Table 1. Nicotinamide-adenine dinucleotide of *M. tuberculosis* $H_{37}Rv$ grown in the absence and in the presence of INH (0.5 μg/ml) and streptomycin (5 μg/ml). Each experiment was carried out with tubercle bacilli from different cultures. STR, streptomycin.

Time of exposure to INH (hr)	NAD content of bacteria (μg/mg of protein)			
	Control	Exposed to INH	Decrease below normal (%)	Exposed to STR
4	1.37	1.2	10.5	1.42
6	1.62	0.74	54.4	1.67
6	1.4	.58	58.6	1.17
24	1.6	.5*	58.8	
24	1.3	.6	53.9	
48	1.63			1.5
48	1.54	.57	63	
48	1.11	.6	56	

* The bacteria in this experiment were exposed to 0.1 μg of isoniazid per milliliter.

Table 2. Nicotinamide-adenine dinucleotidase activity of extracts from *M. tuberculosis* H_{37}-Rv in the absence and in the presence of isoniazid. Each experiment was carried out with extracts of tubercle bacilli from different cultures. Results are expressed as the number of millimicromoles of NAD cleaved per hour per milligram of protein.

Native extract		Heated extract	
Control*	INH	Control	INH
2.6	5.9	29.5	38.1
2.0	12.6		
7.3	14.6	26.0	
1.6	11.6	9.0	
1.6	18.0	12.5	
0.0	13.7	13.7	16.0
14.9†	29.1	32.0	43.5

* Control tubes were given preliminary incubation for the same duration of time (30 minutes) as the tubes containing the drug. † In this experiment, extract from tubercle bacilli stored at −20°C for about 6 months.

ide was added. Absorption was read at 390 mμ.

There was a clear-cut decrease in the NAD content of tubercle bacilli that had been incubated with isoniazid (Table 1) compared with those incubated without the drug. The change could be observed after 4 hours of incubation. In some experiments the decrease of NAD was more than 50 percent. This action of isoniazid was specific toward tubercle bacilli; no such effect was seen with *Escherichia coli* and *Staphylococcus albus*. Another potent antitubercular drug (streptomycin) did not affect the amounts of NAD in the tubercle bacilli under the same experimental conditions.

The decrease of the NAD content was not due to the formation of the isoniazid analog of NAD. Experiments in this direction did not reveal any trace of the analog. This result is in accordance with that obtained with other microorganisms (*1, 2*). There is an increased activity of nicotinamide-adenine dinucleotidase of cell-free extracts of the tubercle bacilli (Table 2) in the presence of isoniazid. The enzyme whose activity is nil, or very small, becomes active after prior incubation with isoniazid. Furthermore, isoniazid increases the activity of extracts partly activated by heating for 1 minute at 85°C. Storing of a batch of tubercle bacilli at −20°C for about 6 months activated the bacterial enzyme, but in the presence of isoniazid this activity was much stronger when compared with that in the absence of the drug.

Since there is no effect of isoniazid on the purified bacterial enzyme (*4*), the observed increase in the activity of the enzyme is apparently due to an inactivation of the inhibitor of the enzyme by the drug.

Under physiological conditions nicotinamide-adenine dinucleotidase is probably part of a regulation system for maintaining an unchanging concentration of NAD in the cells. Its activity is checked by its inhibitor. Isoniazid may cause a decrease in NAD by inactivating the inhibitor. This decrease in NAD content may result in the reported accumulation of carbohydrates (*6*), in lipid synthesis changes (*7*), and in changes of nucleic acid synthesis without affecting the synthesis of proteins (*5*). The decrease in the content of the cofactor may ultimately cause the death of the bacterial cells as it causes the death of tuberculous or cord-factor-treated animals (*9, 13*) or of tumor cells treated with nitrogen mustard or ethyleneimino compounds (*14, 15*).

Contrary to the effect on *Mycobacterium*, in mammalian cells the addition of isoniazid results in the preservation of NAD in the cells (*15*), this being due to its inhibitory effect on animal nicotinamide-adenine dinucleotidases (*1*). The fortunate combination of these two opposite effects on the mammalian and mycobacterial enzyme makes isoniazid the drug of choice in the tuberculous infection.

