INSTITUTE »JOŽEF STEFAN«, UNIVERSITY OF LJUBLJANA
AND
INSTITUTE OF ONCOLOGY, LJUBLJANA, YUGOSLAVIA

MAGNETIC RESONANCE IN THE DIAGNOSIS OF CANCER*

Šentjurc M., M. Schara
Auersperg M., R. Golouh, J. Lamovec

Sadržaj: Da su autori mogli ustanoviti značaj magnetskih resonancija u dijagnostici rakastih tvorevina, merili su spektre elektronske paramagnetske resonancije (EPR), i relaksaciono vreme spin-mreža T_1 za protone vode. Merenja su vršili na seriji pacijenata sa različitim patološkim promenama štitnjače, limfnih žlezda, i sa malignim melanomima.. Prema zapažanjima, autori mogli su zaključiti da su tkiva, u kojima je T_1 veći od 700 msec, sumljiva na rakastu tvorevinu. Istovremeno i tkiva u kojima su nadjene nekroze, pokazuju vrednost T_1 iznad 700 msec, a sa druge strane opet se vrednosti T_1 papilarnih karcinoma štitajače ne razlikuju od vrednosti za nemaligna tkiva.
Pošto se nekroze mogu lako izdvojiti na osnovu histoloških ispitivanja, njihove visoke vrednosti za T_1 ne smanjuju korisnosti metode merenja protonskog spin-mrežnog relaksacionog vremena za karakterizaciju rakastih tvorevina. Ova metoda mogla bi se verovatno upotrebiti za intraoperativnu dijagnostiku, jer su merenja brža od metoda intraoperativne histološke dijagnostike, i ne zavisne od ličnog iskustva.

UDK 616-006.6-07:539.143.43(497.1)

Deskriptori: diagnostika raka, elektronska paramagnetska resonanca (spektri) štitasta žlezda, limfna žlezda, maligni melanom.

Radiol. Iugosl., 4; 319—327, 1974

Introduction. — Recently, there have been several attempts to characterize and specify tissue samples according to their malignancy with magnetic resonances.

By electron paramagnetic resonance methods (EPR) it is possible to follow the changes in the type and concentration of the native paramagnetic centres and the free radicals in tissues (1, 2, 3). These centres are intermediates of the metabolic processes or are bound to the tissue structures. Their nature is only partially known and can change during the neoplastic growth. The changes in the concentration of these centres as well as the appearance of some new centres have already been reported for some malignant tissues (3).

Pulsed NMR technique is used to determine the proton relaxation times that depend on the paramagnetic centres concentration, water content and structural distribution of water molecules in tissues. They reveal the structure of the water molecules environment and the dynamics of the molecular motions. Spin lattice relaxation time T_1 and spin-spin relaxation time T_2 have been found to be longer for malignant than for normal tissues (4, 5). It was also found that T_1 is prolonged not only in the cancerous tissue but in all other tissues of the animals with tumourous growth (6).

Considering all these findings, we have tried to estimate if the increased T_1 is specific for malignant growth or can also be observed in some other pathologically changed tissues, benign tumours, inflammations etc. At the same time we have tried to examine the applicability of the magnetic resonance methods for the diagnosis of cancer. In our previous work (7) measurements have been per-

* This work was supported by the »B. Kidrič« Foundation.

formed on a series of patients with different thyroid gland diseases. Increased T_1 values, above 700 msec, were found for malignant thyroid gland tissue in comparison with the other pathologically changed thyroid tissues, where the T_1 values were bellow 700 msec.

In this work we have tried to extend our observations to some other malignant tumours and increase the series of measurements on the thyroid gland tissue in order to obtain better statistics.

Experimental. — The EPR spectra and proton spin-lattice relaxation time T_1 were performed on the thyroid gland tissue in the series of 47 patients with different thyroid gland diseases, on lymph nodes in the series of 10 patients with different primary tumours and on malignant melanoma — 6 cases. In addition, measurements on one malignant schwannoma, one fibrosarcoma and one breast cancer were performed.

