Table 1. Summary of mortality data.

Time after exposure (days)	Expt. 1 (dose 550 r in air)		Expt. 2 (dose 600 r in air)		Expt. 1 + expt. 2	
	Animals alive (No.)	Mortality (%)	Animals alive (No.)	Mortality (%)	Total animals alive (No.)	Total mortality (%)
			A. Saline (0.4 ml)			
0	40	0	20	0	60	0
5	40	0	20	0	60	0
10	38	5	20	0	58	3
15	30	25	13	35	43	28
20	28	30	12	40	40	33
			B. Spleen (0.4 ml)			
0	30	0	30	0	60	0
5	30	0	30	0	60	0
10	30	0	30	0	60	0
15	26	13	28	7	54	10
20	26	13	28	7	54	10
			C. Spleen (0.2 ml)			
0	10	0	20	0	30	0
5	10	0	20	0	30	0
10	10	0	19	5	29	3
15	7	30	14	30	21	30
20	7	30	14	30	21	30

mortality (*1*). This factor was also found in the spleens obtained from unirradiated donor animals (*2*) but was missing in extracts prepared from the spleens that had been removed 6 days after exposure of the donor mice to an LD 55/15 days (*2*).

We have now been able to demonstrate that such cell-free saline extracts protect not only mice but also guinea pigs against radiation-induced mortality (*3*).

The preparation of the mouse spleen extracts was essentially the same as has been previously described (*2*). Spleens were removed as quickly as possible from unirradiated donor mice that had been killed by cervical dislocation. The organs were frozen on Dry Ice, ground in a mortar, and extracted for at least 24 hours with saline (1 ml per spleen) at refrigerator temperature. The extracts were made cell-free by centrifugation and filtration through both a Seitz and a Selas porcelain filter (*4*). All these procedures took place at low temperatures. The filtrate was lyophilized.

For injection, the lyophilized material was dissolved in sterile saline so that 1 ml corresponded to the contents of five spleens (spleen extract). Amounts of 0.2 and 0.4 ml were injected intramuscularly each day, starting on zero day shortly after irradiation of the guinea pigs, for five consecutive days.

The guinea pigs from a highly inbred Rockefeller strain, bred at the Naval Medical Research Institute, were exposed in a large animal irradiator (*5*) to dosages of 550 and 600 r in air of Co^{60} gamma radiation with a 4 π geometry in groups of ten (*6*). Mortality was observed over a period of 20 days and compared with that produced in animals that had been subjected to the same amount of irradiation and that had received 0.4 ml of saline. The results are presented in Table 1.

As may be seen in Table 1, with each of the two radiation dosages, administration of 0.4 ml of spleen extract caused a reduction in radiation-induced mortality, while the injection of 0.2 ml of extract resulted in a mortality corresponding to that of the saline-injected control animals.

A statistical analysis of these data by chi square was made (*7*); this established the fact that the difference between group *A* (saline) and group *B* [spleen (0.4 ml)] was statistically not significant in experiment 1 (χ^2, 0.8203) but was significant in experiment 2 (χ^2, 4.7482). Since the statistical analysis also proved that neither the saline controls nor the spleen groups in experiments 1 and 2 were significantly different from each other (χ^2, 0.256 and 0.186, respectively), a comparison of the combined mortality data for the two experiments for groups *A* and *B* was permissible. The combined data demonstrated a protective effect of spleen extract (0.4 ml), with a satisfactory statistical significance (χ^2, 5.3788; *P*, 0.05 to 0.02).

The favorable effect of the spleen extract on mortality was also reflected in the weight curves (*8*).

As far as we know, the foregoing data represent the first observations of protection against radiation-induced mortality with a cell-free total spleen extract obtained from heterologous material (*9*).

The importance of these observations consists in the following: (i) They give strong support to the theory that a humoral factor or factors of spleen (*10*) is the essential agent, in the protection against radiation-induced mortality, of spleen shielding, spleen transplantation, or spleen homogenates. (ii) They indicate that this agent is not a species-specific factor. (iii) They offer, therefore, a justified hope for the eventual identification and isolation of the protective factor.

Investigations along these lines are in progress.

