THE EFFECT OF PERCHLORATE ON THE LOCALIZATION OF ^{99m}Tc-PERTECHNETATE IN A MOUSE BRAIN SARCOMA

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Technetium-99m-pertechnetate is a popular agent for scanning brain tumors. Because ^{99m}Tc normally concentrates in the thyroid, salivary gland and choroid plexus, potassium perchlorate is often used to block concentration in these organs. This maneuver appears to prevent interference of these organs during the scan, but the influence of perchlorate on the concentration of technetium by brain tumors has not been studied carefully. This report concerns the concentration of ^{99m}Tc with and without prior administration of perchlorate in a transplantable mouse brain tumor (sarcoma).

MATERIALS AND METHODS

Technetium-99m. Technetium-99m-pertechnetate was obtained commercially in a ready-to-inject form (Pertechtin, Hastings Radiochemicals Works, Houston, Tex.). The dose of ^{99m}Tc was 0.5 μCi/gm body weight. This amount of radioactivity was diluted to a volume of 0.01 ml for injection. At the same time, an aliquot was saved and diluted for counting as a standard.

Perchlorate. Potassium perchlorate was obtained in a chemical grade (Baker Analyzed Reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.). A saturated solution was prepared and given to mice by subcutaneous injection in an amount of perchlorate equivalent to that used in patients for clinical brain-scanning purposes (200 mg/70 kg).

Tumor mice. The mice were Yale-Swiss males, 3–5 weeks old, obtained commercially (Texas Inbred Mice Co., Houston, Tex.). The tumor is a methylcholanthrene-induced brain tumor obtained initially in 1951 (*1*). Each week tumor is harvested from several mice and homogenized, and a small aliquot of tumor (0.01 ml) is injected through the cranium and deposited in the intracerebral cleft just beneath the sagittal sinus. Within 1 week the tumor has grown to a size of about 4 mm^3 (64 mg); the tumors can be detected by the bulging of the head. At this point the animals and tumors are ready for study.

Technique. Mice are injected with the aqueous solution of ^{99m}Tc-pertechnetate intravenously through the tail vein using a 0.25-ml serological syringe and 27-gage, 0.5-in. hypodermic needle. Care is taken to fit the needle securely into the vein to prevent infiltration. The animals are not disturbed between the injection and the time for tissue sampling. At the appropriate time, the animals are electrocuted (*2*); immediately after death, a noose is tied tightly around the neck. This procedure prevents any flow of blood between the head and the body during subsequent tissue sampling. (The difference between the naked-eye appearance of the brains of two mice, one with a noose tied around the neck and the other without, is significant in the relative absence of blood in the brain of the latter.) The heart is then incised, and heart's blood is drained into a vial. An aliquot of 0.1 ml of blood is immediately pipetted into counting vials. The skull is then opened, and the tumor and samples of brain tissue are removed, weighed and placed into separate counting vials containing 1 ml of nitric acid and capped to prevent loss of radioactivity by volatilization.

Speed and coordination are essential throughout the experiment. It cannot be assumed that the concentration of blood in the tissue remains constant after sudden death of an experimental animal for a readily diffusible substance will alter location after cessation of circulation. In previous studies, one of the authors (TK) established that the volume of blood in a brain sample can be altered by a factor of five, depending solely on the method of killing the animal and sampling the tissue. Microscopic examination reveals that electrocution produces no hemorrhages in the brain or brain tumor, and the application of the noose prevents flow of blood from the head.

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Calculations. Percent activities are obtained as percent injected dose per gram of tissue. In addition, the weight of the animal in milligrams divided into the counts per minute injected gives a theoretical value for the expected activity per milligram of tissue if the dose has distributed itself evenly throughout the body. This value is compared to that observed in sample tissue for the percent activity in the tissue at each time interval. Thus, if one expects, with even distribution, 1,000 net counts/min/mg and if one observes 500 net counts/min/mg, then this tissue has 50% of the expected activity.

