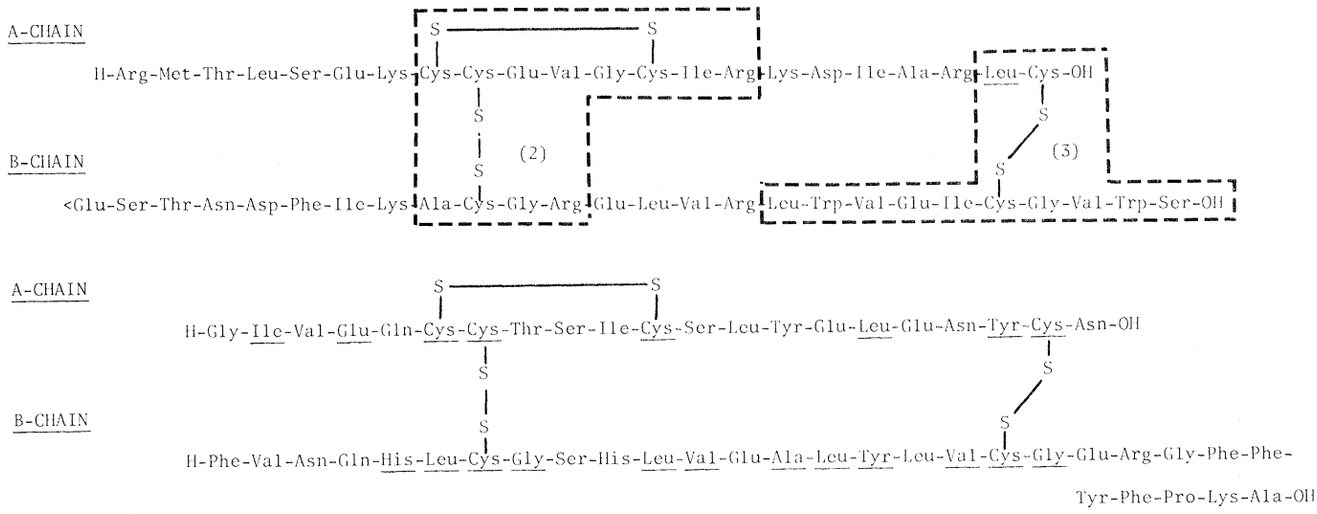

Fig. 2. The primary structures of porcine relaxin (top) and insulin (bottom) with their cysteine residues aligned. The dashed lines in the relaxin structure identify the tryptic peptides designated Nos. 2 and 3 (also Table 1). In the insulin structure, the underlined residues signify those that are either homologous or conservatively replaced with reference to the respective chains in relaxin.

Table 1. Further evidence for the cross-link between the cysteines at A22 and B22 was obtained from experiments in which lysosomal carboxypeptidase B was allowed to act on the intact relaxin molecule. As was recently reported (*3*), lysosomal carboxypeptidase B rapidly removed the COOH-terminal residues Ser, Trp, Val, and Gly as well as the cysteines involved in the cross-link. A time course digest of total relaxin with lysosomal carboxypeptidase B yielded—in addition to serine, tyrptophan, valine, and glycine—leucine and arginine before isoleucine or glutamic acid could be observed. This can only be interpreted as evidence that Leu^{21} and Arg^{20} in the A chain were hydrolyzed before the first glutamic acid (Glu^{20}) and isoleucine (Ile^{21}) could be released from the B chain.

In view of these experiments, we concluded that the cross-linking patterns of insulin and relaxin are superimposable. It is surprising, however, that virtually no homology exists between the insulin and relaxin molecules with respect to the remaining residues. The homology is limited to only five residues in the A chain. In addition, three conservative replacements occur in the A chain. The B chain contains six homologous positions in addition to six conservative replacements relative to insulin. Considering only the two initial bases of the appropriate codons, this would correspond to 51 point mutations. If we assume that the rate of mutation acceptance for relaxin is 1 pauling unit (10^{-9} mutation accepted per amino acid per year) the gene duplication may have occurred as early as 5×10^8 years ago. Insulin accepts mutations at the rate of 0.4 pauling or four residues per 10^8 years (*5*), that is, it has changed its amino acid composition possibly in 20 positions during the same period. This estimate of 5×10^8 years for relaxin seems reasonable when one considers that sharks and bony fishes diverged from the branch of vertebrate evolution which eventually gave rise to the mammals.

It has been suggested (*6*) that the A2 isoleucine in insulin (at which position leucine is found in relaxin), the A16 leucine (which is isoleucine in relaxin), as well as the A19 tyrosine (which is leucine in relaxin), would allow a similar formation of a hydrophobic core in relaxin as it occurs in insulin. Another point of identity or similarity between the B chains is given by the fact that cysteine is followed by glycine in both hormones and is preceded by an aliphatic residue (alanine and isoleucine in relaxin as compared to leucine and valine in the insulin B chain). The identical cross-linking patterns of relaxin and insulin suggest that duplication of an ancestral gene led to the evolution of two hormones with profoundly diverse functions.