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Erythropoietic and Reticuloendothelial Function in Bone Marrow in Dogs

Abstract. *Erythropoietic and reticuloendothelial functions in bone marrow were found to be identically distributed between various bones and within individual bones in the dog.*

The comparative localization of erythropoietic bone-marrow function with ^{59}Fe and of reticuloendothelial bone-marrow function with ^{99m}Tc-sulfur colloid proves to be similar throughout the bones of adult dogs. This knowledge was desired for its use in optimal localization of scintillation detectors for activity counting in organs by external probe, in quantification of bone marrow in individual bones for experiments with radiation shielding, and in shielding and orientation for radiotherapy. Furthermore, the comparative distributions of these functions have implications for basic biology and for the potential use of scintillation-scanning techniques for bone marrow.

Although data have been obtained from individual bones by microscopic examinations, cell counts, weights, or comparison of volume or color (*1, 2*), such studies on every bone of an animal would be prohibitively tedious and subject to considerable error; variability of cellularity within individual bones, damage to cells during preparation for counting, and difficulty in dissociating

aggregates of marrow cells are but a few of the problems. For these reasons and because previous studies showed anatomical rather than functional distribution, we used radioisotopes, which could be easily measured in intact individual bones.

Twenty-four hours after intravenous injection of ^{59}Fe–ferrous citrate almost all the radioactivity resides in hemoglobin-producing erythrocytic precursors in bone marrow—a well-defined functional space in bone marrow. Similarly, intravenously administered gelatin-stabilized colloid of ^{99m}Tc and sulfur (*3*) accurately defines the reticuloendothelial functional space and has the additional advantage of being well-suited for scintillation scanning. Although only 2.8 percent of the injected ^{99m}Tc activity was found in the bones of the one dog for which this value was determined, this amount proved ample for scintillation counting and scanning with the dosage administered. Others have found 2 to 4 percent of ^{198}Au-colloid, particle size 20 mμ, in the bone marrow of dogs after intravenous injection (*4*).

Three male and two female adult mongrel dogs, of from 16 to 25 kg, received 20 to 40 μc of ^{59}Fe-ferrous citrate and, 24 hours later, 200 to 8000 μc of ^{99m}Tc-sulfur colloid, both intravenously. They were exsanguinated under barbiturate anesthesia 15 minutes after they received the colloid. Because simple exsanguination may not remove peripheral blood (containing reticulocytes that have incorporated ^{59}Fe) from the bone marrow (*1*), 0.9 percent saline was administered intravenously, concurrently with exsanguination, to support the intravascular volume. Hemoglobins just before the deaths were 2.1 to 6.2 g/100 ml.

Individual bones or bone groups (Table 1) were immediately disarticulated, debrided of all soft tissue, and then counted in a standard position below a 27- by 7.5-cm sodium iodide (Tl) crystal scintillation detector housed in a shielded room. Photopeak counts of the two isotopes, corrected for radioactive decay and for ^{59}Fe Compton contribution to the ^{99m}Te photopeak, were used in calculations. The only statistically significant differences between the relative distributions of the two isotopes ($P < 0.02$) were in the rear paws (an area having scant biologic significance for the bone marrow) where the count rates were so low that the figures may have been inaccurate. Right and left femurs, counted separately, were virtually identical. The relative distribution of the bone marrow in relation to the weights of individual bones also appears in Table 1.

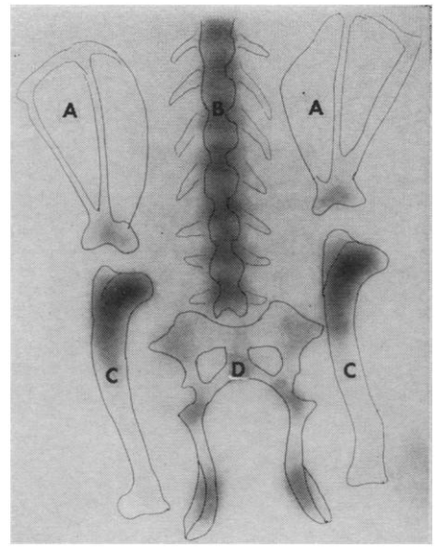

Fig. 1. Example of ^{99m}Tc scanning. *A*, Scapula; *B*, lumbar spine; *C*, humerus; *D*, pelvis.

