the reaction, or possibly to the presence of minute amounts of contaminating glucose in their samples.

The minimum concentration of glucose which reacts with the enzyme stick is about 0.002 percent in water and 0.01 to 0.05 percent in urine. This indicates the small amounts of glucose that would have to be present in the commercial sugar samples to give the results obtained. It is impressive that, after glucose removal, none of the sugars in urine gave a positive reaction at a concentration less than 20 percent. This concentration is much more than is ever encountered in any of the benign meliturias (*3*). Accordingly, the specificity of the test is demonstrated.

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References and Notes

1. These sticks for detection of urinary glucose are supplied by the Ames Company of Elkhart, Ind., under the registered trademark of Clinistix.
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Effect of Bone Marrow Treatment on Mortality of Mice Irradiated with Fast Neutrons

Lethality following acute whole-body x-ray irradiation of laboratory animals can be greatly reduced by injecting a suspension of normal bone marrow cells into the animals after irradiation (*1*). Increases in the probability of recovery of guinea pigs following chronic gamma-ray irradiation and following the mixed radiation from an intravenous injection in mice of a sodium citrate solution containing radon in equilibrium with its decay products have also been reported for the same bone marrow treatment (*2*). A further test of this treatment for mice following irradiation by fast neutrons is described in this report (*3*).

The mice, usually 12 weeks old, were placed in individual perforated plastic tubes at a distance of 15 cm from the target of a Cockcroft-Walton accelerator. They were then irradiated with 14.1-Mev neutrons produced by the $H^3(d,n)He^4$ nuclear reaction. The dose rate in wet tissue was 100 to 200 rad/hr. There was little gamma contamination (*4*). In each experiment, the dose was determined by the use of two simultaneous monitors. Isologous bone marrow was taken from the femurs of normal mature animals, suspended in Tyrode's solution, and injected intravenously into half of the irradiated animals within a few hours after the completion of irradiation. In all but one experiment reported here, each treated mouse received the bone marrow taken from the shafts of eight femurs. Control and test animals were identically caged, 3 to 6 to a cage. Purina laboratory chow and water were supplied *ad libitum*. In all experiments, survival was checked daily up to 30 days, and in some experiments average body weights of treated and control groups were followed.

Table 1 gives the mortality results for seven experiments and the totals for all experiments. In every experiment, the 30-day mortality was greater among the control animals than among the animals treated with bone marrow. The most pronounced effect of the treatment was the 5/1 reduction in the totals in the probability of mortality during the second week of animals surviving the first week. Ninety-seven percent of all deaths occurred within 2 weeks after irradiation.

Applications of the chi-square statistic to the totals indicated that the reduction in mortality following bone marrow treatment was significant at the 1-percent level for 30-day mortality, 14-day mortality, and mortality during the second week, and at the 4-percent level for 7-day mortality, but was insignificant for 5-day mortality. Applications of the three-dimensional chi-square test (*5*) to the totals of LAF_1 and $101 \times C_3HF_1$ mice indicated that: (i) at the 10-percent level, bone marrow treatment of LAF_1 mice was more effective than treatment of $101 \times C_3HF_1$ mice, but this may be because of differences in the mortality levels in controls for the two strains; and (ii) that there was no significant difference in the effectiveness of treatment of males versus females.

Curves of the average weights of the survivors at each day for the first three experiments showed that the control animals reached a minimum of about 73 percent of their initial weight at 8 days postirradiation but asymptotically approached the initial weight at 30 days. The bone marrow treated animals reached a minimum of about 76 percent of their initial weight at 6 days and asymptotically approached their initial weight at 30 days. The effects of smaller doses of bone marrow are being investigated. The mortality among control animals exposed at these dose rates to 14-Mev neutron irradiation was somewhat lower and later than the reported values for equal irradiation by lower energy neutrons at higher dose rates (*6*).