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3. This is a preliminary report. The opinions or assertions contained herein are my personal ones and are not to be construed as being official or as reflecting the views of the U.S. Department of the Navy or of the Naval service at large. The technical assistance of personnel of the Radiation Technology Division, Naval Medical Research Institute, and of B. F. Lindsley, E. L. Silvers, E. L. Schloegel, and R. H. Crutcher, is acknowledged, with thanks.
4. To prove that the filtration method used to make the extracts cell-free truly removed all cellular material, the following tests were performed. A pure strain of A.T.C.C. No. 4157 of *Escherichia coli* in brain-heart infusion broth was filtered after 48 hours' incubation, first through a Seitz, and then through a Selas, microporous filter (No. 03 porosity) under pressure. The filtrate was then streaked out on a blood-agar plate, and, in addition, a brain-heart infusion broth was inoculated. Both samples were found to be sterile at the end of a 72-hour incubation period.
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11 September 1957

Dietary Calcium Levels and Retention of Radiostrontium in the Growing Rat

The contamination of dietary sources of calcium with radiostrontium has led to a consideration of methods by which the absorption and retention of dietary radiostrontium can be reduced. The data reported here do not imply that remedial measures are now necessary and do not evaluate the practical application of such methods. Previous work has been mainly

concerned with methods for removal of this radioisotope after acute or short-term exposure, and this work has been the subject of a recent symposium (*1*) and has been reviewed recently by Comar and Wasserman (*2*). The chronic ingestion of radiostrontium, as from fallout debris, represents a problem different from that of acute exposure.

Procedures usually visualized for minimizing the retention of continuously ingested radiostrontium involve the use of some dietary additive. Necessarily, any such alteration of the diet must not interfere with the over-all nutritional health of the individual. The most obvious approach is to supplement the diet with uncontaminated calcium, a physiological element, for the purpose of diluting ingested radiostrontium. However, data reported by MacDonald *et al.* (*3*) and by Wasserman *et al.* (*4*) have shown that increasing the calcium content of a single oral dose of radiostrontium by a factor of 10 to 1000 did not appreciably reduce the amount of radiostrontium absorbed and retained. The present study was therefore designed to learn whether radiostrontium intake and deposition are in fact governed by the calcium intake and whether, on a longer-term basis, supplementing the diet with uncontaminated calcium would quantitatively reduce the retention of ingested radiostrontium (*5*).

Thirty-two 5-week-old male rats of the Carworth strain, initially weighing 83 g, were divided into three groups according to body weight. The control group was given the basal diet containing 0.5 percent calcium supplied in the following forms: calcium naturally occurring in corn, brewers yeast, and so forth, 0.14 percent; as the biphosphate, 0.04 percent; as the carbonate, 0.09 percent; as the citrate, 0.23 percent. The other groups received this diet supplemented with $CaCO_3$ to contain either 1.0 percent or 2.0 percent calcium; sufficient phosphorus (as KH_2PO_4) was also added to maintain a Ca/P ratio of about 2/1. After either 8 days (period I) or 38 days (period II) on these diets, the drinking water of six rats from each group was replaced with distilled water containing about 20 μc of Ca^{45} and 10 μc of Sr^{85} per liter; both radioisotopes were employed as the chloride and were essentially carrier-free. The rats were continued on their respective diets, and the volume of water consumed over the next 7 days was estimated by use of graduated drinking tubes. All rats consumed about the same amount of water. Then at the 15th or 45th day after they had been on the diet, the rats were killed, and the Ca^{45} and Sr^{85} retained in the pelted, eviscerated carcasses were determined by the usual radiochemical procedures (*6*); the results are expressed as "percentage of ingested dose," but sample values are given in a footnote of Table 1 to indicate the absolute retention of the Sr^{85}.

The pertinent data are summarized in Table 1. It may first be noted that the higher levels of dietary calcium did not greatly alter the total feed ingested, the body weight, or the ash content of the carcass at either time interval. However, it is apparent that, as the calcium level in the diet increased, the retention of Sr^{85}, as well as that of Ca^{45}, was reduced. In the 15-day groups (period I), a fourfold increase in calcium intake resulted in about a threefold decrease in radiostrontium retention; at 45 days (period II), this relationship was nearly proportional, for each twofold increase in calcium ingestion gave about a twofold depression in the retention of Sr^{85}. These differences would suggest that the rat requires more than 15 days and less than 45 days to adapt fully to the different levels of calcium intake.

The comparative effect of added calcium upon both Ca^{45} and Sr^{85} can be conveniently observed by the calculation of the "strontium-calcium observed ratio" (OR), which is defined as (*7*),

$$OR_{carcass\text{-}diet} = \frac{Sr^{85}/Ca^{45} \text{ in carcass}}{Sr^{85}/Ca^{45} \text{ in diet}}$$

It is first noted that the $OR_{carcass\text{-}diet}$ values are about 0.23, which shows that the Sr^{85} was retained only 0.23 as effectively as the Ca^{45}; this is in good agreement with previous findings (*7, 8*). The main point is, however, that the $OR_{carcass\text{-}diet}$ values were essentially the same at each level of dietary calcium. This means that the added calcium influenced the absorption and retention of both Sr^{85} and Ca^{45} to about the same degree.

