one involving the possibility of hold-up in the food chains. For example, dairy cattle fed on stored herbage would contribute less Sr^{90} to dairy products than those on open range.

4) In the case of stillbirths, Sr^{90} data may be distorted by nonnutritional calcium prescribed for many pregnant women.

5) The authors continue the Atomic Energy Commission practice of reporting Sr^{90} concentrations in terms of maximum permissible concentrations (MPC) which are strictly meant to apply only to healthy, occupationally exposed adults. The MPC for children, to be consistent with the recommendations of the National Commission of Radiological Protection, ought to be 1/20 the occupational MPC.

6) Values for MPC have been revised downward steadily during the past two decades as more knowledge of the ultimate biological effect of skeletally retained radioelements has accumulated. In view of the greater radiosensitivity of infants to nuclear radiation, the global exposure involved, and the lifetime irradiation periods, it may well be that the appropriate MPC for evaluating the global Sr^{90} hazard should be 1/100 the occupational value. The MPC for Sr^{90} is based on comparison to the radium MPC, which, in turn, hinges on our experience with radium poisoning in human beings. Practically no data are available for radium retention in children and, in addition, very few radium-retention studies on human beings have been carried out over a period of 40 or 50 years.

7) In projecting their estimates of Sr^{90} retention through 1970, the authors make no allowance for additional nuclear tests. In view of the fact that the British will test weapons in the megaton range within a few months and the Soviets may overcome their continental proving ground limitation so that a Castle series of tests may be undertaken shortly, it seems naive to assume a vacuum in testing from 1956 to 1970.

In addition to these seven points, one should consider the role of concentration factors in fallout, the selectivity of global fallout, the possibility of different fallout patterns for bomb debris injected into the stratosphere at points other than the U.S. and U.S.S.R. test sites as well as the influence of different substrata on fallout phenomenology. Nor should one neglect the possibility of ecological upset owing to concentration of radioelements in nature.

Any meaningful evaluation of the Sr^{90} hazard must seek to assess the risk of excessive radiation exposure to the most radio-sensitive groups of the total population. Because of the global nature of the fallout, the problem of risk-evaluation should be undertaken on an international basis. No governmental group within the United States should undertake to assume or calculate risks for peoples of foreign lands. The United Nations has established a committee to investigate the biological effects of nuclear fallout, and it is to be hoped that technical reports will be forthcoming soon. Then attention may be focused on weighing the probable risks of future bomb tests, and it may be possible to fix a limit to the annual testing of nuclear weapons to keep stratospheric pollution within safe limits.

It is salutary that the Atomic Energy Commission has sponsored such high-quality scientific research and even more hopeful that phases of this work are emerging from the classified category. Independent analyses of the problem, such as those appearing in the *Bulletin of the Atomic Scientists,* now rest on a more solid foundation of fact than was heretofore possible.

RALPH E. LAPP
Arlington Towers, Arlington, Virginia
19 February 1957

Although we share Ralph Lapp's concern for the seriousness of the Sr^{90} problem for the world population, we wish to dissent from some of his interpretations of our data.

1) We quite agree that momentarily the biological hazard is greatest for young children. We do not see how a discussion of the average concentration of Sr^{90} in adults is *misleading* when we give all the individual data, as well as the averages of 10-year-age intervals, and definitely conclude that "children have 3 to 4 times more strontium-90 per gram of calcium . . . than adults."

2) We did not conclude that the present data permit "careful evaluation of the biological hazard" in children. In fact, we made it clear that many more data are urgently needed. The important statistical quantities, of course, are the mean and the standard deviation.

3) We clearly pointed out that the present situation does not represent equilibrium but that reasonable predictions can be made of what the equilibrium situation may be. By examining the steps in the bomb-to-bone chain, we were able to conclude that the quantity of Sr^{90} in human bone is approximately that predicted from our knowledge of the total fallout and the fractionation factors in the chain. Actually, the time scale of importance is on the order of a year, and in this period the milk appears to be fairly well equilibrated with the soil. When milk is the major source of calcium in the diet of young children, the children will likewise approach a transient equilibrium.

4) The data on stillborns did not involve the average predicted for ultimate equilibrium.

5) We most emphatically did not present our data in maximum permissible concentrations for several reasons, not the least of which is the current debate among competent medical scientists on what this value should be. We reported all our data in absolute units of micromicrocuries of Sr^{90} per gram of calcium. We discussed the data relative to the one official Sr^{90} level existing at the time we wrote the article—that is, the maximum permissible concentration for industrial workers stated in the *National Bureau of Standards Handbook* No. 52.

6) The setting of the maximum permissible concentration is not in our sphere of scientific competence. This was not one of our conclusions.

