ments were performed with tubercle bacillus protein, NaMN rather than NaAD accumulated. Yeast autolysates and *E. coli* employ either ammonium ions or glutamine as the source of amide nitrogen(14,15). The former uses glutamine preferentially and cannot utilize either asparagine, aspartic acid or glutamic acid. The latter employs ammonium ions as the optimal nitrogen donor; asparagine was not tested. The BCG strain of *M. tuberculosis* was able to use both glutamine and asparagine but not $(NH_4)_2SO_4$ as nitrogen donors. Imsande(15) has suggested that the preferential use of glutamine as the amide donor by yeast *vs* the use of ammonia by *E. coli* may "represent evolutionary changes in metabolic patterns." It is curious to note that in respect to the optimal nitrogen donor, as in so many other ways, *Mycobacteria* appear more closely related to the fungi than they are to other bacteria.

Summary. The pathway of NAD biosynthesis in *Mycobacteria* was studied with cell-free extracts of *M. tuberculosis* var. bovis strain BCG Tice. The organism converted niacin to NAD with NaMN and NaAD as intermediates. PRPP, ATP, and $MgCl_2$ were shown to be requisite for NaMN formation while glutamine or asparagine were shown to be necessary for conversion of NaAD to NAD. The pathway was further elucidated by the demonstration that NaMN was itself utilized directly for coenzyme synthesis without prior decomposition to niacin.

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Effect of 2,4-Dinitrophenol on Magnesium Metabolism.* (30308)

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Mg^{28} administered parenterally in a tracer dose is rapidly concentrated in tissues(1). This tissue uptake of Mg^{28} is facilitated by simultaneous administration of insulin and glucose(2), and thyroxine also accelerates the uptake of this isotope in certain tissues(3). This effect of thyroxine may be due to the uncoupling of oxidative phosphorylation. The present experiments were performed in an attempt to determine whether the uncoupling of oxidative phosphorylation by 2,4-dinitrophenol could be correlated with alterations in magnesium metabolism.

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Materials and methods. Normal domestic adult male rabbits weighing about 2 kg each were kept in individual stainless steel metabolism cages and were fed a stock diet of compressed pellets which contained 0.1723 meq Mg/g. Tap water, containing 0.6 meq Mg/l, was given without restriction. A 2% solution of *2,4-dinitrophenol (DNP),* made by heating the material gently in 2% $NaHCO_3$, was injected subcutaneously into the interscapular space.

Mg^{28}, with a specific activity of 380 μc/meq Mg, was received as $MgCl_2$ in normal saline. A few drops of 1 N NaOH was added to form a precipitate of $Mg(OH)_2$. The precipitate was dissolved with 1 N H_2SO_4, and the neutral solution was filtered. Distilled water was added to the filtrate to make 60 ml of a solution in which the concentration of Mg was 0.0144 meq/l. Each rabbit was injected intravenously with 0.069 meq Mg.

Serum and *urine magnesium* determinations were performed by a modification of the molybdivanadate method for phosphate(4). Tissue magnesium determinations were performed by the method of Stutzman(5).

Radioactivity assay. Samples of plasma and tissue were assayed for gamma-ray activity with a scintillation counter, a total of 10,000 counts being made on each sample. All determinations were corrected for physical decay of the isotope.

Preliminary observations. Eight rabbits were given DNP, 30 mg/kg, interscapularly. The mean rectal temperature rose from a control value of 39.3°C to 41.6°C at 3½ hours, and 4 of the rabbits died within 4 hours. Four other rabbits were placed in individual plastic spirometers, and the oxygen consumption of each one was measured serially before and after administration of DNP, 5 mg/kg, twice a day for 4 days. Each rabbit showed an increase in oxygen consumption following administration of DNP at some time during the period of observation, the maximum increase ranging from 17 to 70%.

