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Low Paramagnetic-Ion Content in Cancer Cells: Its Significance in Cancer Detection by Magnetic Resonance Imaging

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Abstract: In previous publications, one of us demonstrated that variation in paramagnetic-ion contents is a major contributing factor to the different NMR relaxation times, T_1 and T2, of water protons among normal mouse tissues; and between normal tissues and cancer cells. The nature of the paramagnetic ions involved was not determined.

In the present communication, we report results of analysis of the contents of three biologically prominent paramagnetic ions (manganese, iron and copper) in 9 normal mouse tissues (brain, heart, small intestine, kidney, liver, lung, voluntary muscle, spleen and stomach); one strain of rat cancer cells (As-30, rat hepatoma); and 6 strains of mouse cancer cells (Ehrlich mammary adenocarcinoma, LSA lymphoma, Krebs carcinoma of the inguinal region; sarcoma 180; Klein TA3 mammary adenocarcinoma; P815 mast cell leukemia).

Our data indicate that manganese and iron are by far the two most important paramagnetic ions contributing to the diversity of NMR relaxation times. The average manganese content of all the normal mouse tissues studied (29.6 ± 4.99 μmole/kg) is 24 times higher than the average manganese contents of all the cancer cells studied (1.22 ± 0.27 μmoles/kg) and there is essentially no overlap between the two groups of data. The average iron content of the normal mouse tissues (281.6 ± 51.2 μmoles/kg) is 4 times the average in cancer cells (66.7 ± 7.74 μmoles/kg) but there is some overlap here. The observed differences in both the manganese and iron contents are statistically highly significant, with P's below 0.0001.

The copper contents of the cancer cells is lower than the average of normal mouse tissues but only by some 20%. The difference is statistically insignificant at the 0.05 level but significant at the 0.2 level.

THE DISCOVERY OF LONGER NMR RELAXATION TIMES, T_1 and T_2, of water protons in malignant tumors than those in normal tissues led to the development of a new, noninvasive way of detecting cancer and other diseases: magnetic resonance imaging, or

MRI (Damadian, 1971, 1972). Already a powerful diagnostic tool, future developments of new capabilities of MRI requires deeper understanding of the physiological basis of what a radiologist sees on the MRI screen: images reflecting primarily the density as well as the T_1 and T_2 of water protons in living cells.

To further our understanding of the physiological basis of MRI, we must continue to search for more accurate answers to the key question: "Why do water protons in cancer cells exhibit longer T1 and T2 than those of most normal cells?"

The first answer cited by Damadian (1971) was that of Szent-Gyorgyi (1957, footnote on p. 136): "cancer (has) less water structure". However, Szent-Gyorgyi offered no specific theory of water structure in living cells beyond that it is more like "liquid ice" (loc cit. p. 37), nor ***how*** water in living cells has become like "liquid ice."

In contrast, the polarized multilayer (PM) theory of cell water (Ling, 1965, 1972) offers an explicit suggestion on both how and why cell water assumes a dynamic structure different from normal liquid water: the bulk of cell water exists ***aspolarizedrnultilayers*** (Ling, 1965; 1972), in consequence of interaction of the water molecules with a matrix of fully extended protein chains in the cells. In this theory, the ***intensity of water polarization*** is not a constant, but varies among different cellular and subcellular systems and varies with time. Within this theoretical framework, one deduces naturally: water in cancer cells exhibits longer T1 and T2, because (among other reasons, see below) water in cancer cells is ***less intensely polarized*** than water in most adult normal cells.

Other investigators offered a different interpretation. In their view, the different T_1 and T_2 of water protons in normal and in cancer cells are simply, and exclusively the consequences of the *different* (extracellular and intracellular) ***water contents*** of the tissues (Belton ***et al.,*** 1972; Kiricuta ***et al.***, 1973; Hollis ***et al.,*** 1973; Inch ***et al.,*** 1974; Fung ***et al.,*** 1974, 1975; Eggleston ***et al.,*** 1978; Kodama ***et al.,*** 1978). Though once popular, this view was soon challenged.

Ling and Tucker (1980) demonstrated that pure cancer cells with near-zero extracellular-space fluid content and with intracellular water contents made equal to those of normal tissues, retain their relatively longer T_1 and T2. This finding does not signify that variations of cell water contents have no influence on NMR relaxation times; it does. But only in a modest way. The variation of the cell-water contents accounts for no more than 5 to 15% of the observed T1 differences, even when the comparison was made between, on the one hand, the highly hydrated cancer cells, and on the other hand, three of the least hydrated normal mouse tissues: kidney, liver and spleen.

