production. The effect of pancreatectomy on the serum cholesterol of the eel is the exact opposite of that found in mammals, including man, in which the operation is followed by increased concentrations of serum cholesterol (*18, 19*).

Since the animals had no food intake for months, one would assume that the drop in serum cholesterol is due to the removal of the endocrine pancreas. However, in severe human diabetes (*19*), as well as in experimental diabetes after B-cell cytotoxins (*20*), cholesterolemia is increased. Also, glucagon injections lower the cholesterolemia in mammals and lizards (*21*), and stimulates cholesterol breakdown into bile acids and glucose (*22*). From these findings in mammals and lizards, one would expect hypercholesterolemia in cases of insulin or glucagon deficiency, or both. On the other hand, it remains to be seen if in the eel the bile secretion, mucus, or sloughing cells of the digestive tract provide esterified cholesterol, whose uptake would partially depend on a pancreatic cholesterolesterase (*23*). In mammals the cholesterol in bile is found mainly in the free state (*24*), thus capable of recirculating via the enterohepatic pathway without esterase action, and most of the sloughing cells of the digestive tract are lost in the feces (*25*).

In mammals, only a very small amount of the pancreas is necessary to maintain the exocrine and endocrine secretion at effective levels (*26*). Since in all partially pancreatectomized animals we removed less than 50 percent of the gland, the tissue involved in the control of the cholesterolemia must have a rather small functional reserve. Further studies must show if the elevated concentrations of cholesterol in the partially pancreatectomized eels 5 to 6 months after the operation are due to regeneration of pancreas tissue or other compensatory mechanisms.

Finally, our results show that the total serum cholesterol of the eel is comprised of two fractions, one of which is pancrease dependent. The level of serum cholesterol at 12 to 20 days postoperative suggests that the pancreas-dependent fraction amounts to approximately 70 to 80 percent (Fig. 1).

THOMAS L. LEWIS
AUGUST EPPLE

Daniel Baugh Institute of Anatomy, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania 19107

References and Notes

1. W. Chavin and J. E. Young, *Gen. Comp. Endocrinol.* **14**, 438 (1970).
2. A. Epple, *Endocrinol. Jap.* **15**, 107 (1968).
3. ———, in *Fish Physiology*, W. S. Hoar and D. J. Randall, Eds. (Academic Press, New York, 1969), p. 275.
4. S. Falkmer and G. Patent, in *Handbook of Physiology*, sect. 7; vol. 1, *Endocrine Pancreas*, J. Field, Ed. (Williams & Wilkins, Baltimore, 1972), p. 2; S. Falkmer and M. Marques, in *Monograph on Glucagon*, P. J. Lefebvre and R. M. Unger, Eds. (Pergamon, Oxford, England, in press); M. C. Minick, thesis, Wayne State University (1971).
5. A. Caparelli, *Arch. Ital. Biol.* **21**, 398 (1894).
6. S. Falkmer and A. J. Matty, *Acta Soc. Med. Upsal.* **71**, 156 (1966); S. Falkmer, *Acta Endocrinol. Copenhagen* **37** (Suppl. 59) (1961); N. A. McCormick and J. J. R. MacLeod, *Proc. Roy. Soc. Ser. B* **98**, 1 (1925); W. N. Simpson, *Amer. J. Physiol.* **77**, 409 (1926).
7. J. Brinn and A. Epple, *Anat. Rec.* **172**, 277 (1972).
8. L. L. Sulva, B. E. Box, G. Gunter, *Amer. J. Physiol.* **199**, 1177 (1960).
9. A. Epple, *Stain Technol.* **42**, 53 (1967).
10. W. Chavin and J. E. Young, *Comp. Biochem. Physiol.* **33**, 629 (1970).
11. P. F. Nace, M. L. Moule, J. E. Schuh, *Can. J. Physiol. Pharmacol.* **42**, 225 (1964).
12. N. L. Buchner, P. Overrath, F. Lynen, *Fed. Proc.* **18**, 20 (1959).
13. T. H. McCartney, *Fish. Res. Bull.* **29**, 72 (1966); Y. Schimizu, T. Morio, S. Higasa, *Nippon Suisan Gakkaishi* **29**, 219 (1963); E. W. Shell, *Bur. Sport Fish. Wildl. (U.S.) Res. Rep. No. 57* (1961), p. 1.
14. T. H. McCartney, *Fish Res. Bull.* **29**, 40 (1966); T. H. McCartney, *ibid.* **30**, 35 (1967); B. L. Umminger, *Amer. Zool.* **7**, 731 (1967).
15. G. E. Pickford, F. B. Grant, B. L. Umminger, *Trans. Conn. Acad. Arts Sci.* **43**, 25 (1969).
16. T. H. McCartney, *Fish Res. Bull.* **29**, 49 (1965).
17. M. A. Siddiqi, *Naturwissenschaften* **53**, 561 (1966); M. D. Siperstein and L. J. Luby, in *Fish in Research*, O. W. Neuhaus and J. E. Halver, Eds. (Academic Press, New York, 1969), p. 87.
18. S. S. Chernick and R. O. Scow, *Amer. J. Physiol.* **196**, 125 (1959).
19. V. S. Sailer, F. Sandhofer, H. Braunsteiner, in *Handbuch des Diabetes Mellitus*, E. F. Pfeiffer, Ed. (Lehmanns Verlag, München, 1971), vol. 2, p. 775.
20. A. Epple, *Diabetes* **20** (Suppl. 1), 354 (1971); ——— and J. Car, *Arch. Mex. Anat.* **39**, 39 (1972); J. H. Gans and M. R. Carter, *Life Sci.* **10** (part 1), 301 (1971); R. K. Wong and J. T. van Bruggen, *J. Biol. Chem.* **235**, 30 (1960).
21. S. A. Suryawanshi and P. V. Rangneker, *J. Anim. Morphol. Physiol.* **18**, 134 (1971).
22. M. R. Malinow, P. McLaughlin, I. Pierovich, *Amer. J. Physiol.* **222**, 256 (1972); *Primate News* **10**, 14 (1972).
23. G. V. Vahouny, C. R. Borja, C. R. Treadwell, *Arch. Biochem. Biophys.* **106**, 440 (1964).
24. G. B. Philips, *Biochim. Biophys. Acta* **41**, 316 (1960).
25. H. J. Weis, *Klin. Wochenschr.* **48**, 1203 (1970).
26. W. E. Creutzfelt, in *Handbuch des Diabetes Mellitus*, E. F. Pfeiffer, Ed. (Lehmanns Verlag, Munich, 1971), vol. 2, p. 239; R. O. Scow, *Endocrinology* **60**, 359 (1957).
27. Supported in part by NIH grant RR-5414. We gratefully acknowledge the technical assistance of Miss Mary L. Pitcher and Mrs. Mary C. Hamill.

