attenuation along the axis of the uranium sleeve as most of the slow neutrons were expected to stream in from the ends.

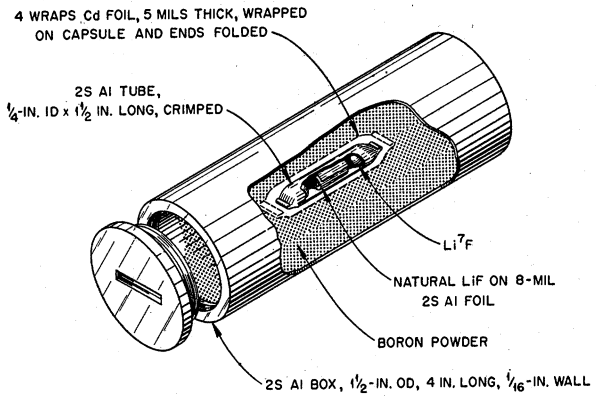

FIG. 1. Experimental arrangement for pile exposure.

Two pills of fused Li^7F (0.2 g each) were irradiated for 10 hr, with a small sample of natural LiF between them, along the axis of the uranium sleeve. The axial attenuation at one end of the assembly was slightly too low and in consequence one of the samples was not usable. The central natural LiF sample indicated a $Li^6(n,t)$ contribution equivalent to 4.2 mb. This is probably several times the effective value at the well-shielded sample, but, in any case, negligible.

Polonium-Beryllium Irradiation Procedure. The high energy of polonium-beryllium neutrons (maximum near 4.5 Mev) made $Li^6(n,t)$ interference less important than in the pile irradiation and, therefore, allowed the use of 9988 percent Li^7 which was available in larger quantities.[2] The capacity of the tritium extraction apparatus (about 0.5 g per run) limited the exposed sample to 2 g Li^7F; 2 g of natural LiF were exposed at the same time as a $Li^6(n,t)$ monitor. The powdered samples were wrapped in aluminum foil and strapped to a thin-walled aluminum tube without shielding. The neutron source was slipped inside the tube and the assembly hoisted up in an attic about 5 m from possible neutron moderating walls or beams for 9 days.

Tritium Yield and Cross Sections. The tritium was recovered by fusing the samples repeatedly in a 1-percent hydrogen-argon atmosphere. The activity was detected by introducing the gas into a proportional counter and adding 10 percent methane-argon as the counting gas. This apparatus had been previously calibrated (*2*), giving 1 c/s for 6.70×10^8 tritons. The pile sample gave 377 c/s, and portions of the Po-Be irradiated sample gave 1.5 c/s. Accidental contamination on unexposed samples ran about 0.5 c/s, so the second result has a rather large uncertainty.

The pile flux above 2.8 Mev, the calculated threshold for $Li^7(n,t)\alpha+n$, has been computed as 2.0×10^{10} n/cm² sec (*3*), with an uncertainty of perhaps 25 percent. This flux decreases exponentially with energy (*4*), so the computed cross section, 72 ± 18 mb[3] is essentially that near 3 Mev. The average neutron loss in the boron shield is about 7 percent at this energy.

The total Po-Be flux computed from the manufacturer's measurements was 2.7×10^6 n/cm² sec. The computed cross section is 30 ± 20 mb.

A more complete investigation of the $Li^7(n,t)$ cross section should be possible through the use of monoenergetic neutrons from charged particle reactions, and much larger samples of Li^7 isotope. The poor recovery of tritium from large samples might be circumvented by fusion as a thin layer or by stirring.

[2] Courtesy of J. Schenck, ORNL Physics Division.

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[3] Tritium produced by the reactions $Li^7(\gamma,t)\alpha$ and $F^{19}(n,t)N^{17}$ is considered negligible.

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Intracellular Distribution of Acid and Alkaline Ribonuclease in Normal Rat Liver[1]

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While investigating the intracellular distribution of ribonuclease (RNase), the presence of RNase activity at both acid and alkaline pH was observed. Extensive survey of the literature[3] revealed that alkaline RNase was never reported present in rat liver.

RNase activity was determined by the spectrophotometric method of Schneider and Hogeboom (*1*). The succinate buffer was replaced by ABC (acetate-borate-cacodylate) (*2*) and PBC (phosphate-borate-cacodylate) buffers, as these buffers could be employed over a broad range of pH and did not seem to affect the RNase activity.

The pH-activity curves (Fig. 1), determined with constant concentrations of ions in ABC and PBC buffers, reveal two peaks of activity, one at 5.8 and another at 8.2. The peak at pH 8.2 is beyond the optimal pH and the isoelectric point of crystalline RNase, which are pH 7.7 and 7.8 respectively (*3*). A proportional relation of enzymatic activity to the amount of tissue used as well as to the length of incubation was found at both pH 5.8 and 8.2. Optimal concentration of substrate was also determined. For 0.1 ml of 10-

[1] This investigation is being supported by grants from the National Cancer Institute of Canada and the Damon Runyon Memorial Fund for Cancer Research, Inc.

[2] Fellow of the Damon Runyon Memorial Fund for Cancer Research, Inc.