Having thus shown that variation in water contents is not the only cause of the observed differences in the NMR relaxation times of normal and cancer tissues, Ling and Tucker pointed out that there are three other potentially important causes for the observed T_1 and T_2 differences between normal and cancer cells:

1) **The amount and nature of cell proteins:** because cell proteins, and possibly a small amount of water tightly bound to the proteins, shorten T_1 and T_2 (for review, Cooke and Kuntz, 1974).

2) Different intensity in the polarization of cell water: As briefly mentioned earlier, although the bulk of water in ***all*** resting living cells assumes the dynamic structure of polarized multilayers, cancer cells have longer T_1 and T_2 because the intensity of polarization of cancer cell water is weaker when compared to that of water in most normal adult living cells.

3) **Different concentrations of paramagnetic ions:** Due to their unpaired electrons, paramagnetic ions in aqueous solutions hasten the relaxation of bulk-phase water protons, reducing T_1 and T_2 (Bloch et *al.,* 1946; Bloembergen *et al.,* 1948). Ling and Tucker pointed out that earlier rejections of the idea that paramagnetic-ion content plays a significant role in NMR relaxation times of water protons in living tissues was based on limited observations and not really justified (see also Lewa and Baczkowski, 1976 for a similar view expressed earlier).

Even before the publication of Ling and Tucker's paper, evidence had been gathering, confirming the earlier suggestion that water in cancer cells is less intensely polarized than in normal tissues. This completed work has not yet been published in full, but will be soon (Ling, 1984, p. 342,709; Ling and Fu, 1991; Ling et *al.,* 1991).

More recently, evidence has also been accumulating in support of another suggestion Ling and Tucker made in 1980: lower paramagnetic ion contents might offer another cause for their longer Ti and T_2 of water protons in cancer cells (Ling, 1983). In Ling's 1983 study, tissues and cells were incinerated in a muffle furnace. The solubilized ashes were dissolved in a 10.4% ovalbumin solution and their T_1 and T_2 measured. The data obtained showed that the ashes from all the normal tissues contained more materials which cause rapid NMR relaxation of water protons than ashes from all 6 highly malignant cancer cells studied.

Since all organic materials had been burnt away, only ions were preserved in the ashes. Ions known to be present in living tissues at non-trivial concentrations and possessing strong effects on NMR relaxation of water protons are the paramagnetic ions. This finding suggests that cancer cells of widely diverse tissue origins, all contain less paramagnetic ions than the normal mouse tissues studied.

There was one shortcoming in this set of incineration experiments: some of the NMR activity observed in the ash-extracts might be due to an artifact: The paramagnetic ions in the dissolved ashes might include those which in their natural state within the cells do not cause significant water proton relaxation. Thus, the 4 iron atoms contained in the cytoplasmic protein, hemoglobin, are so well shielded by the protein part of the molecule that they have no influence on the relaxation of the surrounding water protons. Yet after ashing and dissolution in water, the protein part of the hemoglobin is removed; the iron atoms, thus removed from the shielding influence of the protein, might have become effective in causing water proton relaxation. To test the significance of this defect in the original ashing procedure, Ling (1989) carried out some additional investigations.

Ling's newer studies showed that extracting the paramagnetic ions from normal tissues and cancer cells with a mixture of hot 15% trichloracetic acid (TCA) and 80 mM ethylene diamine tetracetic acid (EDTA) — which does not liberate iron from hemoglobin — produced similar results as those produced from ashing (see Discussion). The concurrence of these two sets of data show that liberation of shielded paramagnetic ion(s) plays only a minor part in the observed differences of the water proton relaxation times observed.

While both ashing and TCA-EDTA extraction have successfully demonstrated a key role of paramagnetic ions in the observed NMR relaxation times of living tissues, normal and cancerous, neither study could tell us *what* paramagnetic ion(s) are responsible for the shortened NMR relaxation times in the living cells. To answer this question, we carried out new studies to be described in the present report.

