into the tissues. The microbarbs probably allow the eggs to cling to the vascular endothelial lining and facilitate their penetration into adjacent tissues. The microbarbs could not be removed by incubation in thioglycollic acid or pepsin.

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Reversal of Streptozotocin Diabetes with Nicotinamide (33707)

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Streptozotocin (*N*-nitroso derivative of glucosamine) (1) can be produced by *Streptomyces achromogens* and has been reported to have both antitumor (2), and tumor inducing activities (7), antibacterial (3, 4)and diabetogenic activities (2, 5, 6, 8). The diabetogenic effect of streptozotocin is caused by irreversible damage to the beta cells of the pancreas (6, 8). Nictoinamide treatment will prevent the diabetogenic action of streptozotocin but not its antitumor activity (9). The present studies showed that nicotinamide will prevent "streptozotocin diabetes" also when given as long as 2 hr following the diabetogenic compound.

Methods. Streptozocin (Upjohn lot no. 6742-DEG-30-5) was injected as a single rapid i.v. dose in citrate buffer (pH 4.5) within 5 min after it was in solution. The volume was adjusted so that each animal received the equivalent of 0.5 ml/150 g of body weight. Animals were intact males weighing 135–175 from the pathogen-free colony of The Upjohn Company. Drug injections were given following an overnight fast. Nicotinamide was given intraperitoneally in 0.5 ml of saline 15 min before and 30, 60, 120, and 240 min following streptozotocin. NAD[1] and nicotinic acid were given i.p. in saline, 15 min before streptozotocin. In one study, the pH of NAD solution was adjusted to 6.0 with 1 *M* NaOH. Blood sugars were determined by a microferricyanide method utilizing the AutoAnalyzer on blood obtained from the orbital sinus of animals 1 week after treatment. The animals were not fasted prior to bleeding.

[1] Nicotinamide adenine dinucleotide or DPN.

Results. Nicotinamide, at doses of 250 and 500 mg/kg, completely inhibited the diabetogenic activity of 50 mg/kg of streptozoic when injected 15 min before or 30, 60, and 120 min after the streptozotocin (Table I). Nicotinamide was ineffective in blocking the diabetogenic action of streptozotocin when it was given 240 min following the diabetogenic compound. Nicotinic acid (250 mg/kg) and NAD (1,500 mg/kg) were both ineffective when injected 15 min prior to streptozotocin (Table II).

Discussion. Nicotinamide was an effective inhibitor of streptozotocin diabetes since it blocked streptozotocin activity when given as late at 2 hr following streptozotocin injection. Results showing that nicotinamide was effective when given 15 min prior to streptozotocin agree with the results of Schein *et al.* (9). Results show that changes observed 1–2 hr following streptozotocin injection (6) are reversible. It is also noteworthy that nicotinamide and nicotinic acid both inhibit alloxan diabetes if given prior to alloxan, but not if given following alloxan injection (11, 15). The fact that streptozotocin diabetes

TABLE I. Effects of Nicotinamide on Streptozotocin Diabetes.

Treatment	Dose (mg/kg)	Before or after administration of streptozotocin (min)	Blood sugar (mg/100 ml ± SE[a]) 1 week post-treatment
Buffer[b]	—	—	139 ± 6.4
Streptozotocin	50	—	300 ± 14.7
Streptozotocin + nicotinamide	50 + 250	15 Before	134 ± 3.8
Buffer[b]	—	—	132 ± 4.0
Streptozotocin	50	—	323 ± 10.4
Streptozotocin + nicotinamide	50 + 50	15 Before	357 ± 12.4
Buffer[c]	—	—	131 ± 2.3
Streptozotocin	50	—	354 ± 10.3
Streptozotocin + nicotinamide	50 + 250	30 After	141 ± 5.7
Streptozotocin + nicotinamide	50 + 250	60 After	139 ± 5.1
Buffer[b]	—	—	124 ± 3.2
Streptozotocin	50	—	302 ± 11.1
Streptozotocin + nicotinamide	50 + 250	120 After	126 ± 0.9
Streptozotocin + nicotinamide	50 + 250	240 After	350 ± 13.4

[a] Standard error of the mean.
[b] Five rats/group.
[c] Ten rats/group.