Plan of experiment. External balance studies for magnesium were performed for 8 days in 8 rabbits. Each morning before the rabbits were fed, body weight, total food consumption, and 24-hour urine volume were measured. Blood from the marginal ear vein was collected into heparinized test tubes. Control data were obtained during the first 3 days. On the 4th day and for 3 days thereafter, each animal received 2 doses of DNP, 5 mg /kg. On the 8th day, 30 minutes after a dose of DNP (5 mg/kg), 0.069 meq Mg tagged with Mg^{28} was injected intravenously into each animal. Four control rabbits which had not been given DNP also received the intravenous injection of radioactive magnesium. The animals were killed by exsanguination exactly 4 hours later, and from each animal 2 aliquots of the tissues listed in Table I were prepared for radioactivity assay. Plasma was obtained at the same time.

Specific activities of the tissues and plasma and the relative specific activity were determined as follows:

$$\text{Specific activity of tissue} = \frac{\text{cpm/g wet wt of tissue}}{\text{meq/kg wet wt}}$$

$$\text{Specific activity of plasma} = \frac{\text{cpm/ml plasma}}{\text{meq/l plasma}}$$

$$\text{Relative specific activity} = \frac{\text{specific activ. of tissue}}{\text{specific activ. of plasma}}$$

Statistical methods. In the analysis of the balance data, each animal served as its own control. The significance of the mean difference was determined by use of the "t" test, a "P" of less than 0.01 being considered significant(6). In the analysis of the tissue data, the mean value for each tissue was determined for both test and control groups, and the significance of the difference between group means was then tested(6).

Results. The animals given 2,4-dinitrophenol showed a significant increase in body weight (from a mean of 1.987 kg to 2.064 kg). The balance data, however, revealed no significant alterations in magnesium intake, excretion, or serum concentration (Table I).

Four hours after administration of radioactive magnesium, the relative specific activities of the following tissues were significantly greater in the animals given DNP than in the controls (Table II): kidney, heart, stomach, liver, lung, bone marrow, skin, brain, and bone cortex.

Comment. When administered under prop-

TABLE I. Effects of 2,4-Dinitrophenol on Magnesium Metabolism in Rabbits.*

	Mean baseline values	Mean values after DNP
Wt (kg)	1.987	2.064†
Serum Mg (meq/l)	2.41	2.29
Mg excretion (meq/day)	5.2	4.7
Mg intake (meq/day)	17.6	22.4
Urine volume (ml/day)	165	139
Body temp (C)	40.1	39.8

* 2,4-dinitrophenol given interscapularly, 5 mg/kg twice a day for 4 days, and 5 mg/kg on the last day.

† Statistically significant difference when compared with mean baseline value.

TABLE II. Relative Specific Activities* for Magnesium of Tissues in Rabbits Given 2,4-Dinitrophenol† and in Control Animals.‡

	Control animals‡	2,4-dinitrophenol†
Kidney	1.35 ± .17§	2.36 ± .14‖
Heart	1.12 ± .17	2.16 ± .14‖
Testis	.93 ± .18	1.32 ± .17
Stomach	.86 ± .08	1.30 ± .07‖
Liver	.78 ± .12	1.72 ± .12‖
Lung	.66 ± .10	1.11 ± .06‖
Spleen	.58 ± .01	2.25 ± .35
Bone marrow	.49 ± .07	1.34 ± .18‖
Skin	.46 ± .02	1.25 ± .12‖
Brain	.11 ± .004	.19 ± .013‖
Bone cortex	.07 ± .005	.11 ± .008‖
Muscle	.07 ± .01	.11 ± .008

* Relative specific activity =

$$\frac{\text{cpm/g wet weight of tissue/meq/kg wet weight}}{\text{cpm/ml plasma/meq/l plasma}}$$

† Mean values in 8 rabbits given a total of 45 mg/kg of 2,4-dinitrophenol subcutaneously and killed 4 hr after intravenous injection of Mg^{28}.

‡ Mean values in 4 rabbits not injected with 2,4-dinitrophenol.

§ mean ± standard error.

‖ Statistically significant difference when compared with control means.

er experimental conditions, 2,4-dinitrophenol not only increases oxygen consumption sufficiently to cause a rise in body temperature, but also prevents the uptake of inorganic phosphate by cells and the production of energy-rich phosphate(7). It is generally believed that a displacement reaction causes rapid decomposition of an energy-rich intermediate, thus leading to hydrolysis of adenosine triphosphate(8). In the present experiment, DNP increased the relative specific activity of magnesium in almost all the tissues studied. This increase is interpreted as indicating increased turnover of magnesium in these tissues. The increased turnover of magnesium is probably related to the increased oxidation induced by administration of DNP rather than to decreased phosphorylation.