Materials and Methods

All normal tissues studied are from noninbred ICR mice from Ace Animals, Inc,. Boyerstown, PA. All cancer cells studied are pure, "maximally deviated" (Potter, 1961) cancer cells in ascites form. With the exception of one strain of rat hepatoma (AS-30) carried on Sprague-Dawley rats, all ascites cancer cells were carried on ICR mice unless otherwise indicated: Ehrlich (mammary adenocarcinoma); LSA (lymphoma) carried on CD1 mice; Krebs (carcinoma of the inguinal region); Sarcoma 180 (sarcoma); Klein TA3 (mammary adenocarcinoma); P815 (mast-cell leukemia) carried on DBA mice.

To avoid contamination, metallic instruments were avoided in isolating the tissues and in any other steps involving direct contact between the instrument and either the isolated tissues or their extracts. Glass knifes, for example, were used instead of metallic scalpels or scissors.

An hour before sacrifice, mice were injected subcutaneously with heparin (1 unit/gram of body weight). The animals were decapitated after etherization, and as much as possible of the blood was allowed to drain from the body in order to minimize the inclusion of blood in the isolated tissues. After isolation, the tissues were very briefly rinsed in an isotonic NaCl solution before blotting between sheets of ash-free filter paper to remove all adhering fluids. The tissue was then separated into two portions.

For the determination of the water content, one portion of the isolated (normal) tissue was weighed on aluminum weighing pans before and after drying at 102°C. The water contents were obtained from the weight losses in consequence of drying.

For ion analysis, the second portion of tissue was extracted by heating in a 10% solution of trichloracetic acid (TCA), following what was described as Extraction procedure A earlier reported (see Ling, 1989). Due to the extremely low concentrations of paramagnetic ions in the tissues, the volume of 10% TCA added to each sample must be kept at a minimum. Our final choice was a volume of TCA equal to 3 times the wet weight of the sample. After having been ground in a heavy-walled glass homogenizer (centrifuge) tube, the tissues-extract mixture was heated in the same homogenizer tube in a boiling water bath for 20 minutes. The top of the tube was covered by a glass marble during heating. Great care was exercised in not permitting the level of boiling water in the water bath to rise much higher than the level of TCA in the tubes; or else significant loss of water (vapor) might occur.

After cooling, the condensate on the inner wall was carefully washed down by tilting and twisting the tubes. With their tops firmly sealed with "Parafilm M", the tubes were centrifuged at about 1000 g for 5 minutes. The clear supernatant fluids were collected. For paramagnetic ion analysis, enough concentrated "universal buffer and extractive" (UBE) solution was added to the supernatant fluid to provide a final concentration of 97 mM LiCl and 3 mM of $NH_4H_2PO_4$. The same concentrations of these salts were included in all standards and the blank.

Since the normal tissues were not extensively washed in Ringer or other media free of paramagnetic ions, the isolated cancer cells were not washed either. Since the extractive procedure does not liberate iron from hemoglobin (Ling, 1989), the inadvertent inclusion of a small number of red cells would produce no significant error.

The ascites fluids were "harvested" from the mice as a rule, on the 8th or 9th days after inoculation. The cancer cells were separated in two stages from the serous fluid in which the cancer cells were suspended in their natural state. In the first stage, the cells were

separated from the bulk of the serous fluid by centrifugation in a 3 ml plastic syringe barrel fitted snugly through its nozzle to a plastic 0.5 ml microcentrifuge tube. This assembly was spun for 10 minutes in a horizontally spinning centrifuge at relatively low speed (ca 1000 g). In the second stage, the cancer cells collected in the microcentrifuge tubes in Stage 1 were spun down a second time at a higher speed (15,000g) for another 10 minutes in order to isolate the cells from the remaining serous fluid.

The supernatant fluid collected at the top of each microcentrifuge tube after the second spinning was taken up with a Pasteur pipette. The remaining drops of supernatant fluid were removed by suction. The cell pellet was then blown out of the microcentrifuge tube after the bottom tip of the microcentrifuge tube had been cut off. From here on, the cancer-cell pellets collected were handled in a similar manner as a piece of the isolated normal tissues, both in the assay of the water content and in the estimation of its paramagnetic-ion contents.

Using a Varian Atomic Absorption Spectrophotometer, Model AA 1475, we analyzed the three most abundant paramagnetic ions in living tissues: copper, iron and manganese.