7) We could have calculated the average concentration of Sr^{90} in man in 1970 either by using the known number of atomic tests to date or by assuming some unknown arbitrary number. We chose the former and clearly stated our assumption. The point here was to show what will ultimately get into man from a known quantity of debris produced. It was not our intention to calculate how much might be present in man by 1970 assuming some grave political situation.

It is hoped that current experimental work in this laboratory and elsewhere will make it possible to provide information on some of the other problems which Lapp and others have raised. Although there will remain much area of debate, new data to be published shortly will place some further limits on the area of speculation. In reporting the laboratory data on this controversial and globally important subject, we have tried, and will continue to try, to present it as objectively as possible, so that the scientist-citizen such as Lapp may discuss the sociopolitical problem in as well informed a manner as possible.

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3 April 1957

Role of 5-Hydroxytryptamine in the Inflammatory Process

Benditt *et al.* (*1*) revealed that 5-hydroxytryptamine, as well as histamine and heparin, is present in mast cells. They also found that edema, following administration of egg white, is induced by liberation of 5-hydroxytryptamine from the mast cells and that histamine has a lesser role in the formation of

Table 1. Percentage of macrophages ingesting tubercle bacilli, strain BCG, after 1 hour's incubation.

Injection		Number of bacilli per monocytes				
Compound	Amt. (μg/ml)	0	1–2	3–5	6–10	10
Control		21	24	36	12	7
Histamine	0.10	20	29	34	15	2
Histamine	1.0	12	27	30	20	11
Histamine	10.0	12	25	18	27	18
Histamine	100.0	76	13	8	3	
Heparin	0.10	25	35	26	12	2
Heparin	1.0	24	35	24	14	3
Heparin	10.0	32	30	22	10	6
5-Hydroxytryptamine	0.1	19	30	31	10	10
5-Hydroxytryptamine	1.0	26	27	32	7	8
5-Hydroxytryptamine	10.0	24	29	37	8	2
5-Hydroxytryptamine	100.0	most of the cells disrupted				
48/80	0.1	17	27	36	15	5
48/80	1.0	26	30	36	2	6
48/80	10.0	25	25	40	4	6
48/80	100.0	30	27	35	6	2

edema. On the basis of these observations, Benditt *et al.* consider that 5-hydroxytryptamine participates in the defense mechanism of the inflammatory process.

The compound 48/80 (condensation product of *p*-metoxyphenethylmethylamine with formaldehyde) is considered to be the most potent of a large number of known liberators of histamine in the skin. If it is administered intradermally, 5-hydroxytryptamine does not equal the action of histamine or 48/80 calculated on the basis of weight as observed in skin reactions (hyperemia, edema, necrosis) or the local accumulation of intravenously injected dyes (Pentamin blue, trypan blue).

In our experiments, histamine, 48/80, or 5-hydroxytryptamine was dissolved in distilled water and applied on the previously depilated skin of albino rats (weight 100 g). Immediately afterward, India ink or trypan blue was injected intravenously into the animals. One hour later, the animals were killed, and we examined the intensity and size of the accumulation of India ink or trypan blue on the inside of the abdominal skin, which was removed. We found that if a 2-percent solution of histamine or 48/80 was rubbed for 1 minute on the skin, the local accumulation of India ink or trypan blue was of equal intensity, but only a minimal accumulation was observed following a similar application of a 2-percent dextran solution. While a 0.1 percent solution of histamine or 48/80 could not induce the phenomenon, a 0.1-percent solution of 5-hydyroxytryptamine led to the local accumulation of India ink or trypan blue of an intensity equal to that induced by a 2-percent solution of histamine or 48/80.

In studying the role of histamine in the inflammatory process, we found (*2*) that monocytes revealed an increased phagocytic activity under the influence of small amounts of histamine (1 to 10 μg/ml), but larger amounts of histamine (100 μg/ml) considerably reduced the phagocytic activity of these cells. Loos (*3*) showed that histamine increased phagocytosis of carbon particles by equine leucocytes *in vitro*. Rigdon (*4*) attributed a chemotactic action to the histamine, and Bloom (*5*) considered the histamine to be responsible for the migration of leucocytes into the area of inflammation in the tissues. Wolf (*6*) demonstrated a similar action *in vitro*, and Findlay (*7*) showed the same phenomenon *in vitro* and *in vivo*. These observations substantiate the opinion of Jancso (*8*) that histamine is a physiological activator of the reticuloendothelial system. We produced further experimental evidence (*9*) supporting Jancso's views.

Recent data published by Benditt *et al.* (*1*) suggest that a similar role could be played by 5-hydroxytryptamine. Both histamine and 5-hydroxytryptamine are built into the mast cells, which suffer a serious destruction when injury to the tissues occurs. According to Riley and West (*10*) and Fawcett (*11*), the destruction and regeneration of mast cells are processed simultaneously with the liberation and restoration of histamine, respectively. It is of further interest that the intensity of these phenomena is proportionate to the degree of the injury sustained.