The fast neutron doses administered were chosen to produce more than 50 percent mortality at 30 days in control animals. The dose required to meet this condition is a function of the dose rate, average linear energy transfer and probably the ratio of dose to bone marrow to that to the gastrointestinal tract. An investigation of the relative biological effectiveness for acute lethality in the RF strain of mice as a function of these parameters is in preparation (*7*). The higher concentration of elements with atomic weights greater than 20 (for example, calcium and potassium) and lower concentration of hydrogen in bone than in wet tissue tend to make the ratio of bone marrow dose to tissue dose other than unity. The ratio of the first collision dose rate in a large homogeneous bone to the dose rate in wet tissue exposed to the same 14-Mev neutron flux is estimated as about 0.75. This value is only an approximation to the value ob-

Table 1. Effect of bone marrow treatment on mortality from fast neutrons.

Strain	Sex	Dose (rad)	Treatment	Mortality* during days		
				0–7	8–14	0–30
RF	M	715	Bone marrow†	3/10	1/ 7	6/10
RF	M	715	None	1/10	9/ 9	10/10
$101 \times C_3HF_1$	M	837	Bone marrow	2/12	1/10	3/12
$101 \times C_3HF_1$	M	837	None	3/11	2/ 8	5/11
$101 \times C_3HF_1$	M	906	Bone marrow	7/12	1/ 5	8/12
$101 \times C_3HF_1$	M	906	None	7/12	4/ 5	11/12
$101 \times C_3HF_1$	F	877	Bone marrow	3/11	0/ 8	3/11
$101 \times C_3HF_1$	F	877	None	9/12	2/ 3	11/12
$101 \times C_3HF_1$	F	902	Bone marrow	0/12	1/12	1/12
$101 \times C_3HF_1$	F	902	None	3/12	1/ 9	5/12
LAF_1	M	900	Bone marrow	1/12	3/11	4/12
LAF_1	M	900	None	6/12	6/ 6	12/12
LAF_1	F	800	Bone marrow	1/10	0/ 9	1/10
LAF_1	F	800	None	2/12	6/10	8/12
Total			Bone marrow	17/79	7/62	26/79
Percentage			Bone marrow	21.5	11.2	32.9
Total			None	31/81	30/50	62/81
Percentage			None	38.3	60.0	**76.5**

* The numerator gives the number of mice that died during the interval and the denominator gives the number living at the start of the interval.
† Each of these mice received bone marrow from only 3 donor femurs.

taining in the bone marrow of a mouse because of the uncertainties in atomic composition of bone and bone marrow and because of the lack of homogeneity of composition in volumes encompassing the range (up to about 2 mm) of the recoil protons (the most important and energetic ionizing particles produced by fast-neutron irradiation). In contrast to the fast-neutron case, in typical x-ray irradiations, this ratio in homogeneous bone is perhaps 3 (*8*), and the applicable value of the ratio is subject to the same kinds of uncertainties as for neutron irradiation. Also because of the relative compositions, the ratio of the average linear energy transfer in bone to that in wet tissue is probably less than unity in photon irradiations and greater than unity in neutron irradiations.

In a general way, most acute irradiation deaths have been classed as caused by gastrointestinal or bone marrow damage (*9*). In gastrointestinal damage, deaths in mice tend to occur within less than 7 days after irradiation and in bone marrow damage during the second week after irradiation. Considering the characteristically sharp break in the shape of mortality versus dose curves and the ratios of dose in bone to that in wet tissue, one might anticipate that a change from x-ray to fast-neutron irradiation would markedly increase the percentage of gastrointestinal deaths in irradiations with equal over-all mortality. This has been found by various workers. Furthermore, owing to the increase in gastrointestinal death, against which bone marrow treatment is expected to be ineffective, one might anticipate that bone marrow treatment would be less efficacious against death (from all causes) following fast-neutron irradiation than following x-ray irradiation. At best, the frequency distribution of mortality might be shifted to that of gastrointestinal death alone.