We have previously reported that administration of insulin and glucose(2) and of thyroxine(3) increased the uptake of Mg^{28} in the tissue of rabbits. Since both insulin(9) and thyroxine(10) can act as uncoupling agents, an increased rate of oxidation may be the common denominator for the increase in tissue uptake of Mg^{28} produced by all 3 agents. Exactly what aspect of oxidation, whether the glycolytic or the aerobic phase, is predominantly affected remains to be determined.

In contrast to magnesium, the uptake of potassium by rabbit kidney slices is impaired by DNP. It appears, therefore, that the biochemical mechanism for the tissue transport of magnesium is different from that for potassium(11).

Summary. After receiving interscapular injections of 2,4-dinitrophenol, 10 mg/kg daily for 4 days, rabbits were killed on the fifth day. The balance data revealed no significant changes except an increase in body weight. The relative specific activity of Mg^{28}, determined 4 hours after intravenous injection of Mg^{28}, was significantly increased in the kidney, heart, stomach, liver, lung, bone marrow, skin, brain, and bone cortex. The increased turnover of magnesium in these tissues is attributed to the increased rate of oxidation caused by 2,4-dinitrophenol.

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Occurrence of Natural Antibacterial Antibody in Human Parotid Fluid. (30309)

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Although saliva has been reported to contain antimicrobial factors, little attention has been given to the demonstration of the presence of natural bactericidal antibodies in the fluid(1). The designation "natural" antibodies refers to those specificities found in serum of man in the absence of infection or overt stimulation(2). Antibodies other than bactericidal have been shown in both whole saliva and parotid fluid. Investigators have demonstrated the presence in whole saliva of bacterial agglutinins, isohemagglutinins, and diphtheria antitoxins(3). In addition to these studies others have directly demonstrated the occurrence of immunoglobulins γA, γG, and γM† in saliva(4,5).

The reports of Brill and Bronnestam(6) and Mann and Stoffer(7) would indicate that at least some of the antibody found in whole saliva could be contributed by fluid outflow from the gingival pocket. This fluid, rich in serum proteins, would obscure that portion of antibody which might be derived from the salivary glands. Accordingly, it seemed reasonable to examine the fluid obtained from the parotid gland in humans for the presence of natural antibacterial antibody since this secretion could be obtained without contamination directly from the duct in sufficient quantities for assay.

Materials and methods. Parotid fluid and serum. Paired parotid fluid and serum samples were taken from normal, apparently healthy, individuals. The parotid fluid, obtained by paraffin stimulation, was collected aseptically by use of Curby cups and immediately placed in ice. Parotid fluid is normally uncontaminated by bacteria; however, as a precaution against accidental contamination all samples were passed through Millipore® filters (size HA). Aliquots in amounts suitable for each day's assays were frozen at —20°C. Pooled lyophilized parotid fluid was also used in some studies. Serum samples taken at the same time as the parotid fluid were prepared and stored frozen at —20°C without preservatives until used.

Cultures.‡ *Escherichia coli* 0127, *Salmonella typhosa 0901, Shigella dysenteriae* and *Aerobacter aerogenes* were picked from single colonies and transferred to nutrient agar slants. Subcultures were made to Brain Heart infusion (Difco) and grown overnight at 37°C for the bactericidal assay.

Bactericidal assay. The assay method used was described by Muschel and Treffers(8). A modification was made in their procedure by substituting precolostrum calf serum (Colorado Serum Co., Denver) for the adsorbed guinea pig complement recommended (9). Each lot of complement was tested for bactericidal antibody, and if found free, was used in the amounts recommended by Muschel and Treffers.

Precipitin tests. Capillary precipitin reactions were performed using specific antisera to γA, γG, and γM immunoglobulins (Hyland Laboratories, Los Angeles). Gel precipitin

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† The notations used for human immunoglobulins are in accordance with those suggested by WHO Meeting on Nomenclature of Human Immunoglobulins(15).

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