[3] At the time this paper was sent for publication we were not aware of Roth's results on RNase activity of rat liver at different pH values (*Biol. Bull.*, Oct. 1953, p. 359). These results appear to confirm the presence of two peaks of RNase activity.

percent tissue homogenate (1 g of fresh tissue per 10 ml of suspension) alkaline RNase requires 14 mg of commercial RNA sodium salt,[4] whereas acid RNase requires only 3 mg. Therefore, alkaline RNase needs, for optimal activity, 4.6 times more substrate than the acid enzyme. These results suggest the presence of two forms of RNase, that is, an acid and an alkaline, or of two distinct RNase enzymes. This may be supported by the report of Martin and Porter that a purified RNase electrophoretically homogeneous showed two distinct peaks on chromatographic fractionation, thus indicating two distinct forms of the enzyme (*4*).

Acid and alkaline RNase, moreover, differ in their behavior toward magnesium sulfate and heparin. Magnesium sulfate, at a final concentration of 0.003 M (Fig. 2), activates acid RNase by 25 percent, whereas the alkaline RNase, at the same final molarity of magnesium sulfate, is decreased by 14 percent. At lower final concentration, magnesium sulfate does not seem to affect the alkaline RNase. Heparin,[5] at a final concentration of 0.25 mg/ml in the incubation mixture (Fig. 2), decreases the level of acid RNase activity by 60 percent and alkaline RNase only by 16 percent. The same levels of activity were obtained with higher concentration of heparin. Previous report has shown that heparin does not affect crystalline RNase in the same manner at acid and at neutral pH (*5*).

Preliminary results on the intracellular distribution of acid and alkaline RNase are summarized in Table 1.

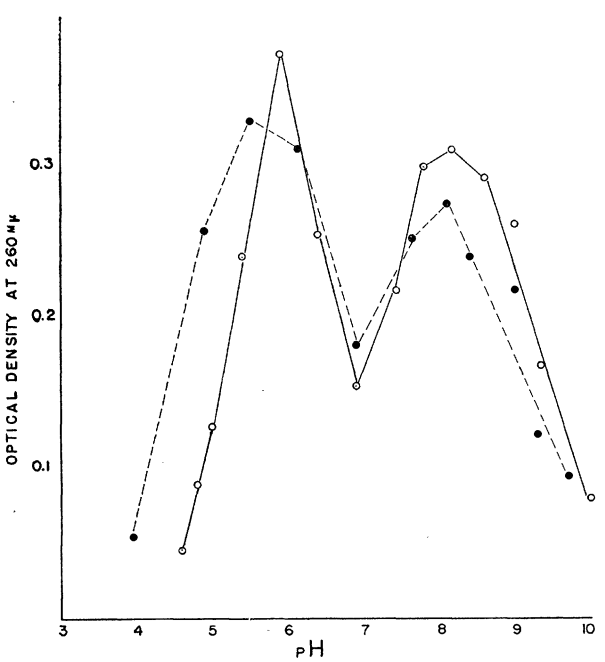

FIG. 1. pH-Activity curve of rat liver homogenate RNase. Optical density corresponds to the acid soluble material liberated from RNA during 30 minutes of incubation at 37° C. ● ABC buffer, ○ PBC buffer.

[4] Sodium Nucleate (from yeast), Schwarz Laboratories Inc., New York City.

[5] Depo-Heparin Sodium, Upjohn Co., Kalamazoo, Mich.

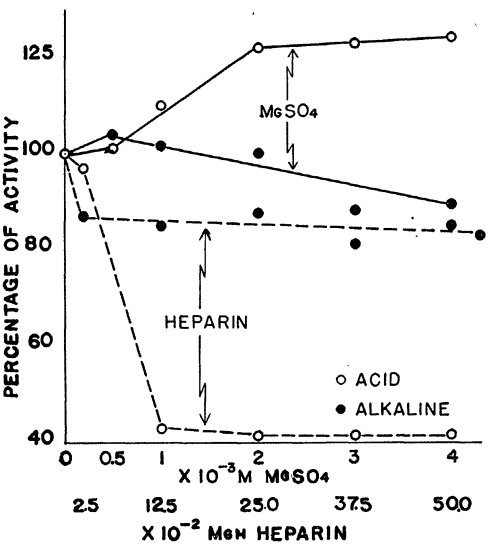

FIG. 2. Effect of magnesium sulfate and heparin upon acid and alkaline RNase activity. Homogenate value is taken as 100% activity.

The values, given in arbitrary units (optical density reported for 1 g of tissue or tissue equivalent) per gram wet weight and as percentage of the whole homogenate activity, are the mean of four different assays. For each assay, homogenates were prepared from pool of two normal perfused rat livers. Cell particulates, that is, nuclear, mitochondrial, microsomal, and supernatant fractions, were isolated from these homogenates by the usual method in 0.25 M sucrose (*6*).