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15 April 1977

A New Satellite of Saturn?

Abstract. *Analysis of all available observations of faint objects near Saturn during the 1966 passage of the earth through the plane of Saturn's rings suggests the existence of at least one previously undiscovered satellite of Saturn. The data support the previously published orbit for Janus. These satellites may be major members of an extended ring.*

In 1966 the earth passed through the plane of Saturn's rings. Since the ring system is only a few kilometers thick (*1*), there was very little scattered light from the rings at that time, which enabled Dollfus (*2*) to discover a tenth satellite of Saturn (S10), commonly referred to as Janus. Dollfus calculated an orbit based on his own observations as well as those by J. Texereau at McDonald Observatory, Fort Davis, Texas. A later analysis by Franklin *et al.* (*3*) included an additional observation by R. Walker at the U.S. Naval Observatory, Flagstaff, Arizona.

We have reexamined photographs tak-

en at the Catalina Observatory of the Lunar and Planetary Laboratory (LPL) during the 1966 apparition (*4*). Careful scrutiny showed that there were more possible satellite images. We have measured these and all other available images taken at that time, using known satellites as references. Because most of the LPL images are close to the photographic detection threshold, we required that the suspected images repeat on several frames of sequences covering several minutes, that they appear on the best-quality frame of the sequence, and that the frames be relatively free of defects. Many of the objects were established as real telescopic images because they were affected by telescope shaking or atmospheric dispersion in the same way as the other satellite images. The images in the LPL data repeat on from 4 to 12 frames within a sequence, and we could thus make composites to improve our ability to measure their positions. It should be emphasized that almost all of the images are marginal. One of the better images is reproduced in Fig. 1.

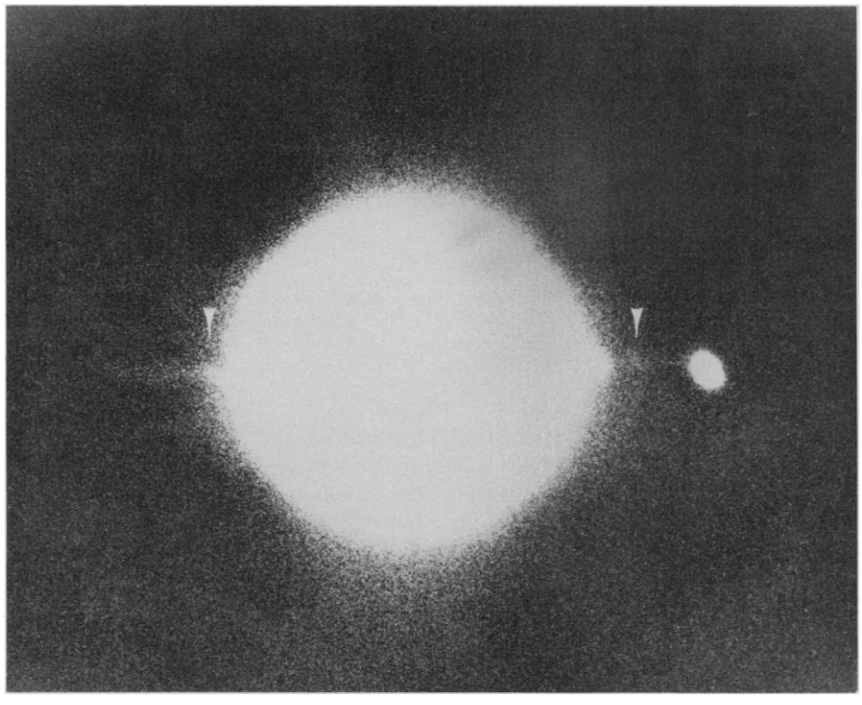

Fig. 1. Composite of five images of Saturn taken with the 154-cm reflector at the Catalina site of the Mount Lemmon Observatory on 18 December 1966 at 2.823 hours Universal Time. The faint satellites are indicated. Enceladus (nearer the planet) and Tethys are visible on the right, Janus on the left.

Table 1. Summary of observations and their identification. The observers were T, Texereau; D, Dollfus; LPL, Lunar and Planetary Laboratory; and W, Walker. The quoted position errors are standard deviations for our measurements; the measurements supplied by Dollfus did not include errors. East is indicated by + and west by −. For the December observations, 1 arc second = 6.9×10^3 km. Middle time of exposure is in Universal Time.