Scintillation scanning of ^{99m}Tc (Fig. 1) revealed the distribution of reticuloendothelial function within individual bones. After the ^{99m}Tc had decayed to imperceptible levels, scintillation scanning and autoradiography of the ^{59}Fe showed the distribution of the two isotopes to be the same. This fact demonstrates that erythropoietic and reticuloendothelial compartments are distributed, both functionally and anatomically, in a similar manner in the normal dog.

Most activity in the cervical spine was in the more caudal vertebrae, whereas activity was spread evenly throughout the thoracic and lumbar vertebrae. Within individual vertebrae the scans revealed more activity in the body than in the spine, although this difference may be a function of the relative thickness of the bone-marrow space. Most activity in the humerus was in the proximal one-third of the bone; in the scapula, in the glenoid fossae; in the pelvis, in the ilium and acetabulum; in the tibia, proximally; and in the femur, in the head and neck, though some activity was also noted distally. Activity was uniform throughout the sternum, was greatest cephalad in the ribs, and was greatest in all ribs near their articulations with the vertebrae. Interestingly, ^{99m}Tc was detected in the chondral portions of the ribs, confirming our observations of bone marrow in these areas.

Keene and Jandl (*5*) found signifi-

Table 1. Relative distribution of erythropoietic (^{59}Fe) and reticuloendothelial (^{99m}Tc) activity in bones of five normal adult dogs.

Site	Compartmental activity (% total skeletal)				Rank (%:%)	
	Erythropoietic		Reticuloendothelial			
	^{59}Fe ± SD	Rank	^{99m}Tc ± SD	Rank	^{59}Fe:wt	^{99m}Tc:wt
Ribs	20.54 ± 1.70	1	18.71 ± 2.33	1	5	7
Thoracic vertebrae	17.60 ± 1.25	2	16.92 ± 1.13	2	2	2
Lumbar vertebrae	14.99 ± 3.07	3	14.33 ± 3.57	3	4	4
Humerus (2)	10.82 ± 1.18	4	12.15 ± 0.15	4	7	5
Pelvis	8.89 ± 1.21	5	8.87 ± 1.15	5	6	6
Femur (2)	7.20 ± 1.15	6	8.20 ± 2.15	6	9	8
Cervical vertebrae	6.71 ± 1.07	7	6.53 ± 1.15	7	10	10
Scapula (2)	5.08 ± 0.75	8	4.48 ± 0.84	8	8	9
Sacral vertebrae	3.09 ± .67	9	3.01 ± .67	9	1	1
Sternum	2.79 ± .67	10	2.73 ± .67	10	3	3
Skull	0.97 ± .54	11	1.25 ± .52	11	14	15
Tibia (2)	.58 ± .30	12	1.24 ± .71	12	13	12
Coccygeal vertebrae	.19 ± .05	13	0.28 ± .05	14–15	12	13
Rear paws (2)	.13 ± .08	14	.35 ± .08	13	19–22	17–20
Mandible	.11 ± .05	15–17	.17 ± .10	17	18	17–20
Radius (2)	.11 ± .04	15–17	.27 ± .18	16	15–17	16
Front paws (2)	.11 ± .08	15–17	.28 ± .27	14–15	19–22	21
Ulna (2)	.06 ± .02	18	.12 ± .08	18	19–22	17–20
Hyoid	.04 (1 dog)	19	.04 (1 dog)	19–21	11	11
Os penis	.02 (2 dogs)	20	.04 (2 dogs)	19–21	15–17	17–20
Fibula (2)	.01 ± .01	21–22	.04 ± .05	19–21	19–22	14
Patella (2)	.01 ± .01 (3 dogs)	21–22	.01 ± .01 (3 dogs)	22	15–17	22
All vertebrae	42.58 ± 3.7		41.07 ± 4.27			

cantly more erythropoietic (^{59}Fe from transferrin) than reticuloendothelial (^{51}Cr from erythrocytes treated with antibody) function in rat tibiae and fibulae and the opposite in rat vertebrae. This variation from our findings may reflect a difference in species or the fact that some reticuloendothelial cells phagocytose or sequester small particles differently from large particles; and Keene and Jandl did not compare these functions in the same animals.

