In no case in which conditions were properly controlled was there evidence of a decrease in excystment time when cysts were irradiated with dosages below the threshold dose causing increased time of excystment.

The results indicate that (1) while radiations of λ3,130Å failed to produce visible effects or kill protozoans after prolonged exposures, when the dosage is sufficiently great, slight retardation of certain cell processes may occur. This can be demonstrated if the test material is sufficiently delicate. (2) The fairly large dosages of radiations of λ3,660Å had exceedingly slight or no retarding effects upon even the more sensitive cell functions here tested.

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THE PRODUCTION OF LYMPHOMATOSIS IN MICE OF KNOWN GENETIC CONSTITUTION[1]

LYMPHOMATOSIS, with or without a leukemic blood picture, has been observed in mice after painting the skin with 1:2:5:6-dibenzanthracene[2] and following the subcutaneous injection of sodium 1:2:5:6:-dibenzanthracene-9:10-endo-α:β-*succinate*.[3] An atypical leukemia followed the intrasplenic injection of 3:4-benzpyrene in a mouse of the S strain.[4] The chemical production of leukoses has been criticized because of the high spontaneous incidence of mouse leukemia and the alleged failure to adequately control the genetic factors.[5] We wish to record the appearance of lymphomatosis in mice of known genetic constitution that followed painting with methylcholanthrene.

The mice used were of the dilute brown strain developed by Little. They were obtained from the Roscoe B. Jackson Memorial Laboratory, where the strain has been followed for more than twenty-five generations. Leukemia has occurred rarely and never before the animals were eighteen months old.[6] Breeding females have a very high incidence of spontaneous mammary cancer.

Forty-eight mice were used. They were not allowed to breed. On November 1, 1937, when the animals were four to six weeks old painting was commenced. The material used was 0.5 per cent. methylcholanthrene in commercial benzene. It was applied with a No. 6 camel's hair brush. The site was changed with each painting so that the same area was not painted twice during the same month. Contiguous regions were not painted in succession. The order was: head, left hind leg, right hind leg, left foreleg, right foreleg, sacral region, abdomen, interscapular region, anterior thorax. Three mice died of infection before the fifty-third experimental day.

When the animals had received their tenth painting (sixty-ninth day) a subcutaneous inguinal mass was found in a female mouse. Three days later bilateral inguinal and axillary lymphadenopathy was present. The animal died on the seventy-fifth day of the experiment. At autopsy the axillary, inguinal, abdominal and tracheo-bronchial lymph nodes were greatly enlarged, attaining a diameter of more than 1 cm. The liver was pale. The spleen was large and gray.

Microscopically the architecture of the lymph nodes was obliterated. The nodes were filled with atypical lymphoid cells which were closely packed together. The cells were larger than normal lymphocytes. They had scanty basophilic cytoplasm and large, round, vesicular nuclei with prominent nucleoli. Mitotic figures were numerous. Capsular infiltration was noted. The normal structure of the spleen was lost. The pulp was replaced by cells similar to those found in the lymph nodes. The liver showed marked leukemic infiltration, particularly in the peri-portal areas.

Nine more mice, four females and five males, have subsequently developed the same picture. The disease is first noted as a cervical, axillary or inguinal lymphadenopathy which is bilaterally symmetrical in distribution. The mice die within two or three weeks of the onset and present more or less generalized lymphoid enlargement. Some of the nodes have measured more than 2 cm in diameter. Subcutaneous edema, ascites and hydrothorax have been found. The fluid is usually clear. Leukemic infiltration has been seen in lung, skin, coagulating gland, bladder, kidney and skeletal muscle. The myeloid cells of the bone marrow are replaced by lymphoid cells.

Blood studies have been done on two mice. The leucocytes numbered 90,000 and 139,000, respectively. A secondary anemia was present. The white cells were almost all of the lymphoid series. Many immature forms were seen.

Five skin tumors have been produced thus far (one hundred and third day), one of which is malignant. None of these animals have shown any evidence of lymphadenopathy.

No tumors have appeared in fifty control mice.

Although the experiment has not been completed, the production of lymphomatosis with a leukemic

[1] From the Department of Surgery, the University of Rochester School of Medicine and Dentistry. This investigation was aided by grants from the International Cancer Research Foundation and from Mr. Simon Stein.

[2] I. H. Perry and L. L. Ginzton, *Am. Jour. Cancer*, 29: 680, 1937.

[3] H. Burrows and J. W. Cook, *Am. Jour. Cancer*, 27: 267, 1936.

[4] W. A. Barnes and J. Furth, *Am. Jour. Cancer*, 30: 75, 1937.