Samples were taken from different parts of pathologic and macroscopically normal tissue and cut in two for magnetic resonances and histologic characterization.

About half an hour after the removal of the tissue, the samples for EPR were frozen in liquid nitrogen. The EPR spectra were taken at —160° C on an E-9 Varian spectrometer. The microwave power 100 mW and 1 mW was used in order to resolve free radicals from the other paramagnetic centres. Modulation frequency 100 kHz and the modulation amplitudes up to 10 gauss have been used. In this study we are only looking for the appearance of some new EPR lines or some distinct changes in the paramagnetic centres concentration, since only pronounced differences can be used for cancer diagnosis.

The intensity of EPR lines was compared to the ruby standard in a double microwave cavity. An equal quantity of tissue was used in all sample tubes.

About one hour after the removal of the tissue, the proton spin-lattice relaxation time was measured at room temperature on a pulsed 32 MHz NMR spectrometer IJS—2—72 with pulse sequence $\pi/2$—$\pi/2$. A retrospective comparison of the magnetic resonance data and definitive histologic diagnosis for the same samples were made.

Results. — A typical change between the EPR spectra of the malignant and nonmalignant thyroid gland tissue is presented in Fig. 1. The strong increase in the $g = 1{,}94$ centre concentration was observed in the cancerous thyroid gland tissue as well as in the lymph node metastases. This observation can well be explained by the histological diagnosis of isostructural malignant tissue in the lymph node and thyroid gland. The paramagnetic centre with $g = 1{,}94$ belongs to the reduced state of non-haeme iron protein (8), and coincides with the one previously found in the malignant thyroid gland tissue (9). Increased concentration of this complex is probably due to the anaerobic conditions sometimes observed in malignant tissues. It was shown, namely, that in tissues stored in an anaerobic atmosphere concentration of the $g = 1{,}94$ centre increases as the enzyme concentration in its reduced state increases (10).

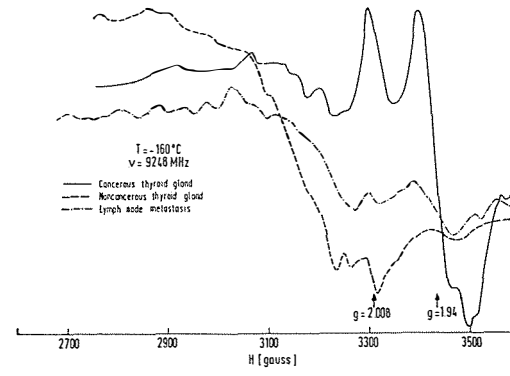

Fig. 1. EPR spectra of the thyroid gland tissue measured at —160° C

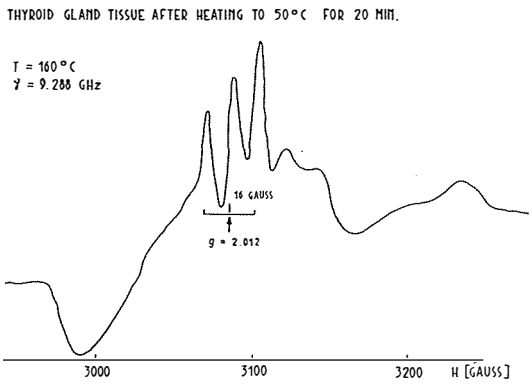

Fig. 2. EPR spectra of the triplet signal which appears during the warming of some tissues up to 50° C for 20 min.

It was also found that the formation of the triplet signal with g = 2,012 and hyperfine splitting constant of 16 gauss (Fig. 2), which appeared during the warming of the samples up to 50° C for 20 min, was more frequent in malignant than in non-malignant tissue. This signal is supposed to belong to NO-haeme iron protein complex (11).

