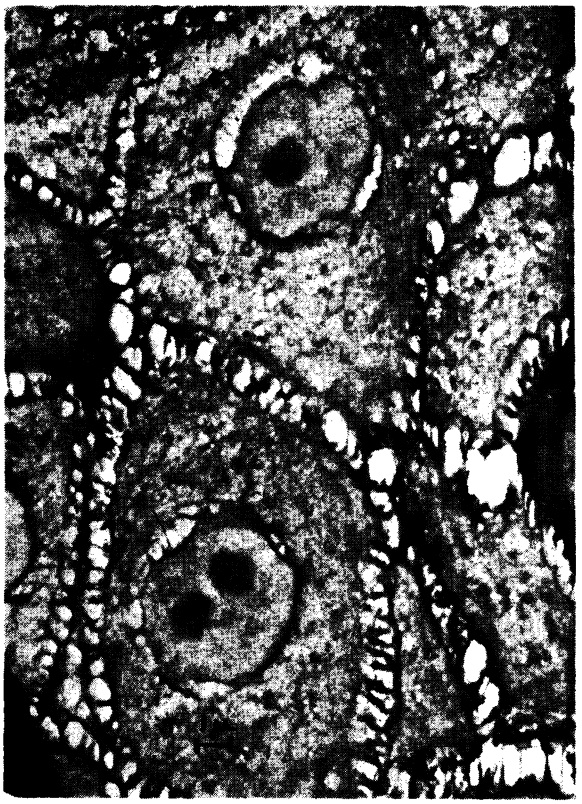

FIG. 1. Electron micrograph of 0.1-μ section of human epidermis (×3540).

ing no apparent relation to the intercellular bridges. The diameter of these fine fibrils in the cytoplasm is more than likely a function of the fixative used, and is at a minimum for osmic acid.

Two interpretations of the intracellular fibers seen in light-microscope preparations suggest themselves.

Experience with fixatives other than osmic acid indicates that the size of the precipitated proteins (meshwork) is often of the same order of magnitude as the fibrils that have been described, and the general visual effect when viewing an ordinary stained section at one focal level would correspond to an appearance of fibrils against a smooth out-of-focus background. But the most likely interpretation is that in any section containing more than a single layer of cells one gets a very definite impression of fibrils as a result of intercellular bridges lying just above or below the plane of focus. Such artifacts arising from the limited depth of field of the light microscope are probably more numerous than is commonly realized. The use of very thin sections with the electron microscope has the disadvantage that it becomes difficult to follow structures that do not lie exactly in the plane of the section, but the large depth of field in the object space, which is characteristic of electron optics, serves to counterbalance this disadvantage to a great extent.

It must be added that preliminary work with pathological material has confirmed a previously recognized fact; namely, that there is a great proliferation of fibers throughout the epidermis. Only with such preparations have intracellular fibers been seen in thin sections.

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Effect of Hibernation upon Survival Time following Whole-Body Irradiation in the Marmot (*Marmota monax*)

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The relationships between reduced body temperature, lowered metabolism, and rate of development of the toxic effects of radiation have hitherto been little studied among mammals. The rate of development of the lethal processes following irradiation is reduced by lowered environmental temperature in frogs (*1*). Similar results have been obtained with chick embryos (*2*) and amphibian eggs (*3*). The reduced metabolic rate that resulted from chilling the irradiated frogs was accompanied by an increased survival time, but no reduction in mortality was observed. Increased metabolic rate resulting from thyroid administration has been found to coincide with an increased mortality in mice following irradiation (*4*). On the other hand, the administration of antithyroid substances, which reduce the metabolic rate, does not alter radiation lethality nor increase the survival time in irradiated mice (*5*).

The metabolic rate in the hibernating marmot averages only about one third that found in the nonhibernator (*6,7*). Marmots were selected for the experiments reported here in order to find out whether sensitivity to radiation would be lower in the hibernator than in the nonhibernator.

Two groups of marmots, equally matched as nearly as possible with respect to number, age,[1] and sex were used for the experiment. The animals of one group were allowed to hibernate in a constant-temperature room held at 3.5° C ± 0.5° C and were irradiated 3 weeks after the onset of deep hibernation. The other group was maintained at room temperature and served as nonhibernating, irradiated controls. The nonhibernating marmots were given 550 r, which was previously found to be approximately the LD_{100}. Higher doses, 650 r and, in one case, 800 r, were given to the hibernating marmots to accentuate a possible decreased radiation sensitivity in the hibernating phase.

[1] Roy Grizell, in a personal communication, kindly provided information, accumulated from several seasons of trapping, indicating that up to about 4 kg these animals increase in weight at a rate roughly equivalent to 1 kg/yr.

