References and Notes

1. J. L. Starr and B. H. Sells, *Physiol. Rev.* **49**, 623 (1969); E. Borek and P. R. Srinivasan, *Annu. Rev. Biochem.* **35**, 275 (1966); P. R. Srinivasan and E. Borek, *Progr. Nucleic Acid Res.* **5**, 157 (1966).
2. D. B. Dunn and J. D. Smith, *Nature* **175**, 336 (1955); J. W. Littlefield and D. B. Dunn, *Biochem. J.* **70**, 642 (1958); J. T. Wachsman and V. Irwin, *J. Bacteriol.* **104**, 814 (1970); B. F. Vanyushin, S. G. Tkacheva, A. N. Belozersky, *Nature* **225**, 948 (1970).
3. S. M. Beiser and B. F. Erlanger, *Cancer Res.* **26**, 2012 (1966).
4. B. F. Erlanger and S. M. Beiser, *Proc. Natl. Acad. Sci. U.S.* **52**, 68 (1964).
5. P. Hackett and P. Hanawalt, *Biochim. Biophys. Acta* **123**, 356 (1966).
6. D. B. Dunn and J. D. Smith, *Biochem. J.* **68**, 627 (1958); E. Theil and S. Zamenhof, *J. Biol. Chem.* **238**, 3058 (1963).
7. A. Yudelevich and M. Gold, *J. Mol. Biol.* **40**, 77 (1969).

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Microwave Absorption by Normal and Tumor Cells

Abstract. *Energy levels exist in mammalian cells which result in the absorption of microwaves between 66 and 76 gigahertz. Many of these energy levels occur when water molecules associate with the various chemical groups of macromolecules. The absorption spectra of cells between 66 and 76 gigahertz, therefore, is determined by the structure of in vivo water lattices, and these seem to reflect indirectly the structural makeup of macromolecules or macromolecular complexes. Tumor cells absorb 66-, 68-, and 70-gigahertz microwaves less strongly and 69-, 72-, and 75-gigahertz microwaves more strongly than normal cells. These differences in the strength of attenuation at each frequency suggest that either the ratio of RNA to DNA or the relative number of certain types of chemical groups in tumor cells is different from that in normal cells.*

Microwave spectrometry has been used to measure the water content of cells as part of investigations into the physiological and genetic role of those bound water molecules which form an integral part of in vivo macromolecular complexes. The use of the apparatus, however, revealed that certain microwave frequencies interfered with the growth of microbial cells (*1*) and inhibited or stimulated the biosynthesis of some macromolecules. In addition, the particular metabolic process affected and the manner in which it was changed was found to be frequency-dependent (*2*). Later, when the absorption spectra of various genera of bacteria were examined, small differences in their spectra were observed. In order to follow up these observations we have conducted investigations into the attenuation of microwaves by different kinds of mammalian cells.

The microwave apparatus we used, which allowed reflection and attenuation to be measured, consisted of a DX151 Klystron coupled with a modified TRG millimeter microwave system, model 6100 (TRG, Boston) and three Tektronix type 502A dual-beam oscilloscopes. The Klystron was operated at 2500 volts, which gave an output of 20 to 103 μwave, depending on the frequency used. The horns of the 6100 system were replaced by a specially designed sample compartment, similar to a sliding short circuit. This was constructed of two pieces of wave guide between which was a slide containing two mica cells of the same dimensions as the wave guide and 1 mm thick. Each mica cell could be located and mated into the total wave-guide system with a loss of less than 0.2 db. By means of hypodermic needles, air or nitrogen of a given relative humidity could be passed through the cells to maintain a given level of water absorbed by the agents studied, including mammalian cells and biochemicals. In addition, as an extra safeguard against sources of error due to the possible setting up of standing waves, a second variable attenuator was placed between the sample cell component and the crystal detector.