From the experimental point, these two paramagnetic centres might be suitable for a rapid characterization of malignant tissue but were found to be poorly correlated with the histological findings as shown in Fig. 3. Here the intensity ratio between the centre with g = 1,94 and free radical, for different tissue samples are

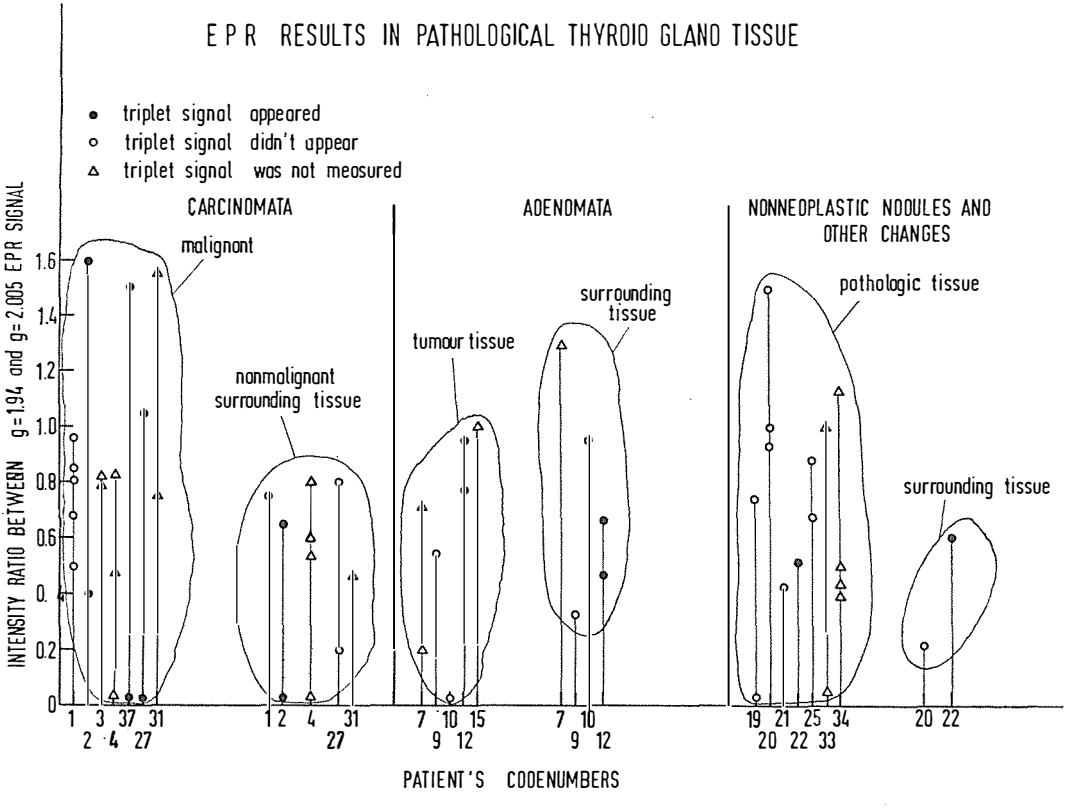

Fig. 3. Intensity ratio between the g = 1.94 paramagnetic centre and free radicals for the series of patients with different thyroid gland diseases

grouped according to their histologic diagnoses. The values corresponding to the samples with the same histologic diagnosis in the same patient are presented in one column. Since the samples were taken from different sites of the surgical specimen with different pathologic changes, the same patient's code number appears in more than one column. Samples with triplet signal are marked with full circles. No additional paramagnetic centres in the whole range from 0 to 4000 gauss have appeared in the malignant tissue as compared to the normal one.

Proton spin-lattice relaxation time of the thyroid gland tissue, malignant melanomas and lymph nodes are presented in Fig. 4 and 5, in the same way as in Fig. 2. The lymph nodes metastases, fibrosarcoma, breast cancer and some cases of malignant melanomas show T_1 values above 700 msec. These values are in accordance with our findings on malignant thyroid glands. In spite of the fact that T_1 values for the malignant samples from th e same patient are scattered, there were always at least some of them exceeding this value.