In an additional study, whole milk containing tracer amounts of Ca^{45} and Sr^{85} was supplemented with different levels of calcium gluconate, and rats were allowed to feed on it *ad libitum* for a period of 1 week. The $OR_{carcass\text{-}diet}$ on the basal whole milk diet was found to be 0.62; when the milk contained either 50 percent, 100 percent, or 200 percent more calcium than the basal diet, the $OR_{carcass\text{-}diet}$ values were 0.61, 0.61, and 0.59, respectively. The constancy of these observed ratios indicates, again, that dietary calcium affected both Ca^{45} and Sr^{85} to the same degree. It is noted that both the dietary vehicle and the calcium salt differed from the previous study in which calcium carbonate was used.

The data from the earlier single-dose studies can probably be accounted for by the fact that, on a short-term basis, the animal tends to absorb more calcium when the calcium level is suddenly raised; if the excessive intake of calcium were continued, it is expected that the animal would eventually revert to its previously established absolute utilization and retention. In addition, the retention of single dosages of radiocalcium and radiostrontium may be complicated by exchange reactions.

The question arises whether increasing the stable strontium concentration of the diet would proportionally decrease radiostrontium retention. In an experiment with rats, similar to those described before, it was found that a fourfold increase in the concentration of stable strontium in a milk diet did not reduce the absorption and retention of ingested radiostrontium. Thus, as expected, the response is related to the total stable calcium-stable strontium content of the diet and, there-

Table 1. Effect of dietary calcium levels on the retention of radiocalcium and radiostrontium in the rat. The basal diet consisted of 68.2 percent ground yellow corn, 19.1 percent vitamin-free casein, 5.7 percent brewers' yeast, 3.8 percent cottonseed oil, and 3.2 percent salt mixture U.S.P. XIV; this diet was supplemented with thiamine, riboflavin, pyridoxine, niacin, calcium pantothenate, choline, inositol, biotin, folic acid, vitamin B_{12}, *p*-aminobenzoic acid, and vitamins A, D, and E at levels estimated to be optimal for the rat (the basal diet contained 0.5 percent calcium).

Level of dietary Ca (%)	Intake		Body wt. at death* (g)	Ash wt. of carcass* (g)	Amount retained in carcass (% of ingested dose)*		OR†
	Food (g)	Ca (g)			Ca^{45}	Sr^{85}	
			Period I (15 days on diet)				
0.5	230	1.2	145 ± 12	3.4 ± 0.2	77.4 ± 1.5	19.3 ± 0.6	0.25
1.0	230	2.3	143 ± 6	3.5 ± 0.2	51.2 ± 1.2	10.5 ± 0.7	0.21
2.0	240	4.8	140 ± 3	3.3 ± 0.1	27.8 ± 1.4	6.5 ± 0.5	0.23
			Period II (45 days on diet)				
0.5	690	3.5	261 ± 7	5.8 ± 0.3	63.0 ± 1.6	17.7 ± 1.1‡	0.28
1.0	680	6.8	266 ± 8	6.6 ± 0.2	32.3 ± 1.1	7.0 ± 0.3	0.22
2.0	730	14.6	261 ± 10	6.9 ± 0.4	18.3 ± 2.5	3.8 ± 0.4	0.21

* Values are mean ± standard error of mean; six rats per group.
† $OR = OR_{carcass\text{-}diet} = (Sr^{85}/Ca^{45} \text{ in carcass})/(Sr^{85}/Ca^{45} \text{ in diet})$.
‡ The following figures indicate the absolute levels in this study: about 4 μc of Sr^{85} was ingested per rat, and the amounts retained at the 0.5-, 1.0- and 2.0-percent calcium levels were 0.72, 0.35 and 0.17 μc of Sr^{85}, respectively.

fore, it is then more useful to focus attention on the more physiological element, calcium.