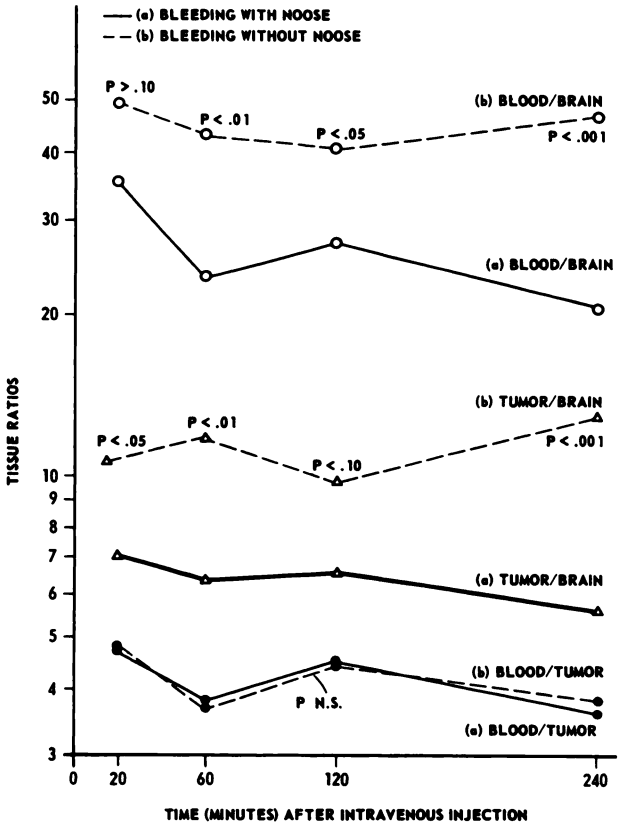

FIG. 1. Comparison of tissue ratios obtained in two groups of mice. In one group, string was tied tightly around neck immediately after death (solid line). In second group, no noose was used. Notice significantly higher blood-to-brain and tumor-to-brain ratios in second group (dotted line) caused by draining of blood from brain when bleeding was performed without noose.

Ratios. The present activity observed at each time interval in tumor, brain and blood is recorded, and blood-to-brain, tumor-to-brain and blood-to-tumor ratios are calculated. Expressed in this manner, all ratios in these experiments are greater than one.

Scintillation camera studies. In selected mice, external scintillation scanning data are obtained using a commercially available scintillation camera (Pho/Gamma III, Nuclear Chicago Corp., Chicago, Ill.).

The mice are first anesthetized slightly with phenobarbitol and are then immobilized on a board with restraints. They have been previously injected with appropriate doses of ^{99m}Tc with or without perchlorate. At the planned time, they are placed about 1 cm below the pinhole of the collimator, which results in considerable magnification of the image on the 11-in. dia scintillation crystal. They are centered by observing the oscilloscope, and a scintillation photograph is made with the collection of 100,000 counts (duration of count is generally 10 min). The photographs are then evaluated for distribution of radioactivity and visualization of the brain tumors.

Statistical evaluation. In comparison studies between ^{99m}Tc concentration with and without perchlorate, groups at each time interval were compared

TABLE 1. PERCENT INJECTED DOSE PER GRAM OF TISSUE WITHOUT AND WITH PREDOSING WITH POTASSIUM PERCHLORATE

Time post-injection	$\frac{\text{Cpm/gm tissue}}{\text{Cpm/body dose}}$ %			$\frac{\text{Cpm/gm tissue}}{\text{Cpm/body dose}}$ % with potassium perchlorate		
	Tumor	Brain	Blood	Tumor	Brain	Blood
5 min	2.65 ± 0.73*	0.61 ± 0.08	20.99 ± 4.28	—	—	—
10 min	3.93 ± 1.30	0.78 ± 0.29	19.44 ± 5.22	—	—	—
20 min	3.01 ± 0.43	0.48 ± 0.22	14.66 ± 1.60	4.74 ± 0.99	0.97 ± 0.44	19.97 ± 3.19
30 min	2.89 ± 0.54	0.51 ± 0.16	13.92 ± 4.42	—	—	—
60 min	3.18 ± 1.38	0.39 ± 0.14	9.76 ± 2.43	4.25 ± 1.13	0.52 ± 0.32	14.36 ± 3.37
90 min	2.08 ± 0.72	0.26 ± 0.08	7.77 ± 2.07	—	—	—
120 min	2.03 ± 0.59	0.26 ± 0.09	6.48 ± 3.43	5.14 ± 1.15	0.69 ± 0.35	15.85 ± 1.64
150 min	1.25 ± 0.62	0.22 ± 0.07	5.37 ± 2.10	—	—	—
180 min	1.52 ± 0.44	0.19 ± 0.07	5.85 ± 1.97	—	—	—
240 min	1.43 ± 0.59	0.29 ± 0.17	4.45 ± 1.65	4.02 ± 1.09	0.55 ± 0.19	13.43 ± 2.99
6 hr	1.42 ± 0.47	0.20 ± 0.06	5.37 ± 1.14	—	—	—
24 hr	0.31 ± 0.15	0.04 ± 0.03	0.65 ± 0.38	—	—	—

* Standard deviation = $[(\Sigma d^2)n]^{1/2}$.

using the Student t-test of significance. The arbitrary level of significance for these studies was chosen as $p < 0.05$.