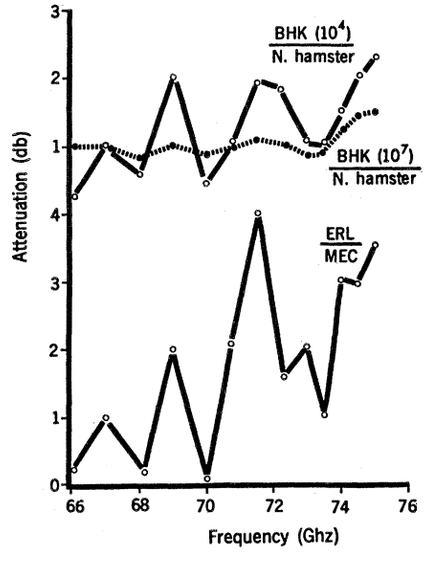
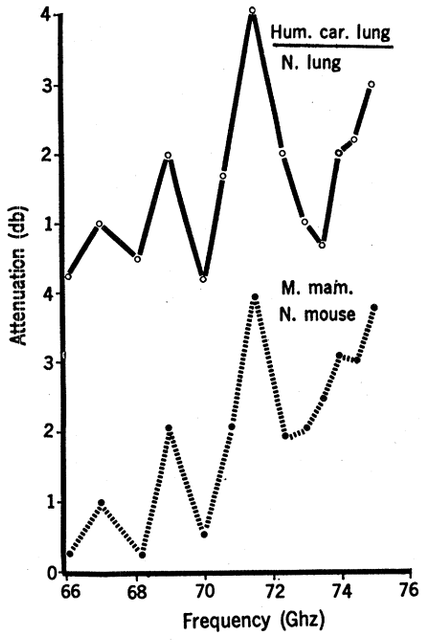


Fig. 1. The differential microwave absorption spectra of tumor and normal cells grown in tissue culture. Spectra presented as *attenuation of tumor* cells with attenuation at 67 as unity. *BHK*, baby hamster kidney cells transformed with mouse sarcoma virus; *N. hamster*, normal hamster kidney cells; *ERL*, Ehrlich ascites cells; and *MEC*, mouse embryo cells. Numbers in parentheses are the minimal numbers of transformed cells of the cell lines used (inoculated per animal) required to produce a tumor.

The tissue culture cell lines used were: baby hamster kidney cells transformed with mouse sarcoma virus (BHK), secondary tissue cultures of normal hamster kidney and mouse embryo cells, and Ehrlich ascites cells. The Ehrlich cells were cultured by transplantation into mice and collected from the ascites fluid after the development of tumors. The other cell lines were grown in tissue culture, washed in Hanks basic salt solution, and sedimented into a pellet by centrifugation. Subsequently, the cells were placed onto one of the mica cells, and their microwave absorption spectrum was determined by using the second cell as a control. In addition, microtome slices of natural human and mouse carcinomas were examined along with sections of normal tissue. The fresh tissue slices and pellets of cells were kindly supplied to us by Dr. R. Bather of our Cancer Research Unit and were examined immediately upon their receipt. No fixation processes were used in these investigations. In later experiments the absorption spectra of many different biochemicals were determined. Initially all spectra were ascertained with the cells or biochemicals held at 90 percent relative humidity and 25°C. However, in some cases the effect of change in relative humidity on the absorption spectrum was determined. In order to compare the spectra obtained from sample to sample of the cells, the attenuation at each frequency was standardized, the attenuation of 67 Ghz being used as unity. In this way error due to possible small differences in the thickness of the films was avoided.