On the other hand, however, in almost all other cases with different pathological changes of thyroid gland: adenoma, reactive nodules, thyroiditis and hormonal hyperactivity, T_1 values never exceed 700 msec, with the exception of a few samples (no. 14, 16 and 45) which are characterised by other preoperative examinations as suspicious for malignancy. Therefore, tissue samples with T_1 values

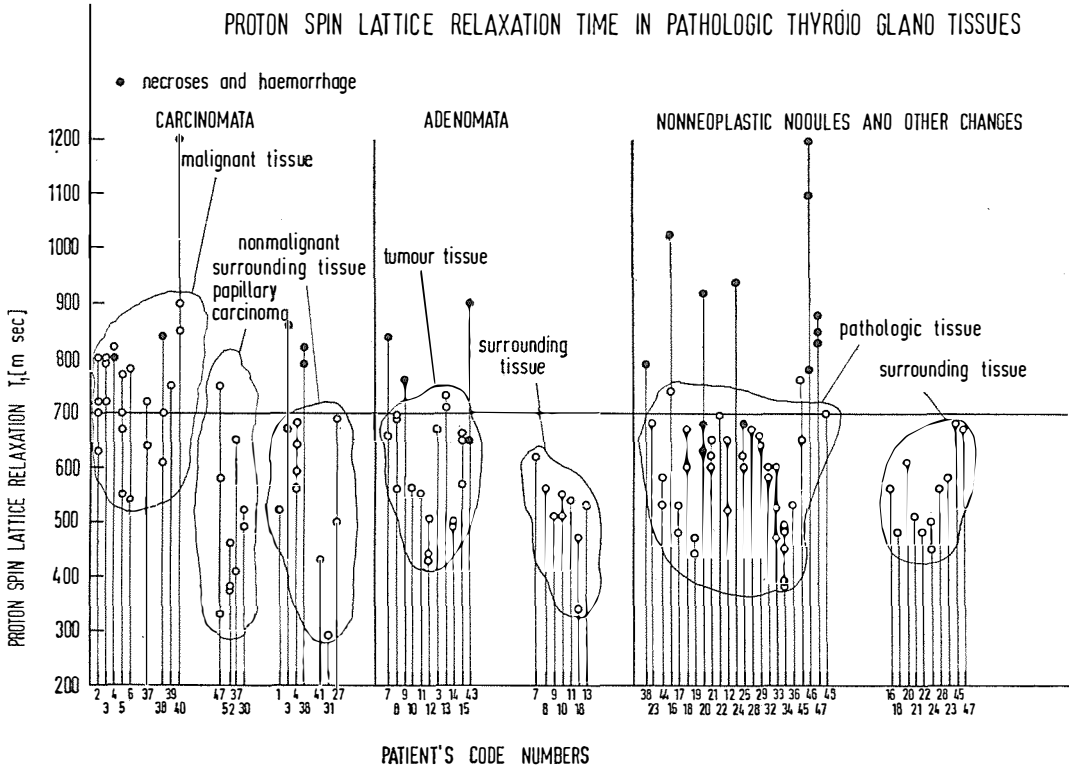

Fig. 4. Proton spin-lattice relaxation time of the thyroid gland tissue for the series of the patients with different thyroid gland diseases

above 700 msec should be considered suspicious for malignant growth.

Necroses and haemorrhages are exceptions which also show T_1 above 700 msec. These values are usually even higher than in malignant samples and even exceed 1000 msec. It has to be stressed at this point, that the critical value 700 msec is valid for T_1 measurements performed at resonant frequency 32 MHz. Recently, similar results were reported by Damadian (12, 13). Their measurements were done at resonant frequency 100 MHz. Since T_1 in the tissues increases with increased frequency (14), the values reported by Damadian are higher than ours.

However, there are some well differentiated carcinomas, like papillary thyroid gland carcinoma, where T_1 values are in the same region as in nonmalignant tissues. In Fig. 4 they are plotted in their own group since they behave specifically.