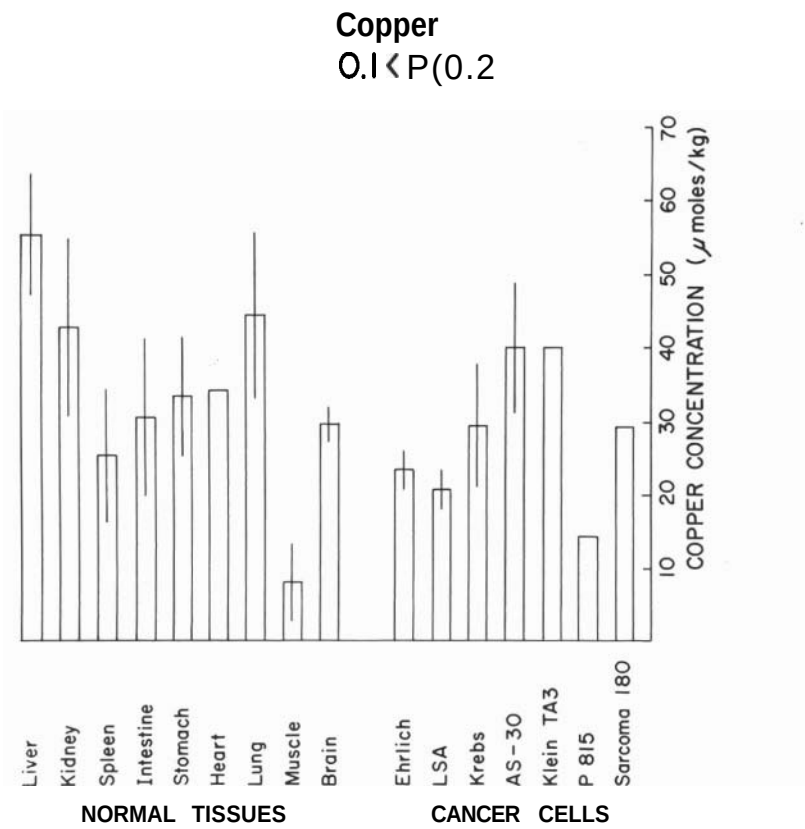

FIGURE 1. The copper contents of normal mouse tissues and maximally deviated cancer cells. For more details see Table I.

Results

Copper: Figure 1 shows the copper contents of 9 normal mouse tissues (liver, kidney, spleen, intestine, stomach, heart, lung, voluntary muscle, and brain) and 6 mouse cancer cells (Ehrlich, LSA, Krebs, Klein TA3, P815, Sarcoma 180) and one rat cancer, AS-30. The copper contents of individual assays, as well as the averages and standard errors of the means (S.E.) are presented in Table I. When the copper contents of the individual assays of all the normal tissues (36.2 ± 3.93 μmoles/kg.) are compared with the individual assays of all the cancer cells (28.6 ± 3.16 μmoles/kg.) the difference is of border-line significance. That is, there is no significance at the 0.05 level, but there is significance at the 0.2 level.

Iron: Figure 2 and Table II show the iron contents of the same groups of normal tissues and cancer cells. The difference between the iron contents of all the normal tissues (281.6

		Copper Contents (μmoles/kg. fresh weight)	
		Individual Assays	Mean ± S.E.
Normal Mouse Tissue	1 – Brain	28; 38; 26.5	27.4 ± 0.9 (3)
	2 – Heart	51; 20	35.5 (2)
	3 – Intestine	14.4; 18.9; 84; 24; 15.3; 26.1	30.4 ± 10.9 (6)
	4 – Kidney	15.6; 13.1; 51.4; 87; 69.8; 19.6	42.7 ± 12.8 (6)
	5 – Liver	25.8; 48.7; 79.5; 26.3; 46.4; 67.3; 95; 55	55.5 ± 8.5 (8)
	6 – Lung	29.2; 69.8; 34	44.3 ± 12.8 (3)
	7 – Muscle	12; 22; 0; 0	8.5 ± 5.3 (4)
	8 – Spleen	43.9; 32; 6.2; 19.2	25.3 ± 8.1 (4)
	9 – Stomach	6.66; 42; 43.3; 41.3	33.3 ± 8.9 (4)
	10 – Plasma	22.3	22.3 (1)
Cancer Cells	1 – AS-30	25; 40; 55	40.0 ± 8.7 (3)
	2 – Ehrlich	13; 35.3; 26.6; 19.1	23.5 ± 2.4 (4)
	3 – Krebs	21.8; 5.2; 21.8; 43; 56	29.5 ± 8.6 (5)
	4 – LSA	24.6; 15.2; 17.2	19.0 ± 2.9 (3)
	5 – P-815	14.5	14.5 (1)
	6 – Sarcoma 180	29.1	29.1 (1)
	7 – TA3	42.4	42.4 (1)