In Fig. 1 the difference is shown in the absorption spectrum between (i) BHK tumor cells and normal hamster cells and (ii) Ehrlich ascites cells and normal mouse embryo cells. In both cases the tumor cells showed a decreased attenuation at 66, 68, and 70 Ghz but increases in attenuation at 69,



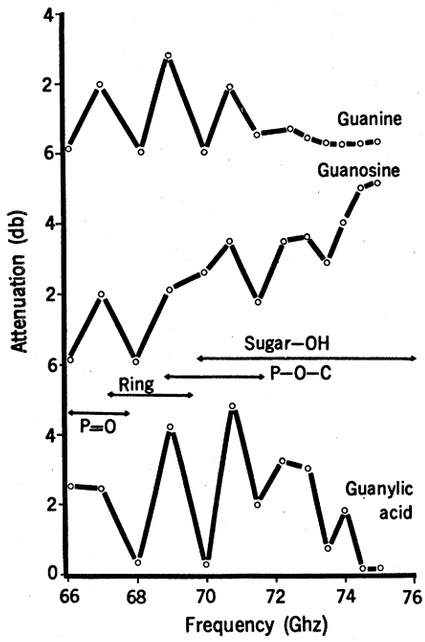


Fig. 2 (left). The differential microwave absorption spectra of tumor and normal tissue slices. *Hum. car. lung*, tissue slices from human lung carcinoma; *N. lung*, tissue slices of normal lung; *M. mam.*, tissue slices of mouse mammary carcinoma; and *N. mouse*, tissue slices of normal mouse mammary tissue. Spectra presented as for Fig. 1. Fig. 3 (right). The microwave absorption spectra at 90 percent relative humidity of guanine, guanosine, and guanylic acid.

72, and 75 Ghz. This same trend was observed in the difference spectra of tissue slices from human and mouse carcinoma and their respective normal tissues (Fig. 2).

In an effort to find reasons for the above differences in attenuation, the absorption spectra of a large number of biochemicals were determined and compared. One such series, for guanine, guanosine, and guanylic acid, is shown in Fig. 3. From this it can be seen that guanine showed no microwave attenuation above 72 Ghz but, when this compound was combined with ribose, attenuation at 70 and 71 Ghz was increased and attenuation of frequencies above 72 Ghz occurred. This suggested that attenuation of frequencies above 70 Ghz was via the rotational energy levels associated with those water molecules adsorbed to C–O–C oxygen or the –OH groups of the sugar. When guanosine was phosphorylated, the attenuation at about 66, 69, and 71 Ghz was increased but the attenuation at 70 and 75 Ghz was reduced. These findings indicated that attenuation at 66, 69, and 71 Ghz was due to the rotation of water associated with phosphate groups, while the loss of attenuation at 70 and 75 Ghz suggested that the attenuation of these frequencies by guanosine was due to the rotation of water associated with the hydroxyl group of the sugar phosphorylated in the formation of guanylic acid. Similar comparisons of the spectra of many other biochemicals tentatively have suggested that the attenuation of the various frequencies studied was due to the rotation of water associated with the groups shown by the horizontal arrows in Fig. 3.

Infrared spectrophotometry has shown that as biological macromolecules are dehydrated certain chemical groups give up their water more easily than others. The adsorption-desorption isotherm for each of these groups, therefore, is different (*3–6*). Since the relative desorption patterns of these groups is known, the attenuation at 66, 71, and 75 Ghz was measured when the cells were held in atmospheres of from 90 to 20 percent relative humidity. Loss of attenuation at 66 Ghz occurred rather sharply, but only when the cells were held below 50 percent relative humidity. At 75 Ghz, loss of attenuation was rapid between 90 and 80 percent relative humidity, while the attenuation at 71 Ghz was lost more gradually between 90 and 60 percent relative humidity. The desorption pattern at 66 Ghz strongly resembled the H_2O desorption pattern from the P=O groups of DNA molecules, that at 71 Ghz resembled desorption from C–O–C, C–O–[P], and some –OH groups, while that at 75 Ghz was unrecognizable (Fig. 4). It appeared possible, therefore, that the increased attenuation of some frequencies by tumor cells was due to the presence of extra C–O–C, C–O–[P], and perhaps –OH groups. To check this supposition, the microwave absorption spectrum of potassium phosphate was determined and, as is shown in Fig. 5, attenuation at 69 and 71 Ghz was observed.

