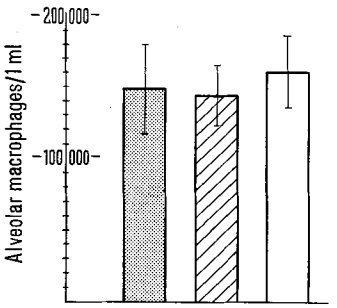
aspirated back into the syringe. The second filling was aspirated back from the lungs after a 10 min interval. After mixing both reaspirated fluids we counted the alveolar macrophages (MAXWELL et al.[7], MYRVIK et al.[8]).

Results. By lavation of the lungs of the first group in which full metabolic acidosis had been evoked (pH 7.04), we obtained on an average 149,813 ± 31,426 alveolar cells/1 ml. From the lungs of the second group, where only the control operation was performed, i.e. ligation of both kidneys, catheterization of the arteria carotis commun. sin. and of the vena renalis sin. (pH 7.40), we obtained on an average 144,500 ± 21,451 of alveolar cells per 1 ml. By lavation of the lungs of the totally healthy animals (pH not measured) we gained on the average 161,500 ± 25,464 alveolar cells/1 ml. As it can be seen from the Figure, there is no statistically significant difference between the amount of delivered alveolar cells gained by lavation from the lungs of animals in the 3 particular experimental groups.



The number of alveolar macrophages obtained by lavation of rat lungs. ■, acidosis; ////, control operation; □, control.

Conclusion. The experiments showed that the entire metabolic acidosis evoked by the infusion of 1 *N* HCl, which produces the decrease of pH on the average to 7.04, could neither increase nor decrease the amount of the cells delivered into alveolar spaces owing to the influence of denatured calf plasma dissolved in the saline. So we can say that metabolic acidosis itself does not influence the ability of the alveolar cells to enter into the alveolar spaces.

Zusammenfassung. Tierexperimentell wurde festgestellt, dass die metabolische Azidose keinen Einfluss auf die Fähigkeit der Alveolarzellen hat, in die Alveolen auszuwandern.

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Radioprotective Effect of Phytohemagglutinin in Mice

Phytohemagglutinin (PHA) has been found to induce blastoid transformations, mitoses[1], and radioresistance[2] in human blood lymphocytes in vitro. Similar morphologic transformations have also been observed in suspensions of blood and tissue lymphocytes obtained from laboratory animals[3]. MEKORI et al.[4] hold the view that phytohemagglutinin (PHA) acts in vivo in a manner similar to its established action in vitro, i.e., by stimulating the change of lymphocytes into dividing blast cells which can initiate lymphoid colonies in the spleen of an irradiated host. Moreover, HUMBLE[5] observed regeneration of bone marrow function in 6 patients with aplastic anemia, treated with repetitive i.v. injections of PHA. HUMBLE holds the view that the small lymphocytes of the blood normally enter the bone marrow and become transformed into precursor cells of the red and white series, as Maximow claimed. Based on these observations, a series of experiments was designed to test whether PHA had any protective effect on mice exposed to dosages of irradiation which normally cause death from bone marrow failure.

Methods. Twelve-week-old CF_1 mice (Carworth, Inc.) weighing 20–25 g were used in these experiments. Vials containing 100 mg of phytohemagglutinin-P (Difco) were diluted with 33.3 ml of isotonic saline. This resulted in a final PHA-P concentration of 3000 μg/ml. For the serial dosage experiments, dilutions were made from this preparation. Two groups of control and PHA-treated animals received i.p. 1 ml of saline or diluted PHA, respectively. Two other groups with the same number of animals in each, treated with either saline or PHA alone, were retained as non-irradiated controls. Whole body irradiation was performed by a 300 KeV, 20 ma X-ray machine with target-mouse distance of 50 cm, HVL 2 mm Cu and 150 R per min dose rate.

Results. Table I presents the percent survival of the mice exposed to 600 R whole-body irradiation as a function of PHA dose. The drug was injected 30 min before irradiation. The degree of radioprotection appears to be a linear function of the PHA dose used. At the highest dose of 3000 μg or approximately 120 mg/kg, 53.3% versus 16.7% survival was observed in favor of the experimental group. This dose was used in the additional experiments. Subsequently tested was the radioprotective effect of PHA as a function of time between drug injection and irradiation (600 R). Table II shows that the protection

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was most marked when PHA was injected 30 min after irradiation. Some radioprotection was also observed when PHA preceded irradiation by 24 h or 30 min. Several experiments (20–30 animals in each group) were also performed to test the radioprotective effect of PHA with various doses of irradiation (Figure). The PHA (3000 µg) was injected 30 min before X-ray exposure. Irradiation doses ranged from 500–800 R. A certain degree of radioprotection could be detected at any dose level through 750 R. At 800 R no mice in either group survived 30 days; most of them died before the 12th day, the majority with signs of rectal bleeding. A single i.p. injection of 3000 µg of PHA-P was not lethal within 30 days to any of the PHA-treated non-irradiated control animals.