Another type of malignant tissue with T_{1x} below 700 msec are some cases of malignant melanoma. Low T_1 values for malignant melanoma were also reported in the already mentioned work (13). In our series of malignant melanoma, however, T_1 values are ranged from 350 to 1100 msec. In order to explain this scattering of results, specimens were carefully examined for the presence of necrotic areas. In Fig. 5 necrotic samples are marked with full circles. From Fig. 5 it can be seen that T_1 values in some malignant melanomas with no necrotic areas are high. Therefore, we have tried to determine the histologic grade of malignancy

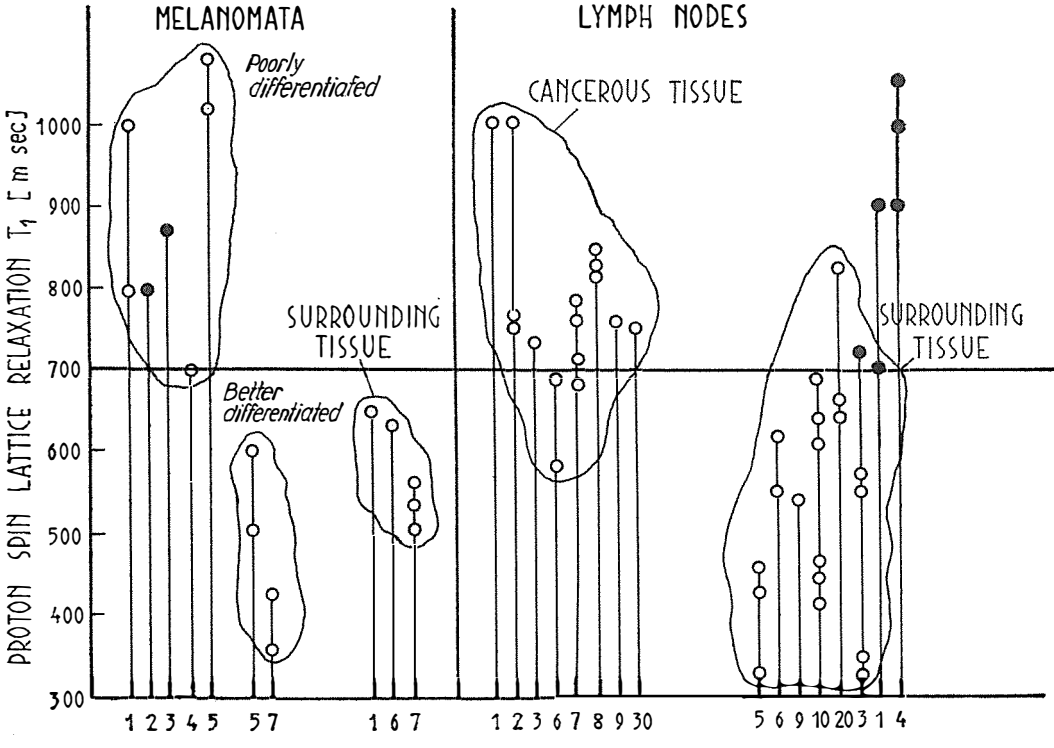

Fig. 5. Proton spin-lattice relaxation time of the lymph nodes and malignant melanoma. Necrotic samples are noted with full circles

and the amount of melanin in melanomas. It seems that well differentiated melanomas have lower T_1 values as undifferentiated types (Fig. 5). At the same time the amount of melanin was higher in the cases with low T_1 values. Both before mentioned characteristics of melanoma could be the reason for decreased T_1 values. The series of 6 cases studied in this work is too small to allow any definite conslusion. Therefore, further studies are in progress.

Discussion. — There are two important questions to be answered: what is the reason for T_1 prolongation in some pathologic tissues and why this prolongation is most pronounced for malignant tissues and necroses.

For the time being, prolongation of the proton spin-lattice relaxation time is still a matter of discussion concerning the relaxation mechanisms in tissues, and only by explaining this part, reliable conclusions could be made about the changes of the intra and extracellular water in malignant tissues. Now the model is being used according to which separation of water protons in two fractions is supposed — those which relax fast, the so called modified water protons which are influenced by protein surfaces and ions dissolved in intra and extracellular water, and protons of free water which are not influenced that way. Therefore, their relaxation time is longer. Assuming the exchange of molecules between the two fractions of water is fast; the relaxation rate $1/T_1$ is:

$$1/T_1 = x/T_1 \text{ (mod)} + (1—x)/T_1 \text{ (free)},$$

where x is a fraction of modified water. Using this model, some possibilities arise for T_1 prolongation in malignant and necrotic cells.