We have investigated the effect of histamine, 5-hydroxytryptamine, heparin, and 48/80 (*12*) on the phagocytic activity of monocytes in order to ascertain whether a similar response could be detected on the action of these substances on surviving macrophages *in vitro*. Materials and methods were used as previously described (*2*). Monocytes were taken from the peritoneal cavity of guinea pigs. Exudate, containing more than 90 percent of monocytes among the cellular elements, was induced by previous intraperitoneal injection of 0.1 mg of glycogen in saline solution. Washed tubercle bacilli (strain BCG) were added *in vitro* to the suspension of monocytes, and, 1 hour after incubation, phagocytized numbers of tubercle bacilli were counted in 100 monocytes on stained preparations (Table 1).

In the given experimental conditions, 1 μg/ml and 10 μg/ml of histamine increased phagocytosis of tubercle bacilli, while 100 μg/ml inhibited the same phenomenon. 5-Hydroxytryptamine had no effect on the phagocytosis; however, 0.1 and 1 μg of heparin per milliliter did not influence phagocytosis of tubercle bacilli, but 100-μg/ml quantities inhibited the ingestion of the bacilli; 48/80 did not at all influence the ingestion of tubercle bacilli.

As injury occurs to the organism, histamine and 5-hydroxytryptamine are liberated from the mast cells, but the two substances show a different behavior if their role is studied in the defense processes. 5-Hydroxytryptamine was found to be the most potent agent, inducing an accumulation of India ink or trypan blue at the site where they were administered percutaneously. These findings confirm those of Benditt *et al.* (*1*), who observed that a considerably smaller amount of 5-hydroxytryptamine than of histamine was necessary to provoke the same intensity of accumulation. In our experiments, not only a quantitative difference was found in the effect of the two substances on the accumulation, but a qualitative one was seen on the phagocytic activity of monocytes *in vitro*, for this activity was influenced by histamine but not by 5-hydroxytryptamine. It would appear that the action of histamine is more general on the reticuloendothelial system, while 5-hydroxytryptamine has no effect on the monocytes.

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References and Notes

1. E. P. Benditt, S. Bader, B. L. Kai, *Arch. Pathol.* 60, 104 (1956).
2. B. Gözsy and L. Kátó, *Can. J. Biochem. Phys.* 34, 571 (1956).
3. H. P. Loos, *Z. ges. exptl. Med.* 75, 462 (1931).
4. R. H. Rigdon, *J. Lab. Clin. Med.* 27, 598 (1941).
5. W. Bloom, *Bull. Johns Hopkins Hosp.* 33, 185 (1922).
6. E. P. Wolf, *J. Exptl. Med.* 34, 375 (1921).
7. G. M. Findlay, *J. Pathol. Bacteriol.* 31, 633 (1928).
8. M. Jancsó, *Nature* 160, 227 (1947).
9. L. Kátó and B. Gözsy, *Am. J. Physiol.* 184, 296 (1956).
10. J. F. Riley and G. B. West, *J. Pathol. Bacteriol.* 69, 269 (1955).
11. W. Fawcett, *J. Exptl. Med.* 100, 217 (1954).
12. Compound 48/80 was provided by Burroughs

Wellcome and Co. through the courtesy of Edwin J. de Beer. Dextran was provided by Abbott Laboratories; molecular weight 79, 400, low fraction, 28, 300, high fraction 180, 800. 5-Hydroxytryptamine was serotonin creatine sulfate (Nutritional Biochemical Co.). This work was partially aided by grants from the Ministry of Health of the Province of Quebec (Federal-Provincial Health Research Grants).

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Protection against X-irradiation by 3-Amino-1,2,4-triazole

Heim, Appleman, and Pyfrom (*1*) recently demonstrated that after intraperitoneal injection of 3-amino-1,2,4-triazole (AT) into rats, the liver and kidney catalase activity was sharply reduced. Our first conclusion, of course, was tentatively to consider this compound to be a catalase inhibitor. Since such radiation protectors as azide (*2*) and cyanide (*2, 3*) have also been shown to inhibit catalase *in vitro* (*4*) and, at least in the case of azide, *in vivo* (*5*), it was decided to test AT also as a protective agent against ionizing radiation (*6*). Injection of a catalase inhibitor may appear to be paradoxical in attempting to protect against irradiation lethality. However, one must consider such possible mechanisms as the formation of a catalase-inhibitor complex more radiation-stable than the uncomplexed catalase (*7*).