Table I. Survival of 12-week-old CF_1 ♀ mice after 600 R whole-body X-irradiation and phytohemagglutinin (PHA)

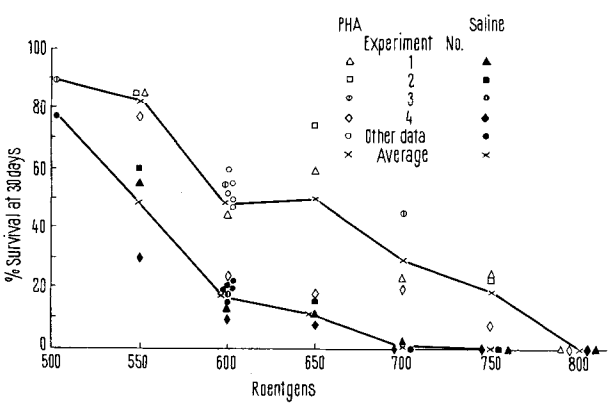
Dose of PHA	Survivors/total number[a] at 30 days	% survivors at 30 days	Mean survival time (days) of non-survivors
None (saline)	10/60	16.7	9.3
3 µg	18/60	30.0	9.5
10 µg	22/60	36.7	10.1
30 µg	21/60	35.0	10.5
100 µg	25/60	41.7	9.8
300 µg	24/60	40.0	12.2
1000 µg	28/60	46.7	12.8
3000 µg	32/60	53.3	11.0

PHA injected 30 min before irradiation. [a] 3 separate experiments with 20 mice in each group.

Table II. Survival of 12-week-old CF_1 ♀ mice after 600 R whole-body X-irradiation as a function of time of injection of phytohemagglutinin (PHA-P, 3000 µg)

Time of injection of PHA	No. survivors/ total number[a]	% survivors at 30 days	Mean survival time (days) of non-survivors
None (saline)	20/90	22.2	10.8
2 days before X-ray	9/60	15.0	11.2
1 day before X-ray	28/59	47.5	8.1
30 min before X-ray	28/60	46.7	9.1
30 min after X-ray	34/60	56.7	11.1
1 day after X-ray	17/60	28.3	8.5
2 days after X-ray	19/60	31.7	11.3

[a] Three separate experiments with 20 mice in each group.



Radioprotective effect of a single i.p. injection of PHA-P (3 mg i.p.) on 12-week-old CF_1 female mice. PHA was administered 30 min before whole-body irradiation. Open figures correspond to PHA-treated groups; closed figures correspond to saline-treated groups. Each point represents a group of 20–30 animals.

Discussion. The results presented above indicate that PHA-P exhibits protective effect on mice exposed to near-lethal doses of whole-body irradiation, the effect being more pronounced when PHA was administered 30 min after irradiation. Whether this compound could be used to restore the bone marrow of humans exposed to accidental or therapeutic doses of irradiation remains to be demonstrated. Preliminary investigation of the effect of 50 mg of PHA (Burroughs-Wellcome and Company) given by i.v. infusion 5 times in the week preceding local irradiation in 2 patients with bronchogenic carcinoma has shown a marked increase in the relative percentage of bone-marrow lymphocytes[6]. Phytohemagglutinin has also been used in patients with aplastic anemia for restoring their bone-marrow function with discordant results[5,7]. One explanation for this inconsistency could be that the doses arbitrarily selected (usually 50 mg i.v. daily for 5 days) may have been sufficient to help only some of the patients.

The mechanism of the observed radioprotection is not well understood. Present findings in this laboratory suggest that PHA, at the dose used to induce radioprotection, has a cytotoxic effect on the hemopoietic organs[8]. The exhibition of this hematologic depression and associated radioprotection recalls similar observations with various mitotic inhibitors[9], when they are introduced before irradiation. In view of this, the radioprotection could be explained as a compensatory mechanism involving an increase in number of the relatively radioresistant cells involved in myelopoiesis. However, this theory does not seem to be consistent with the radioprotection observed when PHA was injected after irradiation. A radioprotective effect with injections of bacterial extracts either before or after irradiation has been observed by SMITH et al.[10]. One may suspect that the radioprotective effect of PHA is of a similar nature. Whether the protection of mice by PHA occurs through stimulation of their lymphocytes still remains to be answered[11].

Zusammenfassung. Es wird ein bemerkenswerter Strahlenschutz durch Phytohämagglutinin (PHA-P, Difco) bei mit nahezu tödlich wirkenden Röntgenstrahlendosen exponierten Mäusen beobachtet. Der Grad des Strahlenschutzes scheint eine Linearfunktion der angewandten Dosis zu sein.

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