18 September 1972

Recognition of Cancer in vivo by Nuclear Magnetic Resonance

Abstract. *Pulsed nuclear magnetic resonance has been used to differentiate in vivo between normal mouse tail tissue and a malignant transplanted melanoma, S91, located on the tail. The tumor displayed a nuclear (proton) spin-lattice relaxation time of ~0.7 second contrasted with the simultaneously measured normal tail tissue relaxation time of ~0.3 second.*

Measurements of the proton nuclear spin-lattice relaxation time T_1 have been shown by Damadian (*1*) and subsequently by others (*2*) to have different values in some normal biological tissues, benign tumors, and malignant tumors observed in vitro. These results raise the possibility of the application of nuclear magnetic resonance (NMR) to nondestructive detection and monitoring of tumors and their progressive growth in live animals, including humans. We report here the first studies in vivo with pulsed NMR to differentiate between the normal tissue of a mouse's tail and a transplanted malignant melanoma (known as Cloudman S91) without any obvious harm to the live animal. The data obtained were associated with two types of tissue, the normal with T_1 ~0.3 second and the malignant tumor with T_1 ~0.7 second. The trend of these numbers is in qualitative agreement with the earlier results in vitro (*1, 2*). Important differences between NMR measurement techniques in vitro and in vivo are geometric arrangements, motion of the animal, and the filling factor associated with the different tissues present.

It is necessary to place the part of the body being tested within a coil of wire (the "probe" coil), which in turn is situated in a strong magnetic field (*3*). Because of the size limitations of the available magnet, it was most convenient to perform this initial experiment on a mouse's tail, which had the advantage of being long and narrow

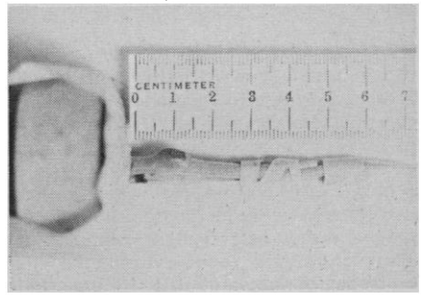

Fig. 1. Typical tumor on tail of DBA mouse. The mouse is in the small cage at the left (obscured).

and easily inserted in a small probe. A photograph of a typical tumor on a mouse's tail is shown in Fig. 1. With the use of magnets with larger sample space experiments could be performed on other animals and on other parts of the body. It should then not be difficult to make measurements on human limbs, breasts, or other protuberances; we have thus, for example, seen a strong NMR spin echo from a human finger.

