

Five more animals showed a definite general lymphomatosis at 75, 76, 77, 82 and 89 days respectively after the first painting. These animals died from 8 to 15 days after the appearance of lymphadenopathy. In each mouse there was bilateral enlargement of the cervical, axillary or inguinal nodes with accompanying hepatomegaly and splenomegaly. Edema was not so pronounced in these animals and there was little or no hydrothorax or ascites. Histological findings of the lymph nodes, liver and spleen were identical with those described for ♀$D_2$5.

The spleen seemed to be secondarily involved in these animals as there were some regions where the architecture had been preserved. Lymphocyte counts made from blood smears ranged from 87 to 99%. Leukemic infiltration was noted in the mammary gland and connective tissues.

Of the remaining mice, 2 died of infection. ♀$D_2$9 and ♀$D_2$10 showed a moderate lymphadenopathy at 74 and 76 days respectively after the first painting, with an apparent slight subsequent regression. These animals were sacrificed at the age of 133 days. Definite areas of leukemic infiltration were noted in the axillary and inguinal nodes, with a definite capsular infiltration. The liver sinusoids showed the beginning of infiltration, but the architecture of the spleen was normal. These animals probably represent early cases of general lymphomatosis.

Not a single case of lymphomatosis has been observed among the 30 C57 brown mice and 10 A strain mice similarly painted for 145 days, nor among 35 dba, subline 212, control mice.

Summary. A general lymphomatosis has occurred in mice of the dilute brown (dba) strain (subline 212) following painting of the skin with a 0.3% solution of 9:10 dimethyl-1:2 benzanthracene in benzene. Bilateral lymphadenopathy of the axillary, inguinal or cervical lymph nodes appears as early at 95 days after birth, death ensuing within 2 weeks.

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On the Free and Combined Silica in Silicotic Lungs.

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While more analytical results for total silica in lungs are needed, there is a much greater need for values for the concentration of free silica, since it is so definitely known to cause silicosis. In the literature on the subject only several publications in the analytical line have to do with free silica in lungs and these reports are limited either to semi-quantitative results[1-4] or to calculations of silica by difference.[5, 6]

By modifying and extending the method of determining free silica in dusts, to lung residues, we have been able to determine minimum free silica. Since the chemical method involves digestion of the finely-divided lung ash residue in hydrofluosilicic acid (H_2SiF_6) in order to dissolve away silicates, and since finely-divided free silica is appreciably soluble in the above acid, we can, however, report only *minimum* free silica values.

The solubility of free silica in hydrofluosilicic acid varies with particle size, among other factors. Particles of silica as long as 10 microns may gain entrance to lungs, although the great majority

1 Sweany, Henry C., Klaas, R., and Clark, G. L., *Radiology*, 1938, **31**, 299.

2 Hicks, Victor, *Instruments*, 1936, **9**, 133; *Ind. Med.*, 1936, **5**, 173.

3 Hicks, Victor, McElroy, O., and Warga, M. E., *J. Ind. Hyg. and Toxic.*, 1937, **19**, 177.

4 Jephcott, C. M., Gray, W. M., and Irwin, Dudley A., *Canadian Med. Assn. J.*, 1938, **38**, 209.

5 Badham, Charles, and Taylor, Harold B., *Med. J. Australia*, 1933, **1**, 511.

6 Jones, William R., *J. Hyg.*, 1933, **33**, 307.