RESULTS

Bleeding with and without the noose. In Fig. 1 the results of evaluation of tissue ratios with and without the application of a neck noose are demonstrated. The blood-to-brain and tumor-to-brain ratios both were affected significantly by the application of the noose; the blood-to-tumor ratio did not appear to be altered. Tumor-to-brain ratios varied from 5:1 to 7:1 between 20 and 240 min with the noose. Without the noose, these ratios were significantly higher, being greater than 10:1. Blood-to-brain ratios with the noose varied from 20:1 to 35:1, whereas without the noose, they ranged between 40:1 and 50:1. It appears from these findings that when the noose is not applied immediately after electrocution, significant amounts of blood drain from the brain but not from the tumor. This occurrence leads to a falsely high impression of the tumor-to-brain ratio. For this reason, the noose was used in all subsequent experiments.

Percent activities with and without perchlorate. In Table 1 the percent injected dose per gram of tissue for tumor, brain and blood is shown with and without the administration of potassium perchlorate. Note the considerably higher value for the groups receiving perchlorate. In Fig. 2, the percent activity in blood, tumor and brain at time intervals varying from 15 min to 24 hr is shown. The percent activity represents that amount of radioactivity found in the tissue divided by the amount that would have been expected if the radioactive tracer had been evenly distributed throughout the body. As expected, blood activity initially is considerably higher than the expected (300% of the theoretical amount). Without the administration of perchlorate, the level declines rapidly during the first hour to a level of about 150%; it then declines more slowly. With the administration of perchlorate, the initial activity is the same, but the decline is more gradual, leveling off at about 1 hr to

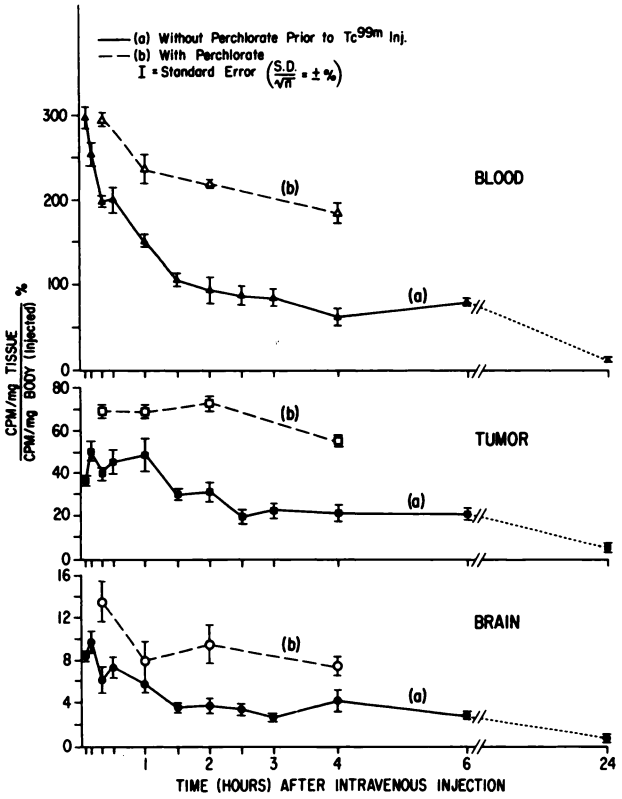

FIG. 2. Comparison of tissue localization of ^{99m}Tc-pertechnetate in two groups of mice: one group with dose of perchlorate preceding injection of ^{99m}Tc (dotted line); one group without perchlorate (solid line). Notice significantly increased amount of radioactivity in blood, tumor and brain in animals receiving perchlorate.