Since mouse sarcoma virus is an RNA virus, it seemed possible that the attenuation pattern of the virus-transformed BHK cells, as well as that of the other tumor cells, might have been due to the presence of extra RNA. However, when the spectra of isolated RNA and DNA were standardized to 67 Ghz and the differential spectrum plotted, the differential absorption at most frequencies was close to unity, while only small differences were observed at other frequencies. Although the general form of this differential spectrum bore some resemblance to that between tumor and normal cells,

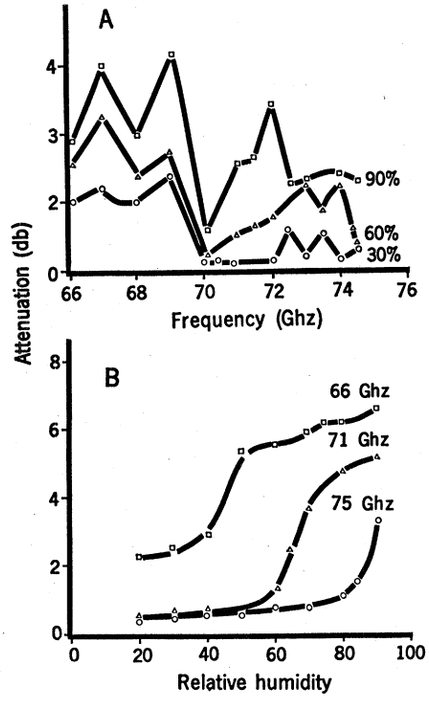


Fig. 4. The influence of water content on the microwave absorption spectra of normal baby hamster kidney cells as determined by holding the cells at different levels of relative humidity. (A) The absorption spectra at 90, 60, and 30 percent relative humidity. (B) Attenuation at 66, 71, and 75 Ghz with the cells held at different levels of relative humidity.

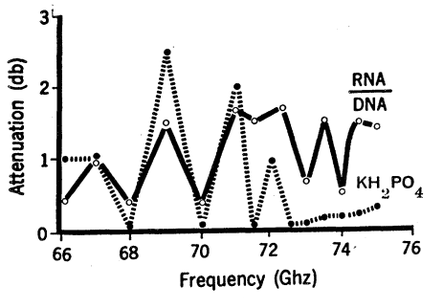


Fig. 5. The differential microwave absorption spectrum at 90 percent relative humidity of isolated RNA and DNA and the absorption spectrum of KH_2PO_4.

it was not exactly the same (Fig. 5). No difference in the absorption spectra of RNA isolated from normal and tumor cells was observed, but extracted tumor DNA, free of RNA (*7*), did exhibit increased attenuations at 69, 71, and 75 Ghz over normal DNA. The magnitudes of these increases, however, were not as great as those observed with intact cells.

The attenuation of microwaves within the frequency band used seems to be due largely to the rotation of water molecules adsorbed to certain groups of macromolecules. Since the makeup of water lattices should reflect the structure of macromolecules, microwave spectroscopy may allow the structure of in vivo individual or complexes of macromolecules to be determined. The results obtained suggest that attenuation between 66 and 76 Ghz is due to the rotation of water molecules adsorbed to P=O, C–O–[P], C–O–C, and sugar hydroxyl groups and, therefore, the absorption spectrum in this frequency band appears to reflect the hydration patterns of nucleic acids within the cell. Since a difference between spectra of RNA isolated from normal and from tumor cells could not be detected, the increased attenuation by tumor cells at 69, 71, and 75 Ghz was thought, at first, to be due to an increase in chromosome number or quantity of DNA per cell. This was ruled out, however, because differences were still apparent after all the spectra of the cells were normalized to the attenuation at 67 Ghz, a frequency attenuated equally by tumor and by normal DNA, and their component parts. There seem to be two possible explanations, therefore, for these results. (i) The RNA/DNA ratio in tumor cells may be different from that in normal cells and (ii) the RNA/DNA ratio may be the same in both cell types but the quantities of specific kinds of macromolecular groups are different. The former supposition does not seem to account fully for the results obtained, for, if it did, the differential absorption of tumor over normal cells at 73 and 74 Ghz would be expected to be less than unity (Fig. 5), not greater than unity as was found (Figs. 1 and 2). Therefore, both factors seem to play some role in the differences observed.