If such similarities in distributions of erythropoietic and reticuloendothelial compartments also occur in animals with altered erythropoiesis, scintillation scanning of bone marrow (with ^{99m}Tc-sulfur colloid) will be an extremely useful tool both experimentally and clinically. Furthermore, combination of this technique with that of Suit (*6*) makes feasible an estimation of the total number of bone-marrow cells in each bone. If stem cells are similarly distributed, this information has important implications for experiments in radiation shielding and for radiation therapy.

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Amniotic Contraction and Embryonic Motility in the Chick Embryo

Abstract. *During part of the incubation period of chick embryos the amnion shows spontaneous contractions. Removal of the amnion on days 9, 10, and 11 has no effect on the amount of, or the cyclic aspects of, motility exhibited by the embryo. These observations question the importance of the amnion and yolk-sac as stimulative factors in the initiation and maintenance of cyclic embryonic motility at the ages studied.*

The amnion of the chick embryo is a nerve-free structure that contains smooth muscle fibers which are capable of spontaneous contraction (*1*). During the 5th day of incubation, the amnion becomes a complete sac within which the embryo and amniotic fluid are contained.

Whether the contractions of the amnion serve as a significant source of stimulation for "active" embryonic movements is a question that has not been answered by previous investigators. Both Preyer (*2*) and Windle and Orr (*3*) report that amniotic contractions and embryonic motility are independent of each other. However, both of these investigations were primarily concerned with problems other than the relation between embryonic motility and amniotic contraction. The first study designed specifically to investigate the relation of the amnion and yolk-sac to embryonic motility was that of Kuo (*4*).

Table 1. Means of percentage of embryo activity, duration of activity and inactivity phases, and percentage of amnion activity at each age for embryos with amnion intact and those with amnion removed.

Age of embryo (days)	No. of embryos	Amnion	Embryo activity (mean %)*	Mean duration (sec)		Amnion activity (mean %)†
				Activity phase	Inactivity phase	
9	10	Intact	46.4	24.0	27.8	94
		Removed	42.8	22.5	29.6	
10	10	Intact	59.3	30.2	21.5	89
		Removed	56.3	31.2	24.2	
11	10	Intact	72.3	44.3	16.5	82
		Removed	75.8	53.8	16.9	

* None of the differences are significant at the .05 level, with the use of the Wilcoxon matched-pairs signed-ranks test or the sign test (both two-tailed). † Means of the percentage of amnion activity derived from only the first recording of each embryo.

Kuo made recordings of the number of movements in 7-, 8-, and 9-day embryos with the amnion intact and with the amnion removed. He found that removal of the amnion reduced the frequency and amplitude of embryonic movements, and he concluded that the amnion (and yolk-sac) serve as significant sources of external stimulation for active embryonic motility. Since no statistical analysis was used and because sufficient details of the experimental procedure were not reported (*5*), the results are not unequivocal.

More recently Gottlieb and Kuo, in a study of development of behavior in duck embryos, reported that, "the action patterns of the embryo are also influenced by nonorganismic factors such as amnion contractions and movements of the yolk-sac" (*6*, p. 187). However, these authors were primarily concerned with specific body movements and patterns of movements, whereas my study is concerned only with the amount and periodicity of general somatic activity.

In earlier reports (*7*, *8*) it was stated that motility of chick embryos during certain stages of development is spontaneous (that is, nonreflexogenic) and cyclic. It was implied that this motility, which included amnion contractions, yolk-sac movements, and self-stimulation, was unaffected by exteroceptive stimulation of the embryo. For at least one stage of development (stage 37, day 11) (*7*), experimentally applied stimulation in no way altered the basic periodicity of motility of the embryo.

My investigation was undertaken in an attempt to replicate Kuo's study and to evaluate quantitatively the possible effects of amniotic contractions and yolk-sac movements on cyclic embryonic motility.

Thirty White Leghorn chick embryos were used. Ten embryos were observed at each of three stages of development: stages 35, 36, and 37 (*9*) which correspond to days 9, 10, and 11. Eggs were incubated in a large, forced-draft incubator at a temperature of 37° to 38°C and a relative humidity of approximately 70 percent. All observations were made in a plexiglass observation box with temperature and humidity held constant at values identical to those in the incubator.

An opening was made in the egg by cutting away a piece of shell over the air space. The inner shell membrane was removed and the embryo was exposed. The amnion was removed by carefully cutting a small opening in the chorioallantois with iridectomy scissors