was identified, absence of the image could be explained by proximity to the planet or to a bright satellite, or by underexposure (*8*). The only satisfactory pair of orbits is: (i) sidereal period 17.9714 hours, corresponding to a semimajor axis of 159,200 km (*9*) and eastern elongation 1966 October 29.583 ephemeris time (ET) (which is very close to the orbit calculated by Dollfus for S10) and (ii) sidereal period 16.6507 hours, corresponding to a semimajor axis of 151,300 km and eastern elongation 1966 October 29.596 ET. While the formal errors imply the stated accuracy, the true errors may well be larger in view of the heterogeneity of the data. The observations and their residuals for the two orbits are given in Table 1. It is possible that some of the observations are not of a single body but of two fainter satellites that are occasionally in near alignment along the line of sight, with a combined light that exceeds the photographic threshold. The near alignment of the observations on 15 December as predicted by these orbits is an example of a similar occurrence. If one admits the possibility that three or more bodies may be involved, it is not clear that a unique solution can be found for this limited data set.

We therefore conclude that Saturn has at least 11 satellites, and that the orbits mentioned above represent a prominent solution which should be tested at the next opportunity. The blue magnitude at a phase angle of 6° is 14.2 ± 0.3 for the objects, implying diameters of 200 to 500 km for albedos of 0.9 to 0.2.

Observations have been reported (*10*) which suggest the presence of a faint extension of the Saturn ring system to 300,000 km. This so-called E ring may well be composed of bodies of which S10 and this new satellite, as well as a population of other small satellites yet to be identified, may be a part. These bodies would have an important bearing on the question of the formation of Saturn's rings and satellites. Meteoroid impact of such satellites could be a source of material for the rings (*11*). Additional observations of these satellites should be made when the ring is again seen edge-on in 1979 and 1980.

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Quantitation of Dimethylnitrosamine in the Whole Mouse After Biosynthesis in vivo from Trace Levels of Precursors

Abstract. *A simple and highly sensitive procedure is described for the recovery and quantitative identification of nanogram quantities of preformed* N*-nitroso compounds in the whole mouse. This procedure has also been applied to the quantitation of* N*-nitroso compounds after they have been biosynthesized from trace amounts of precursors. The whole animal is frozen in liquid nitrogen and homogenized to a frozen powder; the powder is then extracted and analyzed by a thermal energy analyzer interfaced to a gas-liquid and a high-pressure liquid chromatograph.*

The widespread presence of carcinogenic *N*-nitroso compounds in the environment is now well recognized (*1*). Even more ubiquitous are the precursors of *N*-nitroso compounds, secondary, tertiary, or quaternary amines and nitrites or oxides of nitrogen, from which biosynthesis occurs both in vitro and in vivo (*2, 3*).

Most studies on nitrosation in vivo are based on feeding animals large doses of nitrites and precursor amines and subsequently identifying the nitrosated products in the stomach (*2–4*). These studies are complicated, however, by the low yield of *N*-nitroso products caused by losses from absorption (*5*) and metabolism (*2*), and by the high concentration of the precursors tested relative to the levels at which they occur in the environment. For those reasons, we have developed a simple and highly sensitive "frozen animal procedure" (FAP) for the quantitative identification of nitrosamines after they have been synthesized in vivo from trace levels of their precursors.

Groups of two to three male mice (Charles River), weighing approximately 30 g, received successively, by means of gavage, 50-μl saline solutions containing 250 ng of sodium nitrite and then 50 ng of

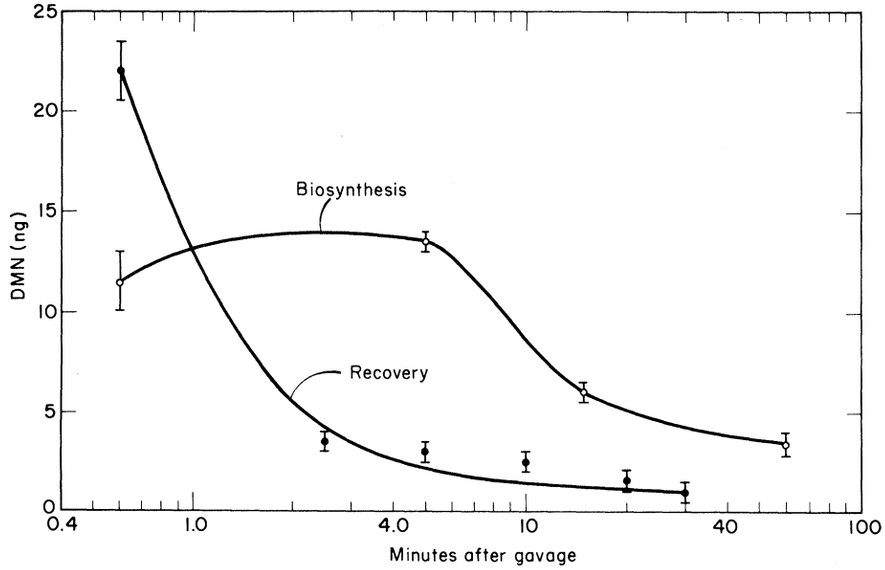

Fig. 1. The time course of recovery of DMN administered to mice by means of gavage, and the time course of DMN biosynthesis after the administration of DMN precursors. Symbols: (●), recovery from mice that received 50 ng of DMN; (○), biosynthesis of DMN in mice that received 50 ng of DMA and 250 ng of nitrite.