1. The ratio of free to modified water molecules alters at the constant amount of total water. Damadian suggested that the prolongation of T_1 could be due to the increased potassium content in malignant tissues. He found that increased K^+ ion concentration can imply increase in the amount of free water in the tissue (15).

2. The total amount of water content increases in malignant cells in favour to the free fraction. It was shown that the prolongation of spin-lattice relaxation time is correlated to an increased water content in tissue (16). The longest T_1 was found in nondifferentiated tissues.

3. The lowering of the paramagnetic centre concentration in malignant cells can result in an increase in T_1 value. This question arose several times (17) but has never been sistematically examined. There are many paramagnetic centres which are not observable by EPR method, and their role on the proton relaxation is not easy to estimate.

4. Nonspecific changes, diminishing of the O_2 and glucose concentration in malignant cells, lowering of pH value down to 6 and other changes could also influence spin-lattice relaxation (18).

None of these factors was completely proved or omitted until now, but the assumption 1. together with 2. seems to be the most probable.

In order to discover, how different factors influence spin lattice relaxation times and EPR data, we have made several experiments on the rat liver tissue homogenate. In these experiments we studied the influence of the animal diet (19), the oxygen content in the atmosphere, where the tissue was stored after removal, and the age of the animal. At the same time we also measured how the time between the removal of the tissue and measurements influences T_1 values and EPR spetra of the rat liver tissue. Significant changes in free radical concentration and T_1 values were found about two hours after the removal of the tissue (10, 19). Therefore, they could not appreciably influence the results of our measurements.

In an experiment with animal diet the rats were fed on avitaminous diet whereas in another one the carcinogen dimethylamino-azobenzene was added to the avitaminous diet. Both diets did not influence T_1 values of the rat liver tissue appreciably. The changes were about 50 msec. On the EPR spectra of the rat liver, however, the increase of the centre at $g = 2{,}03$ was observed. This paramagnetic centre was ascribed to the nitroxy-iron protein complex (20). In a similar experiment with the rats, which were on the diet with different carcinogens, the same centre was found at $g = 2{,}03$ (21). It was supposed to have been a potential tool for an early diagnosis of cancer. In our experiment this signal appears in the liver tissue of the rat fed on the diet containing dimenthylamino-azobenzene and in the liver of the rats fed only on avitaminous diet. Therefore the signal at $g = 2{,}03$ was considered as nonspecific for malignant growth.

In the experiments concerning the influence of the oxygen concentration in the storage atmosphere, an increased concentration of the $g = 1.94$ centre was found in the rat liver tissue when the tissue was in the nitrogen atmosphere (10). In the same samples no changes in the spin-lattice relaxation time were detected.

In figures 6 and 7 the paramagnetic centre concentration and the proton spin-lattice relaxation time in the rat liver and muscle tissue are presented as a function of the animal age. The paramagnetic centre concentration was found to increase up to the third month of the rat's life. This is the time in which the animal developes and the enzymatic activity reaches the optimal value. After this period, the paramagnetic centre concentration remaines unchanged.

T_1 decreases in the first month after the birth, which is in accordance with the intracellular water content decrease in the same period (22). After this time T_1 remains independent of the animal age.

These experiments have proved that T_1 values are influenced mostly by the water content variations in tissue while other influences are not so important. On the other hand, it was shown that the same effects do influence the paramagnetic centre concentration. Taking this into account, the scattered results in Fig. 3 can be better understood.