TABLE I. The copper contents of normal mouse tissues and maximally deviated cancer cells. Data are presented as mean ± standard error of the mean when 3 or more assays were made. Otherwise the data are presented as the mean or a single value. Number in parenthesis indicates the number of assays.

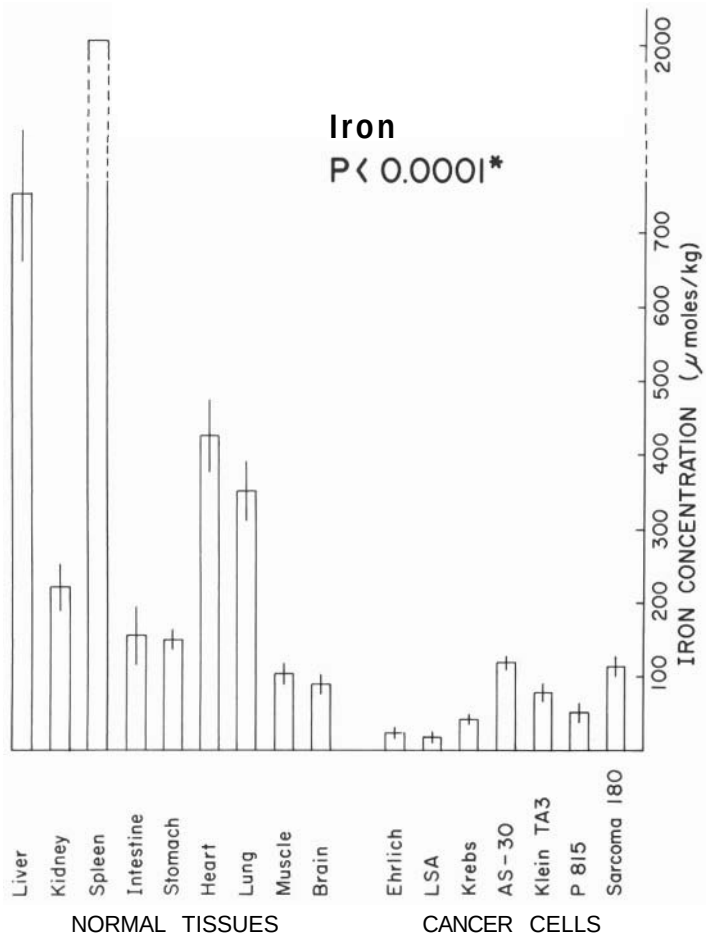

FIGURE 2. The iron contents of normal mouse tissues and maximally deviated cancer cells. For more details, see Table II. For meaning of asterisk, see text.

± 51.2 μmoles/kg.) and those of the cancer cells (66.7 ± 7.74 μmoles/kg.) is more prominent. Indeed when the iron contents of all the individual normal tissue assays are compared with those of the cancer cells, the difference is highly significant. P is less than 0.0001. In both the averaging and in the t test, one single extremely high value of iron for the normal mouse spleen (9384 μmoles/kg.) was excluded.

Manganese: Figure 3 and Table III show the manganese contents of the same collections of 9 normal mouse tissues, 6 mouse cancer and 1 rat cancer cells. There is a striking difference between the manganese contents of the normal tissues (29.6 ± 4.99 pmoleskg.) and those of the cancer cells (1.22 ± 0.27 pmoleskg.). When the manganese contents of the individual assays of the normal mouse organs are compared with those of the individual cancer cell assays, the difference is also statistically highly significant: P is also less than 0.0001.

Discussion

Lower Mn^{++} contents of cancer cells; its significance in cancer detection by MRI.

The variations in intensity in different parts of the MRI image reflects primarily the density, and the T_1 and T_2 of water protons in the specimen under examination. It is well known that the presence of micromolar concentrations of paramagnetic ions with large magnetic moments can exercise powerful influence on the T_1 and T_2 of water protons in an aqueous solution.