Date (1966)	Middle time of exposure (hours)	Observer	Position ($\times 10^3$ km)	Residual ($\times 10^3$ km)		Identification
				S10	Other satellite	
October						
29	2.958	T	−123.1 ± 1.5	−2.7	−60.5	S10
29	3.658	T	−140.5 ± 1.5	+1.5	−44.1	S10
29	3.658	T	−98.0 ± 1.5	+44.0	+0.6	Other
29	5.125	T	−142.7 ± 1.5	+16.3	+5.9	S10, other
December						
11	2.800	LPL	No object			
12	1.578	LPL	No object			
15	18.375	D	+158.4	−0.7	+8.1	S10, other
15	18.650	D	+158.4	+0.4	+7.1	S10, other
15	19.583	D	+142.8	−0.7	+0.2	S10, other
15	19.933	D	+134.4	+0.4	−0.3	S10, other
15	20.267	D	+121.2	−1.9	−3.8	S10, other
16	19.883	D	−144.0	0.0	+6.0	S10, other
17	1.917	LPL	+113.5 ± 1.0	+104.4	+5.9	Other
17	2.499	LPL	+132.7 ± 1.0	+91.7	+4.5	Other
18	1.468	LPL	+136.7 ± 3.5	−7.6	+174.5	S10
18	1.592	W	+134.8 ± 2.0	−6.4	+179.4	S10
18	1.661	W	+143.6 ± 3.9	+4.2	+192.0	S10
18	2.329	W	−89.0 ± 2.0	−206.8	−6.4	Other
18	2.823	LPL	−109.2 ± 5.0	−206.9	−4.5	Other
18	3.346	LPL	−127.9 ± 6.2	−201.1	−3.8	Other

Eighteen observations were identified, eight of which had not been used in previous analyses. On two dates, long-exposure photographs of good quality did not show faint satellite images. Separate images of two distinct satellite candidates were identified on photographs taken on 29 October and 18 December. No star brighter than blue magnitude 20 was found within 30 arc seconds of the planet. No asteroids bright enough to have been a source of confusion were near Saturn during the period of observation (*5*). Our study of condensations observed by Barnard (*6*) and Ferrin (*7*) from light diffusely transmitted by Cassini's division (a gap between rings A and B) and by ring C leads us to conclude that none of the tabulated images may be confused with such condensations. The only images near positions where such condensations might be seen were part of a series showing motion of the object. Motions within a night were incompatible with large bodies embedded in the ring, except in one case when the estimated errors were quite large. There are several observations of the satellites seen outside the ring.

We have attempted to fit the observations to direct circular orbits in the plane of the ring system, since orbits of appreciable eccentricity so near the rings seemed unlikely, and there are insufficient observations to test adequately for eccentricity. Observations of the objects when they were most distant from Saturn showed no measurable deviation from the ring plane. No single orbit would fit all the observations. On the assumption that there are two bodies, an orbit search was made for periods of 14.4 to 19.2 hours in increments of 0.12 and 0.24 hour in epoch, and more than 100 of these orbits were examined in detail. We found many combinations of orbits that would adequately fit subsets of the data. However, all but one of the combinations conflicted with some of the observations. Further, on photographs on which only one or none of the satellites

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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8. In some cases, the added light of the ring system was necessary to bring the brightness of the satellite above the plate limit.
9. In calculating the semimajor axes we included the effects of gravitational multipole moments of J2 and J4 for Saturn. Corrections were made for light time and the longitude of the earth. The radius of the outer boundary of ring A is 136,500 km, and the semimajor axis of the orbit of Mimas is 185,800 km. For both trial orbits, one revolution more and one less between the 29 October and 15 December observations were calculated. For each of these orbits the residuals were about twice as great.
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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

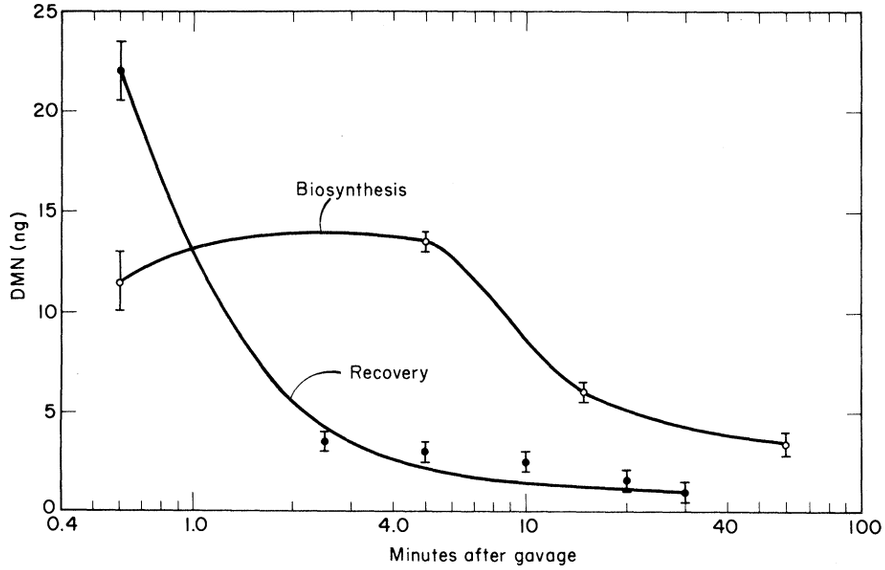

Fig. 1. The time course of recovery of DMN administered to mice by means of gavage, and the time course of DMN biosynthesis after the administration of DMN precursors. Symbols: (●), recovery from mice that received 50 ng of DMN; (○), biosynthesis of DMN in mice that received 50 ng of DMA and 250 ng of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