TABLE 1

WEIGHT CHANGE AND MORTALITY IN IRRADIATED HIBERNATING MARMOTS

No.*	Sex	Average wt before hibernation (g)	r	Days in hibernation after r	Average wt loss after r (hibernating) (g/day)	No. surviving	Days of survival after end of hibernation	Average total survival time (days) of those dying after r
2	♂	1,430	650	28, 28	3.9	1	14.0	42
3	♀	1,946	650	*a*)33, *b*)35, *c*)30	6.6	0	*a*)6, *b*)10, *c*)7	40
2	♂, ♀	2,250	650	♂ 42, ♀ 38	5.7	0	♂ 9, ♀ 4	46
1	♀†	4,120	800	21	14.0	0	(died in hib.)	21

* One animal was aberrant in that it required 72 days in the cold before the onset of hibernation. This marmot died in hibernation at 14 days after irradiation (650 r) and is not represented in the table.

† This animal, included here for convenience, was given 800 r.

Nine marmots, whose mean weight was 2.4 kg, were caged singly and provided with shredded wood for nesting purposes. A combination of low temperature, reduced food supply, darkness, and a minimum of disturbance aided in initiating and maintaining hibernation throughout the experiment. Four marmots were removed from hibernation and returned to room temperature, 2 at 28 days and one each at 35 and 42 days after irradiation. Of the other 4 hibernating marmots given 650 r, one died in hibernation at 14 days after irradiation and 3 spontaneously terminated hibernation and had to be returned to room temperature on the days indicated in Table 1.

Nine nonhibernating control animals averaging 2.2 kg in weight were singly caged, maintained at room temperature of about 27° C, and were given 550 r. One additional marmot which did not enter hibernation even after 70 days in the cold was given 650 r and returned to the cold room (Table 2).

Irradiation was carried out at the rate of 49 r/min with a 200 kvp x-ray unit with 0.08 mm copper added filtration and at a target distance of 70 cm. One half the dosage was applied to each side of the animals. An especially constructed plywood box provided with a device that prevented the animal from rotating was used for the nonhibernators. Care was taken to avoid unnecessary warming of the hibernators at any time. Heavy gloves were worn while handling these animals, and a stream of chilled air was passed over them during irradiation.

Weekly measurements of rectal temperatures averaged 4.4° C during 58 days of hibernation in 2 nonirradiated marmots, whereas the mean rectal temperature in nonhibernating marmots is about 36.5° C.

The marmots completing 28–42 days of hibernation after 650 r survived 40–45 days, as shown in Table 1, compared with a survival time of only 18–27 days for the nonhibernators given 550 r (Table 2). One hibernator recovered and one died in hibernation at 14 days after irradiation (650 r), and 3 nonhibernators recovered. Increases in the length of hibernation time after irradiation did not result in corresponding increases in posthibernation survival time. The differences between posthibernation survival time and the survival time of nonhibernators may be attributed either to the higher radiation dose given the hibernators or to damage accumulating during hibernation or to both these factors.

Weight loss in the hibernating marmots given 650 r averaged 5.6 g/day (range 2.7–8.8 g) during the period between irradiation and the end of hibernation, compared with an average weight loss of 9.2 g/day (range 3.3–15.5 g) during the first 18 days after irradiation in 7 nonhibernators; gains in weight occurred during this period in 2 other nonhibernators. Weight loss averaged 3.0 g/day for 2 nonirradiated control marmots during 58 days of hibernation.

TABLE 2

WEIGHT CHANGE AND MORTALITY IN IRRADIATED NONHIBERNATING MARMOTS

No.	Sex	Mean wt at r (g)	r	Mean wt at r+18 days (g)	No. surviving	Mean survival time and range (days) of those dying
5	♂	2,388	550	2,361	3	21 (19–23)
4	♀	1,855	550	1,705	0	21 (18–27)
1	♂*	2,680	650	1,900	0	12

* This animal, included here for convenience, was maintained at 3.5° C and irradiated 71 days after entry into the cold.

The hibernator receiving 800 r died at 21 days while still in hibernation. During the second week there was a precipitous fall in body weight and in leucocyte and erythrocyte counts. During the hibernating phase the marmots given 650 r showed only those blood changes characteristic of hibernation, but after their return to room temperature both erythrocyte and leucocyte concentrations fell abruptly (*7*).

It is evident from the results of these experiments that a delay in the rate of development of the lethal processes following irradiation accompanies lowered body temperature and reduced metabolic rate in the marmot. The fact that irradiated marmots died in hibernation after only slightly higher doses (one 650 r, one 800 r) than that found lethal for most nonhibernators (550 r) indicates that no great decrease in sensitivity to radiation is attendant upon the change to the poikilothermic state.