Measurements were made at ambient temperature with a conventional phase-coherent, pulse-coherent, 5-kw crossed coil spectrometer. A rotating frame H_1 of ~25 gauss was sufficient to saturate the protons in the mouse tail with a single 180° turning-angle pulse. Signal averaging was accomplished with a gated integrator. Although the motion of the animal was a major source of noise, we were able to obtain significant data without the use of any anesthesia by properly securing the tail with masking tape. No signals arising from protons in the tape were observed.

Concerning the so-called filling factor, the NMR signal is a composite of all the hydrogen nuclei within the probe coil. The in vivo tumor may be only a portion of the material filling the coil. Then, if we assume that a single relaxation time is associated with a particular tissue or tumor, the recovery of the nuclear magnetization $M(\tau)$ after a 180°–90° pulse sequence as a function of the pulse spacing τ can be generalized from the equation used (*1*) for a single relaxation time. Thus, if n is the number of different kinds of tissue in the sample being studied, then

$$M(\tau) = \sum_{i=1}^{n} M_i(1 - 2e^{-\tau/(T_1)_i}) \quad (1)$$

With sufficiently high signal-to-noise ratio, it should be possible to analyze the signal, $M(\tau)$, into its several parts, and to obtain the corresponding values of T_1 for each of these. Such an analysis could result in the detection and identification of one (or possibly more) tumors existing in a host or environment of normal cells, provided that the individual tumors had different relaxation times (*4*). Repetitive measurements taken as a function of time would reveal the growth of the tumor as a relative change in the volume of tumor and normal tissue. A change from benign to malignant might be revealed in the change of T_1 associated with the tumor.

We have performed this type of analysis of data obtained from measurements on the tails of living DBA mice, with and without the Cloudman S91 tumor (Tables 1 and 2). Most of the scatter is probably primarily associated with the variations in experimental conditions, and not necessarily with tissue T_1 variability. There appears to be a frequency dependence (*5*) of T_1. Even with the large variations noted, there is a distinct difference between the T_1 associated with the normal tissue and the T_1 associated with the melanoma. It is believed (*1*, *2*) that the NMR is referable to the protons in the water molecules in the cells. In addition to a T_1 increase, the NMR amplitude increased with tumor volume, as would be expected from the increased filling factor and the well-known fact that cancers, as a class, have greater water content than nearly all corresponding normal tissue. The T_1 associated with the tumor appears to be constant.

Typical relaxation data obtained from a normal mouse's tail are presented on semilogarithmic scales in Fig. 2c. The data fall nearly on a straight line, indicating exponential behavior and a single T_1 (in this case 365 msec). Figure 2b shows data obtained from a tail containing a tumor with a ratio of cancerous tissue to normal tissue of about 2 : 1. The points fall on a curved line, implying more than one time constant. We interpret this curve as being due to the superposition of two exponentials (that is, $n = 2$ in Eq. 1). The longest T_1 (that is, the smallest slope) is from the malignant tumor. Precise values of normal and tumorous tissue T_1 cannot be extracted from the curves in Fig. 2b because of the noise. A best fit would give a T_1 in the range 0.6 to 0.8 second for the tumor and a normal tissue value between 0.1 and 0.3 second, depending on the details of the fitting. In view of the T_1 measured in normal mice (Table 2), it is appropriate to compare the data with a curve synthesized from two exponentials of time constants 0.3 and

Table 1. In vivo time development of nuclear spin-lattice relaxation time (T_1) in tails of tumorous DBA mice. The numbers in parentheses are estimated overall uncertainties. The frequency was 18 Mhz.