Both enzymes are similarly distributed and the mitochondrial fraction contains the highest activity. The low percentage of activity in the nuclear fraction is questionable since it has been shown in previous work

TABLE 1. Intracellular distribution of acid and alkaline ribonuclease activities in normal rat liver.*

	Acid		Alkaline	
	Activity/ g of tissue	Percentage	Activity/ g of tissue	Percentage
Homogenate	410.0 ± 62.0	100.0	786.5 ± 92.0	100.0
Nuclei	46.8 ± 20.0	11.3 ± 3.9	106.5 ± 25.0	13.8 ± 4.3
Mitochondria	222.2 ± 40.0	54.2 ± 3.6	368.2 ± 46.0	46.8 ± 1.7
Microsomes	63.1 ± 13.0	15.5 ± 3.4	146.2 ± 33.0	18.6 ± 4.1
Supernatant	53.5 ± 14.0	13.1 ± 3.4	131.2 ± 22.0	16.7 ± 2.5
Recovery	385.6 ± 55.0	94.0 ± 2.4	752.1 ± 58.0	96.0 ± 3.9

* The values given are the mean of four different experiments with the standard deviation.

that about 11 percent of the total number of mitochondria is present in the nuclear fractions (*7*). The microsomal and supernatant fractions contain about equal amounts of activity. The intracellular distribution of acid and alkaline RNase activity of normal rat liver is similar to the distribution of acid RNase reported in normal mouse liver (*1*). The mitochondrial fraction activity, however, is lower in rat liver than in mouse liver.

It is of interest to mention that the alkaline RNase activity of the whole homogenate, when expressed in arbitrary units, is about twice that of the acid RNase activity. When the alkaline RNase activity is expressed per mg of substrate instead of per weight, it will be 2.5 times less than the acid activity. These results would suggest that acid and alkaline enzymes catalyze the hydrolysis of specific links in the RNA molecule. The possibilities are that the final substrate concentration for each of the acid and alkaline RNase would be an indirect measure of the concentration of chemical bonds specifically affected by each enzyme. Further work is being carried out in this laboratory to investigate the possible role of these enzymes as well as their intracellular distribution in normal and neoplastic tissues.

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Leukocyte Counts in the Blood from the Tail and the Heart of the Mouse[1]

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Law and Heston (*1*) found a marked difference in the total white count of heart and peripheral blood in albino mice. Similar findings were reported by other authors in the rat (*2–4*) and the guinea pig (*5*), but not in the dog (*6*). In order to find whether the concentration of leukocytes in the blood shows the same decreasing trend in various areas of the vascular system from the extreme periphery (tail tip) to the heart, we made counts in the same mouse from the tail tip, tail root, femoral vein, right heart, and left heart. The results from 15 C-57 mice were pooled and the average values as well as variation extremes are recorded in Table 1.

[1] This investigation was supported by a grant, C-2080, from the National Institutes of Health, U.S. Public Health Service, and partly by a grant from the Division of Biology and Medicine, Atomic Energy Commission.

TABLE 1. Blood leukocyte counts from peripheral and central areas of the vascular system in C57-6 mice (av. and variation extremes in 10 mice).

Tail tip	Tail root	Femoral vein	Right heart	Left heart
13,800 (8000–25,750)	6170 (5750–15,250)	4580 (2000–8000)	5720 (1500–8500)	2900 (1250–5500)

It appears from Table 1 that total leukocyte counts from the same vascular area showed considerable variations in different mice, but in no instance was the leukocyte count from the heart higher than the leukocyte count from any peripheral area of the same mouse. Thus, the leukocyte concentration in the blood decreased in the same mouse progressively during the passage from the capillaries to the left heart. This decrease could be estimated for each mouse by calculating the ratio T/H = count from the tail tip/count from the left heart. The average of this ratio and the extreme values for 10 CFW mice are recorded in Table 2. Moreover, this table indicates average T/H ratio and extremes for 4 series each of 10 mice treated by exposure to high temperature, by subcutaneous injection of anticoagulant (heparin, moranyl), and ether narcosis.

It appears from Table 1 that leukocyte concentration in the arterial blood of the mouse withdrawn from the left heart is lower than in any specimen from the venous system; that it increases to a maximum after passage through capillaries of the tail tip, but immediately after this passage (in the tail root) it decreases, and remains approximately on the same level until the blood reaches the right heart. Moreover, a new drop in the leukocyte concentration occurs after

TABLE 2. Leukocyte counts in CFW mice untreated and treated with anticoagulants, heat, or ether (av. and variation extremes in 10 mice).

	Tail tip	Left heart	Ratio T/H
Untreated	22,900 (18,750–33,750)	4230 (2250–6250)	5.7 (4.3–6.5)
Total body heating, 12 min at 94°	11,360 (3250–22,750)	5250 (2000–10,500)	2.1 (1.4–2.8)
Deep ether narcosis	13,790 (8500–20,500)	6500 (3250–11,500)	2.0 (1.1–4.0)
Heparin subcutaneously 100 units (1 mg)	14,750 (8000–22,500)	7900 (5000–10,750)	1.93 (1.15–2.3)
Moranyl (Fourneau 309 = Bayer 205) subcutaneously (5 mg)	31,500 (24,500–48,250)	20,715 (12,000–41,250)	1.7 (1.1–2.8)