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The Hematologic Effect of Folinic Acid (Citrovorum Factor) in Persons with Pernicious Anemia[1]

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A factor in refined liver extract necessary for the growth of *Leuconostoc citrovorum* has been isolated by Sauberlich and Baumann (*1*). The growth-promoting properties of this factor cannot be replaced by vitamin B_{12} or thymidine, but growth of the organism will occur in the absence of this factor if very large amounts of folic acid are provided. Subsequently, Sauberlich (*2*) demonstrated that "citrovorum factor" overcomes the inhibitory effect of aminopterin on *Leuconostoc citrovorum*. The excretion of the "citrovorum factor" in the urine of rats or human beings is enhanced by the administration of folic acid (*3*). Nichols and Welch (*4*) have shown that "citrovorum-factor" activity of liver slices from normal and folic-acid–deficient rats is increased by incubation with folic acid, and that ascorbic acid enhances this effect. These data indicate that folic acid is a precursor of the "citrovorum factor" and suggest that ascorbic acid plays a part in the conversion.

Bond, Bardos, Sibley, and Shive (*5*) isolated a substance called "folinic acid" which overcomes the inhibition of methyl folic acid on the growth of *Lactobacillus casei* more effectively than folic acid. This substance is similar to the "citrovorum factor" in promoting the growth of *Leuconostoc citrovorum*. The same workers (*6*) have described a method for the synthesis of a substance with properties similar to "citrovorum factor" and "folinic acid," and it is probable that these two substances are identical.

May *et al.* (*7*) have reported that crystalline folinic acid is more effective than folic acid in relieving the megaloblastic anemia of monkeys deficient in folic and ascorbic acids. This observation, together with the work of Nichols and Welch mentioned above (*4*), suggests that folinic acid may be a biologically important intermediate in the metabolism of folic acid.

[1] This work was aided by a grant from the Robert Gould Research Foundation.

[2] The authors wish to thank James M. Ruegsegger, of Lederle Laboratories, for generous supplies of crystalline citrovorum factor and folic acid.

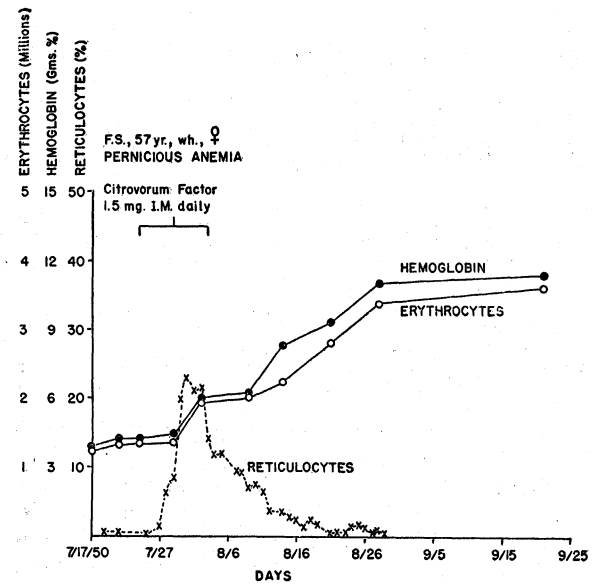

FIG. 1. Hematologic response to citrovorum factor (folinic acid) in patient with pernicious anemia in relapse.

There is evidence that folic acid may be converted to a metabolically active form in persons with pernicious anemia in relapse before it exerts its hematopoietic effect (*8–10*). Such a substance should induce a hematologic effect in doses much smaller than those usually required with folic acid. Since "folinic acid" has the potentiality of being this active form of folic acid, its hematopoietic effect has been tested in persons with pernicious anemia in relapse.

Three such subjects have received intramuscular injections of this substance for 10 consecutive days; the daily dose in 2 of the subjects was 3 mg and in the other, 1.5 mg. In all 3 patients hematologic responses occurred. Erythrocyte and hemoglobin rises were as good as would be expected with similar amounts of folic acid. Reticulocytes increased in all three instances, with peaks of 9%, 13.7%, and 23.1% on the sixth or seventh days of treatment. The megaloblastic bone marrow was converted rapidly to a normoblastic type, as happens after oral or parenteral treatment with folic acid. One of the hematologic responses is recorded in Fig. 1.

Folinic acid was no more effective than folic acid, however. One of the subjects who responded to the daily administration of 3 mg had previously failed to respond to 0.6 mg of the substance daily for 10 days. Another subject with pernicious anemia, previously responsive to refined liver extract, vitamin B_{12}, and folic acid, had relapsed hematologically while receiving 20 mg of folic acid daily. He was given 3 mg of "citrovorum factor" daily for 10 days. There was no reticulocytosis, and erythrocytes and hemoglobin failed to rise following this therapy. Subsequently, he was given vitamin B_{12}, 15 μg daily for 3 weeks and then 20 μg weekly. There was a desultory hematologic response similar to that previously described in similar subjects (*9*).