Mouse	Relaxation time (msec) on day				
	28	25	31	35	36
Tumor 1 ♀	340(10)*† 345(10)*† (mostly normal)		675(75)‡	690(110)	700(20)
Tumor 2 ♀		640*† (mostly tumor)	690(80)		750(50)
	linear fit		800(60)		750(50)

* Pulse sequence repetition rate was slightly fast relative to the time required for recovery of the nuclear magnetization and hence the measured T_1 is 10 to 15 percent shorter than those obtained under less rapid repetition rates. † Nonexponential behavior was observed, but the data were not precise enough to accurately separate the individual relaxation times and only the T_1 corresponding to the best straight line is shown. The relaxation recovery curve is consistent within relatively large experimental error with a superposition of two exponentials having values of T_1 of 0.3 and 0.7 second, respectively. ‡ Relaxation was a superposition of two exponential rates; the longest T_1 is shown.

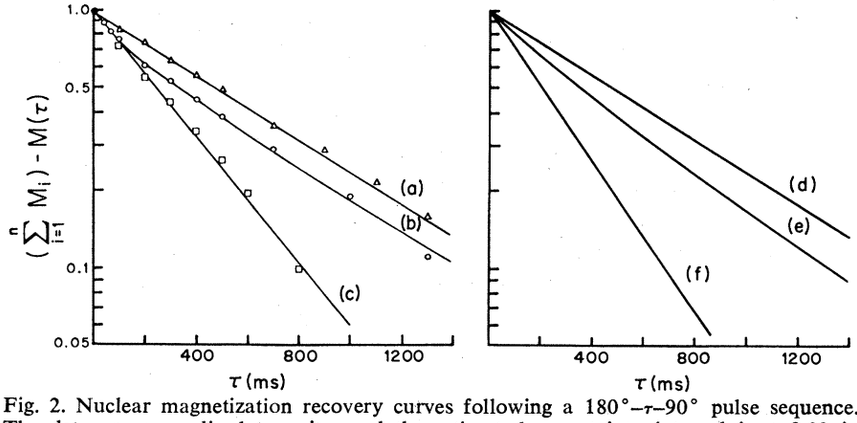

Fig. 2. Nuclear magnetization recovery curves following a 180°–τ–90° pulse sequence. The data are normalized to unity, and the estimated uncertainty interval is ± 0.02 in the units shown on the ordinate scale. (a) Single exponential behavior observed in a tumorous tail. (b) Nonexponential behavior observed in a tail containing normal and tumorous tissue. (c) Single exponential behavior observed in a normal tail. (d) All tumorous tissue, $T_1 = 0.7$ second (theoretical curve). (e) Synthesis from Eq. 1, with $n = 2$; $(T_1)_1 = 0.3$ second, $(T_1)_2 = 0.7$ second, $2M_1 = 0.35$, and $2M_2 = 0.65$. (f) All normal tissue, $T_1 = 0.3$ second (theoretical curve).

Table 2. Nuclear spin-lattice relaxation time T_1 in tails of tumorous mice and of normal mice. Observations on normal mice 1 and 2 were made during the period when tumor mice 1 to 5 were under study.

Test mouse	T_1	Time (days)
	Frequency, 18 Mhz	
Tumor 3 ♀	730(50)	32
Tumor 4 ♀	700(90)‡	32
Tumor 5 ♀	720(60)	37
Normal 1 ♂	285(15)*	
	320(20)*	
	330(20)	
Normal 2 ♂	330(10)*	
	365(25)	
	Frequency, 8 Mhz	
Tumor 6 ♂	500(70)*†, linear fit	28
	527(60)*†, linear fit	28
Normal 3 ♂	195(15)*	
	230(10)*	
Normal 4 ♂	235(25)*	
Normal 5 ♀	250(20)*	

*, †, ‡ See definitions of corresponding symbols in Table 1.

0.7 second. Reasonable agreement is obtained between the data of Fig. 2b and a synthesized curve, Fig. 2e, if we assume a ratio of tumorous to normal tissue protons of 2 : 1.

A potential source of error is due to the pulse sequence repetition rate. If too rapid a rate is used, relative to the rate at which the nuclear magnetization recovers, the observed relaxation time may be shorter than the true value. When relaxation consists of two superimposed exponentials, then the data corresponding to the longer relaxation are more distorted. It should be emphasized that the so-called "null method" used by Damadian (*1*) to obtain T_1 cannot be applied to our composite data. The null method T_1 obtained would be a weighted average of the two T_1's, with the weighting depending on the relative amounts of tissue.

The limit of detectability for tumorous tissue in our work was not less than 10 percent by volume. However this limit could be lowered substantially by taking advantage of digital signal averaging, automatic amplitude calibration (to compensate for instrumental drift), and higher magnetic fields to improve signal to noise.