Among those paramagnetic ions known to be present in living tissues and also possess a large magnetic moment (5.9 Bohr magneton), is manganese.

There are reasons* to believe that most Mn^{++} in living cells exists (loosely) bound to cell proteins and/or other biomacromolecules. In combining with these biomacromolecules, the effect of Mn^{++} in causing water proton relaxation is further enhanced (Eisinger et al., 1961).

The average manganese content of 7 maximally deviated cancer cells studied (1.22 ± 0.27 μmoles/kg.) is only 1/24 of the average of that of 9 normal mouse tissues (29.6 ± 4.99 μmoles/kg.). Not only is there a large difference in the mean values, there is essentially no overlap of the two sets of data.

The effectiveness of the Mn^{++} in causing water proton relaxation in general and the consistent and striking difference between the Mn^{++} contents in cancer cells and in normal tissues leave no doubt that the lower Mn^{++} content of cancer cells is a major cause of the longer NMR relaxation times of water proton in cancer cells. For the same reason, the lower Mn^{++} content in cancer cells must play a significant role in cancer detection by MRI. Stated in more general terms, what one sees as lighter or darker MRI images may be, to a notable degree, due to the differences in the Mn^{++} contents of the various gatherings of cells being observed.

Lower iron content in cancer cells: Its significance in cancer detection by MRI.

The second paramagnetic ions that is found at significantly lower concentration in cancer cells than in normal tissues is iron.

The average iron content of 7 maximally deviated cancer cells (66.7 ± 7.74 μmoles/kg.) is about 1/4 of the average of normal mouse tissues (281.6 ± 51.2 μmoles/kg.). As pointed out under Results, the difference in iron content is statistically also highly significant even though there are some overlaps of the iron contents in the two groups. The interpretation of the iron-content data are complex and requires a careful analysis.

Iron in living cells exists in at least 4 forms: (1) free form (NMR active); (2) "small molecular weight form" (NMR active) include iron complexes to free amino acids and loosely bound to proteins (Jacobs, 1977; Mulligan and Linder, 1982); (3) heme proteins (NMR inactive) include hemoglobin, myoglobin, cytochrome C; (4) "storage iron", including water-soluble femtin, (inactive or very weakly NMR active, Ling, unpublished) and water-insoluble hemosiderin (Harrison et al., 1974). The NMR activity of hemosiderin is to

* Footnote: The level of Mn^{++} in mouse plasma is much lower than those in most cells and tissues studied (Table III). There are two types of explanations for the elevated level of a solute in the cells: an inward pumping mechanism (according to the membrane-pump theory) and selective adsorption on intracellular protein(s) and possibly other macromolecules, according to the association-induction hypothesis (Ling, 1962, 1984). Insufficient energy to cope with one postulated pump (the sodium pump) alone, makes further postulation of another manganese pump pointless. Selective adsorption of Mn^{++} seems to be a more reasonable explanation for the high levels of Mn^{++} in most living cells.

		Iron Content (μmoles/kg. fresh weight)	
		Individual Assays	Mean ± S.E.
Normal Mouse Tissue	1 – Brain	49.3; 12.5	49.3; 122.5 (2)
	2 – Heart	426	426 (1)
	3 – Intestine	129; 131; 201	154 ± 24 (3)
	4 – Kidney	218.5; 234.4; 206.2	220 ± 14.4 (3)
	5 – Liver	574; 693; 972	746 ± 188 (3)
	6 – Lung	384; 240; 415	346 ± 54 (3)
	7 – Muscle	74.1; 119; 155	114 ± 23.7 (3)
	8 – Spleen	9.38	9.38 (1)
	9 – Stomach	162; 133	162; 132.5 (2)
	10 – Plasma	47	47 (1)
Cancer Cells	1 – AS-30	168; 141; 96; 111; 74; 168; 126; 127	126 ± 11.7 (8)
	2 – Ehrlich	0.72; 0; 0; 45; 38; 28; 90	29.5 ± 12.6 (8)
	3 – Krebs	8; 88; 42; 64; 42; 78; 33	52.1 ± 9.6 (7)
	4 – LSA	0.5; 0; 47; 28; 33; 34	23.7 ± 7.8 (6)
	5 – P-815	66; 55	60.5
	6 – Sarcoma 180	133; 115; 124	124 ± 4.32 (3)
	7 – TA3	77; 110; 64; 92	85.8 ± 8.6 (4)

TABLE II. The iron contents of normal mouse tissues and maximally deviated cancer cells. See legend of Table I for explanations of details.

the best of our knowledge still unexplored. Being water-insoluble, it is probably NMR-inactive.