In addition, from the comparison of our EPR and NMR results obtained on the same tissues (see for example Fig. 6 and 7 and ref. 19) we can concluded that the changes of the paramagnetic

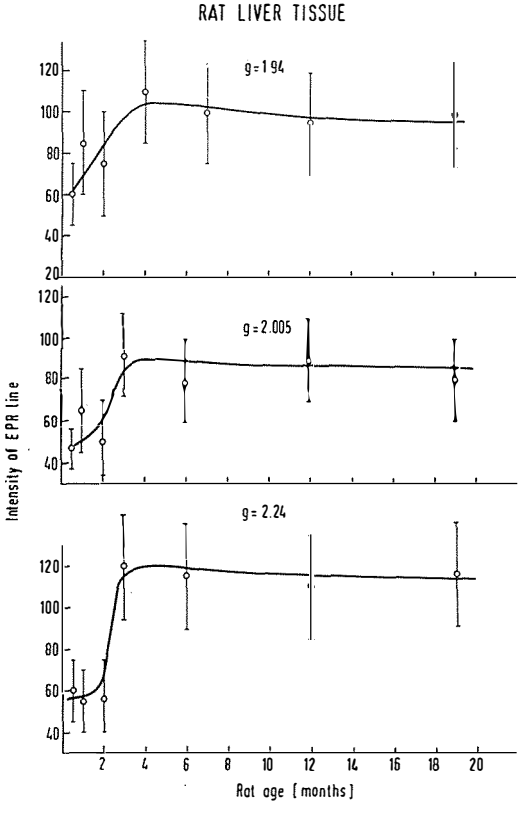

Fig. 6. Paramagnetic centre concentration of the rat liver tissue as a function of the animal age

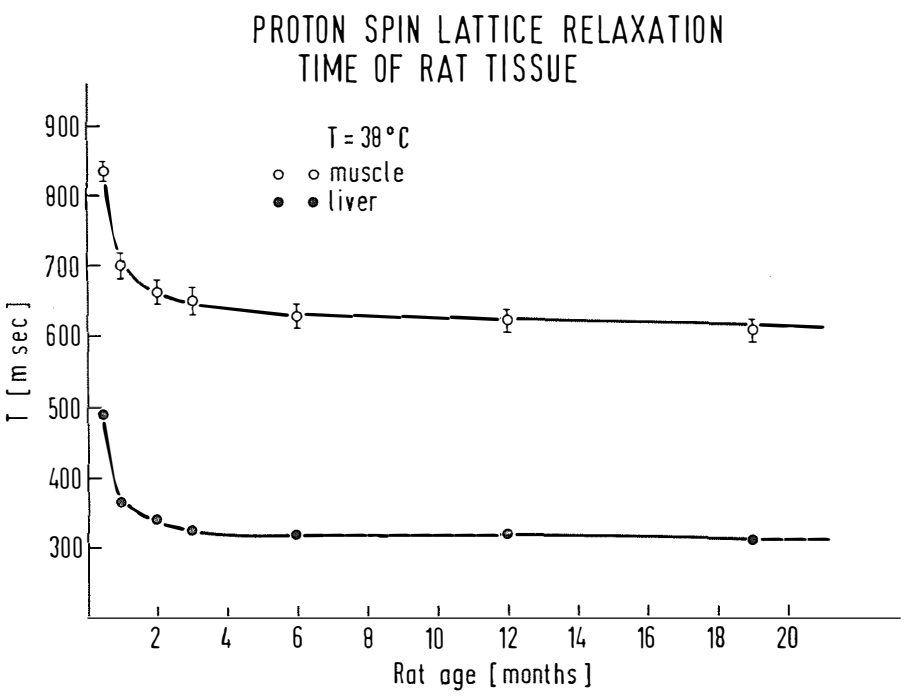

Fig. 7. Proton spin-lattice relaxation time of rat liver and muscle tissue as a function of animal age

centres concentration which were observed by EPR are not influencing proton relaxation.

Conclusion. — It was found that proton spin-lattice relaxation time in almost all malignant tissue we had examined, exceeded the value of 700 msec if measurements were performed at the resonance frequency of 32 MHz. All the other pathologic changes as well as normal tissue provided T_1 below this value. We believe that the proton spin-lattice relaxation measurements can be helpful not only in thyroid gland intraoperative diagnosis, where frozen section technique is often inconclusive, but also in the diagnosis of other malignancies, since it is much faster (about 2 min) and requires considerably smaller samples as frozen section technique. Their interpretation is not dependent on personal experience as the histological findings are.