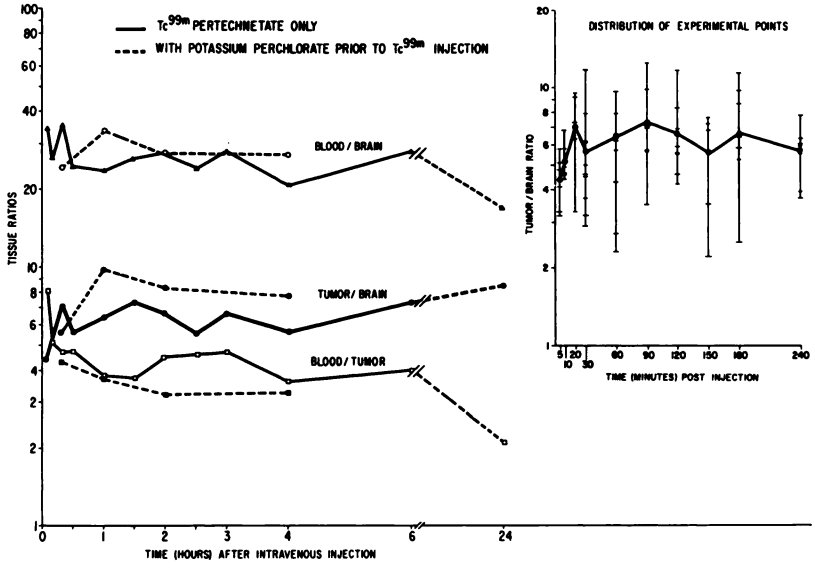

FIG. 3. Comparison of tissue ratios between perchlorate-treated mice (dotted line) and nonperchlorate-treated mice (solid line). Average tumor-to-brain ratio is higher with perchlorate although this was statistically significant only at 4-hr time interval ($p < 0.05$). Inset shows distribution of experimental points for tumor-to-brain ratios without perchlorate and demonstrates variability of results.

about 250% activity, from which it declines at about the same rate as the blood curve without perchlorate.

Tumor levels of activity are not as great as those in the blood and, in fact, have less than theoretically evenly distributed activity. Beginning at about 35% there is an increase at 30 min to 50% activity, a fairly stable level up to about 1 hr, and then a decline, leveling off again at about 3 hr to 20% activity. With perchlorate the amount of activity in the tumor is higher initially, beginning at about 70%; it remains stable for the first 2 hr, then declines slowly. During the fourth hour after injection, there is still approximately 60% activity.

The brain has activity far below that of the blood and the tumor. Initially, it is about 8%, and its decline tends to parallel that of the tumor. With the administration of perchlorate, the activity in the brain is somewhat higher, starting at about 14%, but the decline in the brain is more rapid than is that in the tumor under these conditions.

Tissue ratios. In Fig. 3, the blood-to-brain, tumor-to-brain and blood-to-tumor ratios are shown. With ^{99m}Tc-pertechnetate alone, the blood-to-brain ratio is about 30:1 and remains fairly stable. With administration of perchlorate, blood-to-brain ratios do not show any significant change.

The important tumor-to-brain ratio without perchlorate is initially about 4:1, but it increases rapidly to a peak of about 7:1 at 30 min. From this point there are fluctuations but no significant changes throughout the period of observation up to 6 hr. With the administration of perchlorate, the initial tumor-to-brain ratio is somewhat lower, but within 1 hr, the ratio has reached the higher peak of about 10:1 and remains above the line for pertechnetate alone throughout the 4-hr observation. Tests of significance of these two curves indicate that only the last observation at 4 hr is statistically significant ($p < 0.05$) although at the 1- and 2-hr levels, the difference is of borderline significance ($p < 0.1$). In the panel on the right, distribution of experimental points for tumor-to-brain ratios with pertechnetate alone are shown to indicate some of the variability of the results.

The blood-to-tumor ratio is initially high at 8:1 but declines rapidly and within 30 min reaches the low point of about 5:1 where it tends to plateau. The administration of perchlorate tended to lower this ratio at most points but not to a statistically significant degree.

Lower blood-to-tumor ratios would be favorable for scanning, and high tumor-to-brain ratios would be favorable. Thus, it appears that, on these two important criteria, perchlorate enhances the tissue concentration ratios in favor of detection by external scanning.

Scintillation camera pictures. To test the tissue sampling results *in vivo*, scintillation camera photographs were made of two groups of mice. One group without brain tumors and a second group with brain tumors were studied 1 hr after injection with and without perchlorate in each group. In Fig. 4, representative scans of the groups of mice are shown. Mice without tumors show an area of decreased radioactivity in the region of the brain. The main difference in scans with and without perchlorate is that without perchlorate the bulk of the activity is in the thyroid gland and with perchlorate the activity is more evenly distributed. When brain tumors are present, they are seen on scintillation scan as areas of increased activity in the region of the brain. Without perchlorate, the tumor is seen, but the activity is much less than that of the adjacent thyroid gland; the density of the tumor is reduced, comparatively, on the scan. With perchlorate and blockage of thyroid uptake, much greater activity in the tumor is seen on the scan. These findings are representative of those in several mice in each group and lead us to conclude that our tissue levels and ratios do correctly reflect the *in vivo* situation because administration of perchlorate does improve visualization of brain tumors by scanning in this experimental model.