This detection of an increase in vivo of T_1 in tumorous tissue does not differentiate between the model of tissue-water behavior suggested by Damadian (*1*), in which the intracellular water has less structure in tumorous than in normal cells, and another model in which cancerous tissue contains relatively more intercellular water, as in an edema. More detailed measurements of T_1 in vivo and in vitro and NMR amplitude as a function of magnetic field, temperature, and volume of the tumorous and normal tissue (within the radio frequency coil) might distinguish between models.

We have been able to detect and monitor the growth of a cancer (a transplanted S91 melanoma) in a live animal, using pulsed NMR. Our results suggest that it would be worthwhile to attempt to develop this technique for the detection and monitoring of tumors in humans. Perhaps NMR could take its place beside thermography or radiography as a nonsurgical technique for cancer detection and analysis of cancer growth rate.

IRWIN D. WEISMAN
LAWRENCE H. BENNETT
Institute for Materials Research, National Bureau of Standards, Gaithersburg, Maryland 20760
LOUIS R. MAXWELL, SR.
3506 Leland Street, Chevy Chase, Maryland 20015
MARK W. WOODS, DEAN BURK
National Cancer Institute, Bethesda, Maryland 20014

References

1. R. Damadian, *Science* **171**, 1151 (1971).
2. D. C. Chang, H. E. Rorschach, B. L. Nichols, C. F. Hazlewood, *Bull. Amer. Phys. Soc. Ser. 2* **17**, 328 (1972); N. Iijima and N. Fujii, *JEOL News* **9a** (4), 5 (1972).
3. J. A. Jackson and W. H. Langham, *Rev. Sci. Instrum.* **39**, 510 (1968).
4. H. Strehlow and J. Jen, *Chem. Instrum.* **3**, 47 (1971).
5. S. H. Koenig and W. E. Schillinger, *J. Biol. Chem.* **244**, 6520 (1969).

3 July 1972; revised 2 October 1972

Evidence for Active Immunity to Morphine in Mice

Abstract. *The serum from mice actively immunized with a morphine immunogen contained antibodies that could bind dihydromorphine. Morphine effects were diminished in these "immunized" mice, and the concentration of morphine in their plasma was altered.*

The development of tolerance is associated with the chronic use of many drugs. Two of the most common mechanisms invoked to explain tolerance are adaptation of the cells of the central nervous system and increased peripheral metabolism of the drug. Central nervous system adaptation is believed to account for tolerance to the narcotic analgesics. However, a number of observations indicate that an immune-like mechanism may also be implicated in narcotic tolerance (*1*): (i) some types of narcotic tolerance persist for many months, (ii) factors from the serum and tissues of tolerant animals contain material that can be passively transferred to a second animal and influence narcotic action in that animal, and (iii) tolerance to narcotics can be decreased by drugs that inhibit protein synthesis.

If such an immune mechanism for tolerance to a drug exists, it should be possible to demonstrate (i) proteins or antibodies which can specifically bind the drug and (ii) a modification of the pharmacologic activity or concentration of the drug in the immunized animal. We report here the production of antibodies that bind morphine in mice that have been actively immunized with a conjugate of morphine and protein and the production of an altered effect and biologic disposition of morphine in these mice.

The morphine immunogen, 3-carboxymethylmorphine coupled to bovine serum albumin, was previously shown to be effective in producing antibodies specific for morphine in the rabbit (*2*). Mice are also able to develop antibodies to morphine. Weanling male mice were injected subcutaneously once a week for 16 weeks with 0.1 ml of either buffered saline, 50 percent complete Freund's adjuvant emulsified in buffered saline, or 1 μg of morphine immunogen in 50 percent complete Freund's adjuvant emulsion (*2*). Six mice treated with adjuvant, or six treated with morphine immunogen plus adjuvant, were killed; the serums were pooled within each group, and a portion of the pooled serum was diluted with nine parts of buffered saline. This diluted serum (0.1 ml) was incubated for 20 minutes with 0.4 ml of buffered saline (Grand Island Biological) and tritiated dihydromorphine [15,000 disintegrations per minute (dpm), specific activity 680 mc/12.5 mg (New England Nuclear)] and was assayed for specific narcotic binding by an ammonium sulfate precipitation technique (*2*). The serums from mice injected with the morphine immunogen in Freund's adjuvant bound [^{3}H]dihydromorphine (245,000 dpm per milliliter of serum). The serums from those mice that received only Freund's adjuvant or saline bound no dihydromorphine in this test, an indica-