If femtin and hemosiderin are, like the heme iron, also essentially NMR-inactive and again like the heme proteins, their inaccessible iron is not liberated by hot TCA, then the only iron forms that are extracted by hot TCA are free iron and "small molecular weight form" iron. The high atomic weight of iron (55.85) and the triple positive electric charges it carries (and hence the expected high degree of hydration) make it virtually certain that the ***equilibrium distribution coefficient***, or ***q-value*** of free femc ion is low in the cell water. If so, then the only form of iron that contribute significantly to the T_1 and T_2 of living cells must be the so-called "small molecular weight form" iron. Indeed there are some data on hand which tend to support this view.

Mulligan and Linder (1982) estimated that the normal female rat liver contains 52 pg of "small molecular weight form" iron per gram of fresh liver. This is equivalent to about 1 mM. of iron in this form. Taking into consideration species differences, this level of "small molecular weight form" iron may be able to account for a lion's share, if not all of the iron extracted from mouse liver and reported in Table II (0.746 pmoles per kg.).

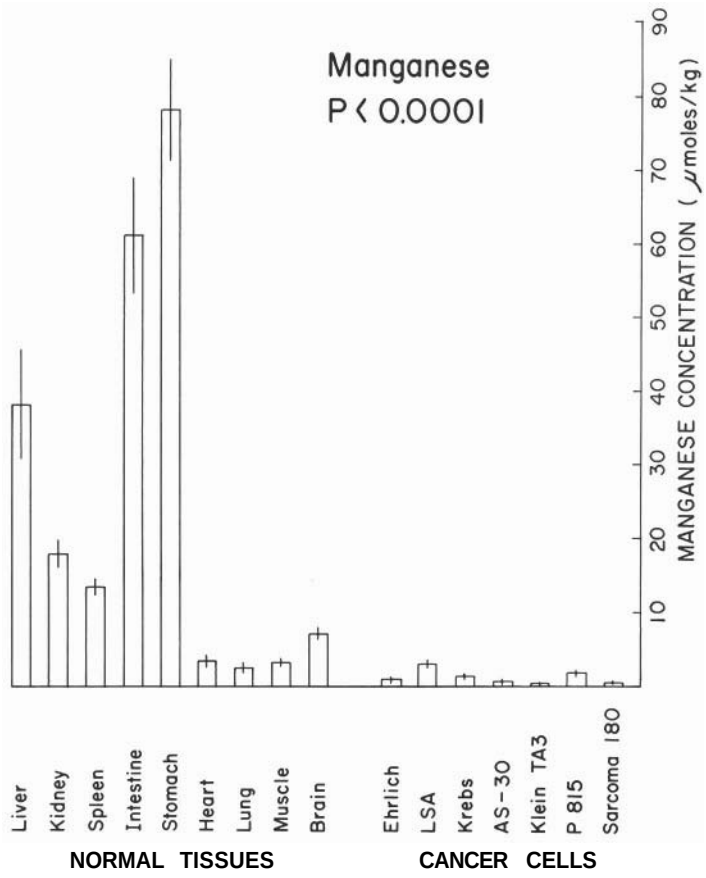

FIGURE 3. The manganese contents of normal mouse tissues and maximally deviated cancer cells. For more details, see Table III.

Thus in a round-about way we have come to the conclusion, tentative though it may be, that like manganese it is primarily the fraction of iron loosely bound to cell proteins that contributes to the diversity of NMR relaxation times of normal and cancer cells. Thus, the lower iron content in cancer cells is another major factor that has made possible cancer detection by MRI.

The significance of cellular copper contents in cancer detection.

The mean copper content of cancer cell (28.6 ± 3.16 μmoles/kg.) is lower than the mean copper contents of the normal mouse tissues studied (36.2 ± 3.93 μmoles/kg.) by some 20%. This difference is marginally significant or insignificant, depending on the level of statistical probability considered significant (see Results). The modest difference in the average copper contents between cancer and normal tissues is a far cry from the striking differences in manganese and iron contents.