T_1 measurements are inconclusive in a few cases of well differentiated carcinomas which provide T_1 values in the same region as nonmalignant samples. On the other hand, T_1 values in the necrotic tissues exceed 700 msec.

Since necroses and haemorrhages can be easily characterized by histologic examinations, the usefulness of T_1 measurements for the characterization of malignant growth has been not diminished.

In further work it would be worthwhile to find out if the same critical value, i. e. 700 msec, for spin-lattice relaxation time is valid for other malignant growth also, and the usefulness of EPR measurements has to be considered for other malignant tissues. A correct inter-

pretation of T_1 prolongation in malignant tissues has still to be found in order to contribute to the understanding of the basic molecular mechanisms of malignant growth.

Abstract. — Electron paramagnetic resonance spectra and the proton spin-lattice relaxation time T_1 have been measured on a series of pathologically changed human thyroid glands, lymph nodes and malignant melanomas. The T_1 values above 700 msec seem to be reliable indicators of malignancy. Necrotic tissues, however, can likewise yield T_1 values exceeding 700 msec. On the other side the T_1 value of the highly differentiated papilary carcinoma of the thyroid gland closely resembles that of the nonmalignant thyroid gland tissue.

The obtained EPR data were found to be inconclusive for characterization of thyroid malignancy.

References

1. Commoner B., J. Townsend, G. E. Pake: Nature *174,* 689 (1954).
2. Mallard J. R., M. Kent: Phys. Med. Biol. *14,* 373 (1969).
3. Swartz H. M.: In Advances in Cancer Research. Eds. Klein G. and S. Weinhause, *15,* 227 (1972). Academic Press, New York.
4. Damadian R.: Science *19* III, 1151 (1971).
5. Hazelwood C. F. et al.: Proc. Nat. Acad. Sci. USA *69,* 1478 (1970).
6. Frey H. E. et al.: J. Nat. Cancer Inst. *49,* 903 (1972).
7. Schara M. et al.: Br. J. Cancer *29,* 483 (1974).
8. Johnson C. E. et al.: Proc. Nat. Acad. Sci. USA *63,* 1234 (1969).
9. Šentjurc M., M. Schara, F. Lukič: Naturwissenschaften *57,* 459 (1970).
10. Schara M., M. Šentjurc, T. Koželj: J. Mag. Res. *6,* 628 (1972).
11. Maruyama T. et al.: Cancer Res. *31,* 179 (1971).
12. Damadian R.: In Ann. N. Y. Acad. Sci., Eds. Lasker S. E. and P. Milvy, *222,* (1973).
13. Damadian R.: Proc. Nat. Acad. Sci. USA *71,* 1471 (1974).
14. Outhred R. K., E. P. George: Biophys. J. *13,* 83 (1973).
15. Damadian R.: In Ann. N. Y. Acad. Sci., Ed. Hazelwood C. F. *204,* 211 (1973).
16. Inch W. R. et al.: J. Nat. Cancer Inst. *52,* 353 (1974).
17. Finch E. D., J. F. Harmon and B. H. Müller: Arch. Biochem. Biophys. *149,* 299 (1971).
18. Shapot V. S.: In Advances in Cancer Research Eds. Klein G. and S. Wienhause *15,* 253 (1972), Academic Press, New York.
19. Šentjurc M. et al.: IVth International Biophys. Congress (Moscow 1972).
20. Vavin A. F., L. A. Blumenfeld, A. G. Četvernikov: Biofizika *12,* 1967 (1967).
21. Vithayathil, A. J., J. L. Ternberg and B. Commoner: Nature *207,* 11246 (1965).
22. Hazelwood C. F.: In Ann. N. Y. Acad. Sci., Ed. Hazelwood C. F. *204,* 593 (1973).

Adresa autora: mgr. ing. Marjeta Šentjurc, Institute J. Stefan, Univerza v Ljubljani, 61001 Ljubljana, Jamova 39, P. O. B. 199, Yugoslavia.