dimethylamine hydrochloride (DMA); negative controls received either saline, or nitrite, or DMA alone. Positive controls received either 5, 50, or 500 ng of DMN; additional positive controls used in preliminary experiments received a saline solution containing a mixture of 5, 50, or 500 ng each of dimethylnitrosamine (DMN), diethylnitrosamine, *N*-nitrosopiperidine, and *N*-nitrosopyrrolidine. The animals were killed, either immediately after gavage or at subsequent intervals, by plunging them individually into liquid nitrogen for 10 seconds. Whole frozen animals were then homogenized in liquid nitrogen in a Waring Blendor for approximately 2 minutes, yielding a fine powder; the approximate time elapsing from initiation of gavage to initiation of homogenization was 40 seconds. The powder was then transferred to a round-bottomed flask and vacuum extracted with mineral oil as described (*6*). Final detection and quantitation of *N*-nitrosamines was by means of a thermal energy analyzer (TEA model 502, Thermo Electron) interfaced to a gas-liquid chromatograph (TEA-GLC). Confirmation of identity was by means of parallel TEA-GLC and TEA–high-pressure liquid chromatography (TEA-HPLC) procedures (*7*). Reproducibility between duplicate analyses was ± 15 percent.

In the negative control mice that received 50 μl of saline, the background DMN level was less than 1 ng per mouse, or 0.03 μg/kg. However, the background levels of DMN in control mice housed for 2 to 4 hours in a biochemical laboratory where nitrosamines were handled in a chemical hood were between 5 and 30 ng per mouse; these levels were unaffected by prior starvation or feeding with a synthetic diet. In positive control mice killed immediately after gavage with the nitrosamine mixture, recoveries ranged between 80 and 85 percent of the administered dose of each nitrosamine; these levels were comparable to the recoveries from mice that were killed under chloroform anesthesia and then immediately injected parenterally with the same nitrosamine mixture.

Rates of DMN loss from the intact mouse were determined by analysis of animals serially killed at intervals up to 30 minutes after gavage with 50 ng of DMN. Figure 1 shows that less than 50 percent of the administered DMN was recovered within 60 seconds following gavage, after which recovery declined by 10 minutes to a steady state of approximately 3 percent, corresponding to 1.4 ng of DMN per mouse or 0.037 μg/kg.

The formation of DMN in vivo was determined in mice killed at intervals up to 60 minutes after they had received 250 ng of sodium nitrite followed by 50 ng of DMA. Approximately 23 percent, corresponding to 11.5 ng of DMA, was converted into DMN within 40 seconds after the initiation of gavage (zero time); this increased to a peak of 28 percent at 5 minutes, which was followed by a decline to 7 percent, corresponding to 3.5 ng of DMA at 60 minutes. No DMN was detected in control experiments with DMA and sodium nitrite in which the animal was omitted. No DMN was detected in negative control mice frozen immediately after gavage with DMA alone. However, approximately 3.5 ng of DMN was recovered from other groups of negative control mice gavaged with sodium nitrite alone and then frozen immediately.

These data demonstrate the high sensitivity of the FAP-TEA technique, which can quantify and confirm nanogram (parts per trillion) quantities of nitrosamines in whole mice. After the administration of DMN and other nitrosamines, the zero time recoveries from the intact animal were high. It is of interest that similar high degrees of recovery have been reported from luncheon meat and cooked bacon to which DMN had been added (*6*). However, there was rapid loss of DMN in mice that received DMN by gavage, and this appeared to be a result of metabolism or exhalation, or both; such losses could not be due to absorption because analysis was based on the whole animal. It is of interest that while mice that received DMA alone did not yield any DMN, administration of nitrite alone resulted in the recovery of low levels of DMN, suggesting nitrosation of dietary or naturally occurring amines in the stomach.

After the administration of low levels of DMN precursors, the level of DMN biosynthesis was high. According to the kinetic expression of nitrosation of amines in a model system (*2*), the DMN levels recovered in the present experiments at 100 seconds, 1.4 ng, were 140 times higher than calculated values, 0.1 ng (*8*). That the conversion of DMA to DMN in whole mice was higher than would be expected from calculations based on model systems is not surprising, because model studies are carried out at relative high reactant concentrations and only approximate conditions in vivo. Furthermore, model studies cannot take into account the many possible competing processes in vivo, including modifying factors such as inhibitors, catalysts, and competing reactions, which may be of particular importance when the reactants are present only at trace levels.

The FAP-TEA technique is simple, highly sensitive, and has other advantages over standard procedures in vivo. First, all metabolic processes are stopped rapidly after death by freezing. Second, the whole animal is reduced to a frozen powder in a form suitable for extraction and analysis. Finally, recovery and analysis are based on the nitrosamine content of the whole animal, rather than of an isolated organ.

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8. According to the kinetic expression in (*2*), the rate = K_2 (amine) (nitrite)^2, and with K_2 = 0.0017 mole^{-2} sec^{-1}, concentrations of 0.022 m*M* DMA and 60 m*M* nitrite, and a reaction time of 100 seconds, the expected DMN content in the stomach should be 0.1 ng, provided that the *p*H in the mouse stomach was optimum for efficient nitrosation, and the total volume of the reactants was equal to the sum of volumes introduced by gavage, that is, 0.1 ml, and that no DMN was lost after formation. In the present experiments, 14 ng of DMN (or 140 times higher than expected) was detected 100 seconds following gavage with precursors.
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