		Manganese Contents (μmoles/kg. fresh weight)	
		Individual Assays	Mean ± S.E.
Normal Mouse Tissue	1 – Brain	6; 8.7	7.4 (2)
	2 – Heart	3.3	3.3 (1)
	3 – Intestine	54; 53; 36; 76.4	61.2 ± 7.6 (4)
	4 – Kidney	16; 16; 18.6; 18.2	17.2 ± 0. 7 (4)
	5 – Liver	37; 31; 54; 24; 45	38.2 ± 5.8 (5)
	6 – Lung	3.1; 3.3	3.2 (2)
	7 – Muscle	2; 3; 4.9	3.3 ± 0.9 (3)
	8 – Spleen	7.5; 8.9	8.2 (2)
	9 – Stomach	74; 90; 69	77.7 ± 6. 3 (3)
	10 – Plasma	0	0 (1)
Cancer Cells	1 – AS-30	0.1; 0; 0	0.03 ± 0.0 3 (3)
	2 – Ehrlich	0; 1.7; 2.3; 0; 0	0.8 ± 0.5 (5)
	3 – Krebs	2.3; 1.1; 1.5; 1.8	1.7 ± 0.25 (4)
	4 – LSA	4.6; 2; 2	2.8 ± 0.9 (3)
	5 – P-815	1.8	1.8 (1)
	6 – Sarcoma 180	0	0 (1)
	7 – TA3	1.2; 0.9	1.1 (2)

TABLE III. The manganese contents of normal mouse tissues and of maximally deviated cancer cells. See legend of Table I for explanation of details.

While the overall differences of copper contents between the two groups of (maximally deviated cancer cells vs. normal tissues) may be insignificant, this does not mean that the copper content of a specific cancer in comparison with that of the specific normal cell type from which this particular cancer cells originate from, is also of no significance. On the contrary, a good correlation was observed between the copper (and iron) contents of squamous cell carcinoma and epidermal tissues on one hand and their respective NMR line widths on the other (The NMR linewidth is a function of T2) (Block, 1973).

In this connection, it is also of interest to recall that 44 years ago, Carruthers and Suntzeff (1945) found that just after one single application of methylcholanthrene to the mouse skin both the copper and iron contents of the skin fell by 50%. The eventual transformation from later hyperplastic stage of the skin to carcinoma entailed a further drop of 30% for copper, but not for iron.

General comment on the multiple causes of T_1 and T_2 differences and the significance for the future development of MRI.

In the conventional X-ray radiographic and computed tomogram images, the formation of an image depends simply upon the varied electron density of the different part of the

specimen examined. This simplicity makes interpretation of the X-ray-based data easy; it also forecasts a limited future.

In contrast, the intensity of MRI images depends on a multitude of parameters, including the density as well as the T_1 and T_2 of the protons of the most abundant component of all living body parts, water.

The T_1 and T_2 of cell water protons, in turn, are influenced by the cell proteins, the cell water and the paramagnetic ions, as either briefly mentioned or more lengthily discussed above.

Therefore, MRI mirrors and thus possesses the inherent ability of informing us on a variety of key parameters whose interplay constitutes life. This complexity in the causes of the magnetic image intensity makes in depth and precise interpretations of the MRI pictures difficult at the present moment. By the same token, the great potential diagnostic insights that MRI can provide about health and disease, are also far-reaching.

However, the expansive uses of MRI is at present no more than a vision, a dream. It will come one day. But only after we have achieved a much higher degree of understanding of how living cells really function and malfunction. MRI can then be further engineered to tell about them in exact terms.

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That different paramagnetic-ion contents contribute to the diferences in the NMR relaxation time, Ti, of water protons in different normal tissues (Ling, 1983, ***Physiol. Chem. Phys. & Med. NMR, 15:***505), and that lower paramagnetic ion contents of cancer cells contribute to the longer Ti and T_2 of water protons in cancer cells than those in normal tissues (Ling, 1988, 1989) received recent confimations also from Negendank et ***al.*** in "Evidence for a contribution of parasmagnetic ions to water proton relaxation times in normal and malignant mouse tissues," ***Soc. Magn. Reson. in Medicine*** 1988, p. ***572,*** (though these authors did not acknowledge the prior work cited above which they confirmed). {See also Renade in "Paramagnetic metal contents and water proton spin-lattice relaxation time in tissues" which appears also in this issue of ***PCP & Med. NMR*** (Vol. ***22,*** No. 1).]