Comparison of the results of the present fast-neutron irradiation experiments, in which bone marrow treatment produced a significant reduction in mortality only after the period assigned to gastrointestinal death, with previous x-ray irradiation experiments (*1*) supports these conclusions.

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New Principle of Preparative Electrophoresis

Electrophoresis is potentially the most elegant and efficient method for protein fractionation and isolation. Even before the adaptation of the method to analytic purposes by Tiselius, there were numerous attempts to utilize it for preparative purposes. The older literature has been well reviewed by Svensson (*1*) and the more recent upsurge of various apparatus designs has also been partially reviewed (*2*).

Some of the older electrophoretic methods were based on the electrodecantation principle of Pauli (*3*). Proteins, and other charged colloids, when exposed to a direct-curent electric field will migrate toward one of the electrodes and, therefore, will accumulate in the immediate neighborhood of a semipermeable membrane placed in their path of migration. This layer of increased colloid concentration will, by virtue of its higher density, settle to the bottom of the vessel, where it can be withdrawn separately from the bulk of the solution. This principle was also adopted by Kirkwood *et al.* (*4*) in the electrophoresis-convection method, using certain elements of the Clusius column.

Based on the fact of the accumulation of charged colloids in the immediate neighborhood of a semipermeable membrane, a new method of preparative electrophoresis was developed, which is best described by the functional name, *continuous free-boundary flow-electrophoresis* (*5*). The method takes advantage of the laminar flow of liquids, and its principle can be illustrated with the aid of the diagram presented in Fig. 1. An electrophoretic cell is constructed of two outer semipermeable membranes *A* and *A'*, defining the size of the cell, and held stretched in a plastic frame (not shown in the diagram). The cell is immersed in a circulating, cooled buffer, and a direct-current electric field can be established across the membranes. The two membranes are kept parallel, 3 to 4 mm apart, while a third semipermeable membrane *B* is inserted between them part way into the cell. The cell is thus divided into three compartments, two at the top and a common one at the bottom. All three compartments have means for continuous input or withdrawal of the colloid solution.

If it is assumed that the solution to be fractionated contains two proteins, differing in their electrophoretic mobility or isoelectric points, then the *p*H of the solution and of the outside buffer is adjusted close to the isoelectric point of one of the proteins. The solution is continuously fed into one of the top compartments, and the polarity of the current is selected so that the other protein, the mobile one, migrates toward the outer membrane of the compartment, as indicated by the short arrows in Fig. 1. The rate of flow of the vertical column of liquid is adjusted so that the migrating protein can reach the outside membrane by the time the liquid containing it reaches the bottom of membrane *B*. This migrating protein will have formed a layer of increased concentration in the immediate neighborhood of the outer membrane, and will be withdrawn quantitatively in that part of the liquid which is collected through the bottom compartment. The other protein, at, or close to the isoelectric point, will not have been influenced by the electric field, and will therefore be uniformly distributed throughout the liquid. It can therefore be withdrawn from the other top compartment.

The inflowing liquid is thus separated into two fractions. The bottom fraction contains all the migrating proteins, but

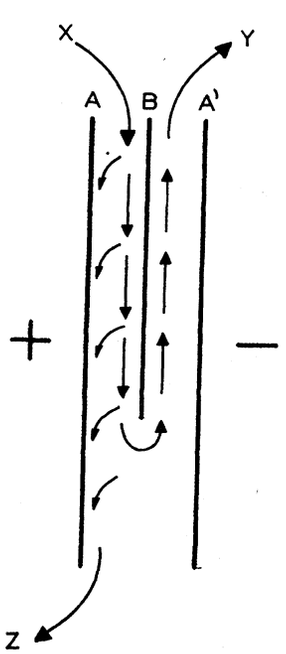

Fig. 1. Continuous free-boundary flow-electrophoresis apparatus. *A, A',* outer membranes; *B,* intermediate membrane; *X,* input of solutions; *Y, Z,* withdrawal of solutions.