pressure changes that tend to increase the gape of the valves (*8*).

The effects of adduction, shown diagrammatically in Fig. 1, are to put the ligament under a strain that subsequently causes the valves to reopen, and to subject the fluids contained within the valves to pressure, ejecting water from the mantle cavity and blood into the pedal hemocoel. Filmed records show that ejection of water liquifies the sand adjacent to the shell immediately prior to retraction, so allowing easier penetration, and that the increased pressure in the foot, together with the relaxation of the transverse muscles distally, causes dilation. Dilation must occur before retraction, in order to give a firm pedal anchorage so that the shell can be pulled down. Thus the bivalve shell effectively operates as a hydraulic machine in which the strength of the adductor muscles is transferred by the body fluids to the distal part of the foot, to be used there during the locomotory cycle.

An important part of the digging cycle is the recovery of the clam during stage *v* (Fig. 3), due to the opening of the valves by the elasticity of the ligament. In the protobranch *Nucula* the ligament is weak and burrowing is both sluggish and superficial, whereas bivalves with more advanced and more powerful ligaments (for example, *Tellina*, *9*) are generally active and deeper burrowers. Some of the hydrodynamic advantages of a hinged exoskeletal structure are thus described; it it hardly surprising that with such a mechanism the Bivalvia have become one of the dominant groups living in soft substrates.

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Nicotinamide-Adenine Dinucleotide in Tubercle Bacilli Exposed to Isoniazid

Abstract. *There is a decrease in the content of nicotinamide-adenine dinucleotide of tubercle bacilli grown in the presence of isoniazid. In extracts of tubercle bacilli, the activity of nicotinamide-adenine dinucleotidase is nil or very small; after incubation with the drug the enzyme becomes active. Isoniazid also increases the activity of the enzyme after it is partially activated by heating. There may be a correlation between the capacity of isoniazid to activate the enzyme and the decrease in the dinucleotide content of the tubercle bacilli.*

Isonicotinic acid hydrazide (isoniazid, INH) inhibits the activity of mammalian nicotinamide-adenine dinucleotidase. This enzyme is able to bring about an exchange between INH and the nicotinamide moiety of nicotinamide-adenine dinucleotide (NAD) and to form an analog of NAD which has no effect on NAD-dependent dehydrogenase reactions (*1*). Some representatives of the genus *Mycobacterium* produce nicotinamide-adenine dinucleotidase, the activity of which can be detected only after heat inactivation of an inhibitor of the enzyme, present in cell-free extracts of the bacteria (*2, 3*). The bacterial enzyme fails to show analog formation from NAD and INH (*1, 2*), and there is no inhibition by INH of the activity of purified enzyme obtained from tubercle bacilli strain $H_{37}Rv$ (*4*). Isonicotinic acid hydrazide is a potent bactericidal antitubercular drug, and, although the drug induces many biochemical changes in the mycobacterial cells, no correlation between these changes and the bactericidal effect has been shown (*5, 6, 7*).

This report deals with changes induced by INH in the NAD content of *Mycobacterium tuberculosis* $H_{37}Rv$ and with the effect of the drug on the activity of nicotinamide-adenine dinucleotidase of these bacteria.

Tubercle bacilli were inoculated into Dubos-Broth Base Medium (Difco) without added albumin and incubated for 6 to 8 days. After that time, the bacteria were harvested, washed once with saline containing 0.025 percent Tween 80, and inoculated into media containing INH (0.5 μg/ml) and into media without the drug. In some experiments the bacteria were also inoculated into media containing streptomycin sulfate (5 μg/ml). After varying incubation periods the cells were harvested, washed once in saline, and treated with high-frequency sound for 5 minutes in 5 percent trichloroacetic acid. After removal of the protein precipitate, NAD was determined by the fluorescent method with methyl ethyl ketone (*8*), according to a procedure already described (*9*). The activity of nicotinamide-adenine dinucleotidase of the tubercle bacilli was measured by the method based on the cyanide reaction of NAD (*10*). Saline extracts of the bacilli were prepared by treatment in saline with high-frequency sound for about 20 minutes. Activity was assayed in the absence and in the presence of INH (*11*) in both native extracts and extracts heated for 1 minute at 85°C (*4*). In one experiment the effect of INH on the activity of the enzyme was measured in extracts prepared from tubercle bacilli that had been stored for several months at −20°C. The reaction mixture (final volume, 0.9 ml) usually contained 0.8 ml of the extract, having about 9 mg of protein, 0.05 ml of 0.006*M* NAD (*12*), and 0.05 ml of 0.045*M* isoniazid in 0.01*M* phosphate buffer, *p*H 6.5. Before the dinucleotide was added the extracts were incubated with and without INH for 30 minutes, in a water bath at 37°C. After 1 hour incubation with the dinucleotide, 3 ml of cyanide was added. The activity of the enzyme was expressed in millimicromoles of nicotinamide-adenine dinucleotide cleaved in 1 hour by 1 mg of protein.

Assays for the formation of the isoniazid analog of NAD were performed with sound-treated tubercle bacilli, prepared as above for measuring the enzyme activity (*1*). The reaction mixture contained 0.4 ml of extract containing per milliliter: protein, about 8 mg; 0.2 ml of 0.015*M* isoniazid in 0.1*M* phosphate buffer, *p*H 7.2; and 0.2 ml of 0.003*M* NAD. After a 1-hour incubation period in a water bath at 37°C, 2 ml of 0.1*N* sodium hydrox-

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