It may be pointed out that the increased dietary calcium levels in these studies caused a decrease in the total radiostrontium retention because (i) the stable calcium was an effective diluent for radiostrontium and (ii) the increased dietary calcium levels did not cause an increased growth of the skeleton. To produce a decreased concentration of radiostrontium per unit of bone, it is only necessary that the stable calcium effectively dilute the strontium. In summary, it has been shown that supplementary uncontaminated calcium in the diet of the rat will proportionally decrease the fractional retention of continuously ingested radiostrontium under the conditions of the present experiment. Further investigations must be made to determine whether these relationships hold over broader ranges of variables that may be involved and to explore the general applicability of the present findings in the rat to man and other species.

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Inhibition of Plant Growth by Root-Drench Applications of Kinetin

The announcement of the isolation of kinetin (6-furfurylaminopurine) and of its stimulation of cell division in tobacco callus tissue (*1, 2*) has led to a search by many investigators for other types of biological activity of this compound. The effects of kinetin reported to date include substitution for red light in promoting expansion of bean leaves and germination of lettuce seed (*3*), prevention of protein degradation in detached *Xanthium* leaves (*4*), stimulation of elongation of *Avena* coleoptiles (*5*), and inhibition of regeneration of tentacles of *Hydra* (*6*). Inhibition of root development by kinetin has been reported by several workers, including Miller (*2*), who stated that the root growth of lettuce seedlings was inhibited severely when the seeds were treated with kinetin. There have been no reports of inhibition of growth with kinetin when intact plants grown to maturity were used. This paper reports such an inhibition (*7*). Our studies were conducted during November and December 1956 in a greenhouse with a temperature range of 72° to 80°F. Twenty-one-day-old seedlings of tomato, variety Bonnie Best, growing singly in sand in 5-inch clay pots were treated by adding aqueous solutions of kinetin to the surface of the sand. Fifty-milliliter portions of a 10-, 50-, or 100-ppm solution were applied to individual pots on the first, third, and fifth days of each week. On the intervening days, the plants were watered with nutrient solution. This schedule was continued for 4 weeks. It is noted that the limit of the water solubility of kinetin at room temperature is approximately 100 ppm. The average increase in height of the plants was used as an index of the over-all growth. The results are given in Table 1. It is apparent from this study that, as the dosage of kinetin was increased, the growth of the tomato plants was more strongly inhibited. Plants treated with 100 ppm of kinetin had small, atypically shaped leaves, drastically reduced root systems, and purple pigmentation similar in appearance to that associated with phosphorus deficiency. They often exhibited loss of turgor on warm days. The plants flowered approximately 2 weeks later than the control plants and produced flowers and fruit of reduced size. Although the fruit were small, seeds harvested from them were viable and produced normal seedlings.

The response of the plants treated with 50 ppm of kinetin was similar, but not so pronounced as that of plants treated with 100 ppm. The growth of plants treated with 10 ppm was significantly inhibited, but only slight indications of the other effects mentioned above were evident.

This experiment was repeated in all essential details, except that the tomato plants were grown in soil. The results are also given in Table 1. Comparatively slight inhibition of growth was obtained by treating tomato plants that were growing in soil. However, a slightly more spindly growth was evident in all treated plants. Several days' delay in flowering and slightly reduced root systems were observed in plants treated with 50 ppm and 100 ppm of kinetin.

Table 1. Average increase in height of tomato plants growing in sand or soil treated with aqueous solutions of kinetin after 4 weeks of treatment. Fifteen plants were included for each treatment. The average initial height of plants grown in sand was 6.7 cm; that of plants grown in soil was 7.8 cm.

Concentration of kinetin* (ppm)	Average increase in height (cm)
Plants grown in sand	
0	20.2
10	12.5
50	5.2
100	3.4
L.S.D.*–0.01	2.1
L.S.D.*–0.05	1.6
Plants grown in soil	
0	23.9
10	18.6
50	16.0
100	17.2
L.S.D.*–0.01	3.5
L.S.D.*–0.05	2.8

* Least significant difference.

The plants growing in soil treated with kinetin recovered—that is, they compared favorably in size with control plants—approximately 2 weeks after the treatments were discontinued. The plants grown in sand made only slight recovery, and this very slowly.

Inhibition of growth in height was observed in similar experiments in which seedlings of sunflower, bean, corn, and wheat were grown in sand. The response of the latter two species was evident only with the 100-ppm kinetin treatment, whereas the other species responded at lower concentrations. Inhibition of root growth was characteristic of all plants treated with the highest concentration.

Experiments now in progress indicate that a single watering with 100 ppm of kinetin significantly inhibits plants growing in sand. Spraying seedlings of several of the previously mentioned species to the point of run-off with aqueous solutions containing up to 100 ppm of kinetin produced no observable effects.

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