Table 1 indicates that intraperitoneal injection of AT before whole body x-irradiation rather consistently protects a large percentage of mice against 650 r of x-rays and significantly prolongs the survival of animals that receive 750 or 850 r of x-rays. (If all data shown are pooled for mice receiving 850 r alone, and for mice receiving AT followed by an 850-r dose, the Student's t value for the difference in survival time is 2.90, with a total population in each case of 59 mice killed. This represents $0.001 < P < .005$.) If administered before a 1700-r dose, or after any dose of x-rays, AT is without effect. Even if AT is administered as long as 24 hours before the irradiation, some prolongation of survival time is conferred. It might be mentioned that the doses of AT employed were well tolerated by the mice.

Even though AT per se has been found not to be an inhibitor of catalase (*8*), the possibility cannot be excluded that a catalase mechanism is in some way relevant to the radiation protection, for a single injection of this compound will cause a 65-percent reduction in liver catalase activity as late as 24 hours after injection (*8*). The mechanism of the biological effects of AT is presently being further investigated.

After this work had been completed, a paper appeared by Friedberg (*9*), indicating no significant effect of AT on mortality rate after 934 r. His data do show, as do ours, prolongation of survival time.

Table 1. Protection of mice by 3-amino-1,2,4-triazole (AT) against whole body x-irradiation.

Amino-triazole (mg/kg)	X-ray (r)	Order of treatment	Number of mice	30-day survivors (%)	Average survival time (days)*
		Experiment 1			
0	450		10	90	9.0
2000	450	AT, x-ray	12	92	13.0
0	650		12	0	12.5
2000	650	AT, x-ray	10	70	15.7
0	850		12	0	8.9
2000	850	AT, x-ray	12	8	12.2
4000	0		11	100	
		Experiment 2			
0	850		8	0	6.3
2250	850	AT, x-ray	12	8	9.7
2250	0		12	100	
		Experiment 3			
0	850		11	0	11.5
1817	850	AT, x-ray	11	0	13.5
1817	850	x-ray, AT	11	0	11.0
0	1700		11	0	4.5
1817	1700	AT, x-ray	11	0	4.4
		Experiment 4			
0	850		12	0	13.4
1817	850	AT, x-ray	12	17	13.3
1817	850	x-ray, AT	12	0	12.1
0	1700		11	0	4.0
1817	1700	AT, x-ray	12	0	4.5
		Experiment 5			
0	650		16	19	13.9
2000	650	AT, x-ray	16	75	19.3
0	750		16	0	11.1
2000	750	AT, x-ray	13	0	13.6
0	850		16	0	10.6
2000	850	AT, x-ray	16	0	12.0
2000	0		16	100	

* Average survival time refers only to those animals that succumbed within the 30-day period.

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References and Notes

1. W. G. Heim, D. Appleman, H. T. Pyfrom, *Science* 122, 693 (1955).
2. Z. M. Bacq and A. Herve, *Brit. J. Radiol.* **24**, 617 (1951).
3. Z. M. Bacq *et al.*, *Science* 111, 356 (1950).
4. H. Blaschko, *Biochem. J.* (*London*) **29**, 2303 (1935); P. Rona, A. Fiegel, W. Nakahara, *Biochem. Z.* 160, 272 (1925).
5. R. N. Feinstein, unpublished data.
6. This work was performed under the auspices of the U.S. Atomic Energy Commission. We wish to express our thanks to T. M. Vial and the American Cyanamid Company for generous samples of technical grade 3-amino-1,2,4-triazole and to John H. Pomeroy and Carolyn A. Craig for its purification.
7. R. N. Feinstein, C. L. Butler, D. D. Hendley, *Science* 111, 149 (1950).
8. R. N. Feinstein, S. Berliner, F. O. Green, in preparation.
9. W. Friedberg, *Proc. Soc. Exptl. Biol. Med.* 93, 52 (1956).

11 February 1957

Use of Δ4-Cholestenone to Reduce the Level of Serum Cholesterol in Man

In 1953 Tomkins *et al.* (*1*) demonstrated that a single feeding of 4-cholestene-3-one (cholestenone) to rats reduced the capacity of their livers to convert acetate to cholesterol. Soon after this observation was made, studies were initiated in our laboratory to test the effects of prolonged administration of cholestenone on the level of plasma cholesterol.

Dogs were fed 1 g of cholestenone every 8 hours for 17 days. In one dog, the concentration of plasma cholesterol fell from an initial value of 100 mg/100 ml to 70 mg on the eighth day of feeding, to 65 mg on the 12th day, and to 55 mg on the 17th day. In another dog, the levels of plasma cholesterol were 115 before the cholestenone feeding was begun and 75, 80, and 70, respectively, on the 8th, 12th, and 17th days of feeding.

Substantial reductions in the levels of plasma cholesterol were also observed in chickens that were fed Purina broiler