As far as the types of groups involved in these phenomena are concerned, the differential spectra of tumor over normal cells suggests that the former cell type has fewer P=O groups and more C–O–[P], C–O–C, or sugar hydroxyl groups than the latter. From infrared spectroscopy, the DNA extracted from tumor cells appears to be a much more rigid structure than normal DNA in that it does not have the same flexibility to alter its structure when its hydration lattice is changed (*7*). One way in which extra rigidity together with a decrease in P=O and an increase in C–O–[P], C–O–C, and sugar hydroxyl groups could occur is by small molecules, or even extra pieces of either RNA or DNA, becoming covalently bonded to the P=O groups present in one of the outer grooves of normal double-stranded DNA molecules.

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References and Notes

1. D. E. Dodds, thesis, University of Saskatchewan, Saskatoon, Canada (1968); S. J. Webb and D. E. Dodds, *Nature* **218**, 374 (1968).
2. S. J. Webb and A. D. Booth, *Nature* **222**, 1199 (1969).
3. E. M. Bradbury, W. C. Price, G. G. Wilkinson, *J. Mol. Biol.* **3**, 301 (1961).
4. M. Falk, K. A. Hartman, R. C. Lord, *J. Amer. Chem. Soc.* **85**, 387 (1963).
5. S. J. Webb and M. D. Dumasia, *Can. J. Microbiol.* **14**, 841 (1968).
6. S. J. Webb and J. S. Bhorjee, *Can. J. Biochem.* **46**, 691 (1968).
7. S. J. Webb and R. Bather, *Phys. Med. Biol.* **15**, 271 (1970).
8. We thank the National Research Council for supplying some of the microwave components and J. S. Webb for technical assistance.

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Morphine Tolerance and Dependence Induced by Intraventricular Injection

Abstract. *Injection of small quantities of morphine into the cerebral ventricular system of awake, relatively unrestrained, monkeys depressed or abolished operant food-reinforced lever pressing. After repeated injections progressively higher doses of morphine were needed to depress responding. Also, dependence could be demonstrated in these animals by precipitating specific abstinence signs with an antagonist.*

Repeated administration of opiate narcotics results in tolerance and dependence. The first is defined usually as a progressive diminution of the effects of successive doses of a drug or, conversely, the need for gradual increases in drug dosage to maintain the original intensity of effects. Dependence is defined by its consequence: the development of a specific physical abstinence syndrome following withdrawal of the agent. This syndrome is readily abolished by readministration of the drug or its surrogates (*1*). While it is generally agreed that most of the functional changes leading to these phenomena probably develop within the central nervous system (*1*), neither the identity of the biochemical disturbances responsible, nor their localization in neural structures, is yet clearly established (*2*). One of the reasons for the relative lack of knowledge concerning these two problems is that most investigators have used methods of narcotic administration (oral, subcutaneous, intravenous, and so forth) which make the entrance of the drugs into brain tissue contingent upon its uptake into extracerebral tissues first, and then upon the uptake properties of the "blood-brain barrier."

We have carried out a series of experiments in monkeys with cannulas implanted permanently in their cerebral ventricular system. Morphine—the prototype of the narcotic opiates—and certain antagonists (*3*) were thus mixed with the cerebrospinal fluid bathing the inner and outer surface of the brain. Our original purpose was to devise a method for efficiently conveying morphine to the brain without the technical difficulties of prolonged vascular catheterization. Soon it became apparent that this approach was not only satisfactory in that regard but that tolerance and dependence developed in these animals