was inhibited by nicotinamide given 2 hr after the streptozotocin but not by nicotinic acid pretreatment, whereas alloxan diabetes was blocked by nicotinic acid and by nicotinamide only when given before alloxan, suggests that alloxan and streptozotocin probably induce diabetes by different mechanisms. It has also been reported that nicotinamide, but not nicotinic acid, is effective in reversing "toxicity" of 3-acetylpyridine (12). It has been suggested that this inhibition is a result of competition of 3-acetylpyridine for nicotinamide in the NAD molecule and, therefore, that the toxicity of 3-acetylpyridine is due to a reduction of effective NAD in the tissues (12). Since nicotinamide is part of the NAD molecule and reverses streptozotocin diabetes, it is possible that streptozotocin may be acting on the beta cells by depleting the NAD content. Although NAD injection has been shown to reverse 3-acetylpyridine toxicity (12), it did not prevent streptozotocin diabetes as shown in this paper. Failure of NAD to reverse the diabetogenic action of streptozotocin may be a result of the inability of NAD to enter the beta cell, while it can enter cells involved in 3-acetylpyridine toxicity. Difference in protective effects of nicotinic acid and nicotinamide against the diabetogenic activity of streptozotocin may be a result of the existence of two separate pathways for the formation of NAD from nicotinic acid and nicotinamide (13, 14). Perhaps only the "nicotinamide pathway" exists in the beta cells.

Summary. Animals given nicotinamide either as a pretreatment or as long as 2 hr (but not 4 hr) after streptozotocin were protected from diabetes. Nicotinic acid and NAD pretreatment were ineffective in preventing

TABLE II. Effect of Nicotinic Acid and NAD on Streptozotocin-Induced Diabetes.

No. of rats	Treatment	Dose (mg/kg)	Blood sugar (mg/100 ml ± SE[a]) 1 week post-treatment
10	Buffer	—	133 ± 3.0
9	Streptozotocin	50	378 ± 13.4
8	Streptozotocin + nicotinic acid[c]	50 + 250	367 ± 14.3
5	Buffer	—	130 ± 3.1
5	Streptozotocin	50	384 ± 10.0
5	Streptozotocin + NAD[b,c]	50 + 1500	471 ± 14.2
5	Buffer	—	127 ± 2.0
5	Streptozotocin + NAD[c]	50 + 1500	368 ± 4.0

[a] Standard error of the mean.
[b] pH adjusted to 6.0.
[c] Injected i.p. 15 min prior to streptozotocin.

streptozotocin diabetes. It was postualted that streptozotocin may interfere with NAD formation in the beta cell and that treatment with nicotinamide reverses the streptozotocin effect by reversing its effect on levels of NAD within beta cells.

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The Role of Mycoplasma in Rat Arthritis Induced by 6-Sulfanilamidoindazole* (33708)

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Periarthritis of the hind paws in rats receiving oral 6-sulfanilamidoindazole (6-SAI) was first described by Mielens and Rozitis (1). They reported the isolation of mycoplasma from the inflamed joints using blind passage techniques. However, the isolates were not characterized and serologic studies were not undertaken. Mice which had been similarly treated succumbed to bronchopneumonia. Because old rats and mice may harbor potentially pathogenic mycoplasma (2–6), Mielens and Rozitis suggested that the effects of 6-SAI were due to activation of latent mycoplasmal infections. In a later study by Sigg *et al.* this hypothesis was accepted although no attempt was made to isolate or characterize the suggested etiologic agents (7). In contrast, Ward *et al.* reported preliminary observations indicating that mycoplasma could not be recovered from rats with arthritis induced by 6-SAI (8).

The present study was undertaken to explore in detail the possible role of mycoplasma in 6-SAI arthritis. The principal purpose was to examine for active infection by mycoplasma as the cause of the arthritis.

Materials and Methods. Animals. Male albino rats weighing 400–500 g were obtained from a local supplier or from Holtzman Company (421 Holtzman Road, Madison, Wisconsin). They were housed in individual cages and fed Purina rat chow and tap water *ad libidum.*

Induction of arthritis using 6-sulfanilamidoindazole (6-SAI). Oral administration was carried out using a 15% (w/v) suspen-

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