[5] M. N. Richter and E. C. MacDowell, *Physiol. Rev.*, 15: 509, 1935.

[6] C. C. Little, personal communication.

blood picture seems to be significant in the dilute brown strain of mice. The general appearance of the animals is different from that observed in the rare cases that have occurred spontaneously in this strain.[6] The incidence, to date, is more than 20 per cent. and the time elapsed in the development of the lymphomatosis is one third of that required for the spontaneous disease to appear. Further studies are being undertaken to evaluate the many factors involved, and other strains of mice are being investigated in the same manner.

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A PARADOX IN THE ADAPTATION OF MARINE BACTERIA TO HYPOTONIC SOLUTIONS[1]

ACCORDING to Korinek[2] marine bacteria are distinguishable from freshwater or terrestrial species on a basis of their salt tolerance. This is in agreement with the observations of ZoBell and Feltham,[3] who found that less than 10 per cent. of the bacteria isolated from the sea at places remote from terrigenous contamination can multiply in nutrient freshwater media, and a smaller percentage of bacteria from freshwater sources multiply in undiluted sea-water media.

In studying the factors which influence the specific salt requirements of bacteria from heterologous environments, attempts were made to acclimatize marine bacteria to hypotonic solutions by gradually diluting the sea-water medium with each successive transfer of the cultures. Twelve recently isolated species were selected which would grow in nutrient sea-water broth but not in similar freshwater media. The media consisted of 0.2 per cent. each of Bacto-peptone, proteose-peptone and beef-extract and 0.025 per cent. of ferric chloride in either sea water, distilled water or various dilutions of sea water. The pH, incubation temperature (22° C.) and other experimental conditions were the same with the different kinds of media. At the beginning of the experiments all twelve cultures multiplied in 75 per cent. sea-water medium (75 parts of sea water diluted with 25 parts of distilled water). Seven of them grew in 50 per cent. sea-water medium, but none of them grew in 25 per cent. sea-water medium.

As soon as the turbidity of the 75 per cent. sea-water broth indicated good growth, a loopful of a dilute suspension of each culture was transplanted to 70 per cent. sea-water broth. If after 24 to 48 hours there was evidence of multiplication, this in turn was used to inoculate 65 per cent. sea-water broth, and so on seriatim, decreasing the concentration of sea water each time by 5 per cent. Under these conditions all twelve cultures were readily adapted to 45 per cent. sea water. Upon the next transfer, however, three of the cultures failed to show any signs of growth in 40 per cent. sea water, even after a week's incubation, although in higher concentrations of sea water they rendered the media turbid with growth in 24 to 48 hours. Therefore transplants of these fastidious cultures were made from 45 per cent. to 42½ per cent. sea-water medium, and in each successive medium the concentration of sea water was decreased by only 2½ per cent.

Most of the cultures could be acclimatized to 25–30 per cent. sea-water media, but below this concentration considerable difficulty and delay were encountered in making them grow, although the concentration of the sea water was changed by decrements of only 2½ per cent. After five months of almost daily attention four of the original twelve cultures were multiplying in freshwater medium containing no sea water. The lowest concentration in which the others grew may be summarized as follows:

2	cultures	in	5	per	cent.	sea water
3	"	"	10	"	"	"
1	culture	in	17½	"	"	"
1	"	"	22½	"	"	"
1	"	"	25	"	"	"

In each case attempts to induce growth in the next lower dilution were negative, and the growth in the above media was very meager after 48 hours.

At this stage the parent stock cultures, which had been maintained in the refrigerator on sea-water agar during the five-month period, were reexamined. Microscopic inspection of stained smears revealed that the acclimatized cultures did not differ morphologically from their parent cultures. Paradoxically, though, it was found that all except three of the parent cultures multiplied when transplanted to freshwater medium and these three multiplied in 10 per cent. sea-water medium. Similar results were obtained with the original sea-water broth cultures, which had been maintained at room temperature since the beginning of the experiment, although the salt content of the medium had actually increased due to the evaporation of water. In other words, the old stock cultures were more euryhaline and better adapted to grow in hypotonic solutions than their progeny, which had been rejuvenated almost daily in attempting to gradually acclimatize them to lower salinities.

A repetition of the experiment with other recently isolated marine bacteria yielded similar results. In fact, tests on over a hundred different pure cultures of bacteria, which upon initial isolation from the sea

[1] Contributions from the Scripps Institution of Oceanography. New Series, No. 16.
[2] J. Korinek, *Centralbl. f. Bakt.*, Abt II, 71: 73, 1927.
[3] C. E. ZoBell and C. B. Feltham, *Proc. Fifth Pacific Sci. Cong.*, 3: 2097, 1933.