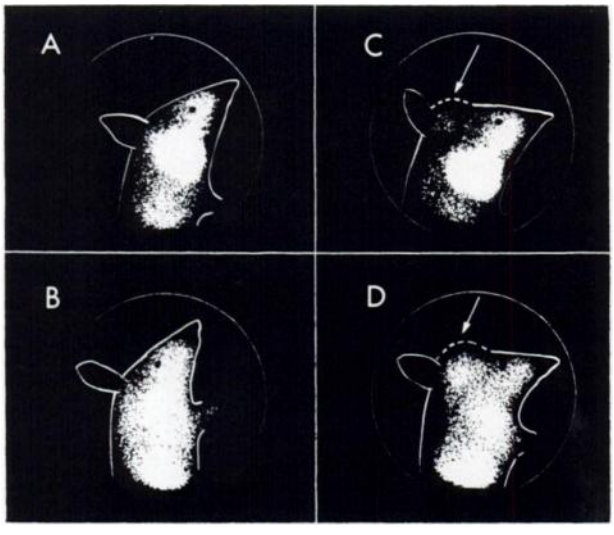

FIG. 4. Scintiphotos of four mice representative of results in four groups studied 2-hr after injection. A is of mouse with no brain tumor and no perchlorate. Notice thyroid concentration of ^{99m}Tc. B is of mouse with no brain tumor with perchlorate showing blocking of thyroid. C is of mouse with brain tumor without perchlorate. Tumor is visualized (arrow), but thyroid concentration is greater. D is of mouse with brain tumor with perchlorate. Tumor is visualized (arrow) with greater amount of radioactivity than before. Thyroid is blocked. (In all pictures, 100,000 counts were collected in approximately 10 min.)

DISCUSSION

Technetium-99m-pertechnetate was introduced for brain scanning by Harper *et al* (*3*). He administered 5–10 mCi of ^{99m}Tc-pertechnetate intravenously along with 1 gm of sodium iodide to minimize localization in the thyroid. Although he did not report any animal tumor-to-brain ratios, he did mention a single case of metastatic melanoma found at autopsy in which the tumor-to-brain ratio ranged from 2:1 to 3:1. The interval between injection and tissue sampling was not given.

Studies done by McAfee and coworkers in mice with ependymomas transplanted into the subcutaneous tissues revealed tumor-to-brain concentration ratios ranging from 15:1 to 29:1 (*4*). No significant difference was demonstrated between oral and intravenous administration. Somewhat higher concentration ratios were obtained at 3 hr compared to 1 hr. McAfee introduced the use of perchlorate to decrease the gastric and thyroid concentration of pertechnetate but did not report on the effect of perchlorate on tumor localization.

Matthews and Mallard also studied tumor-to-brain concentration ratios using rats and two subcutaneously implanted sarcomas (*5*). They indicated that the thyroid was not blocked in their experiments. In this tumor, the mean tumor-to-brain concentration ratio was 12:1. The time interval for achieving this ratio was not specified.

Clinical experience has indicated a need for the use of some type of blocking agent. Primarily, perchlorate has been used although some authors prefer Lugol's solution. The primary use of perchlorate has been in blocking the choroid plexus to prevent positive scans in that area (*6*). In early experiences, some authors encountered false-positive brain scans caused by salivary secretion of technetium and extracranial contamination. The problem appears to have been largely eliminated by the administration of perchlorate. Varying doses of perchlorate have been used. Witcofski mentions "large oral doses" of potassium perchlorate (*7*). Quinn *et al* administered 1 gm of potassium perchlorate 1 hr before brain scanning (*8*). Croll and colleagues used 200-mg doses (*9*). There is little mention in the literature of the effect of this predosing with perchlorate on the visualization of brain tumors.

Andrews and Pope attempted three visual trials to assess the effect of the blocking agents on the region of the choroid plexus (*10*). Normal scans in six patients who had not received perchlorate were intermixed with scans of six patients who had, and the scans were examined independently without prior knowledge of the administration of perchlorate to six of the 12. Apparently, the authors could not reliably distinguish the scans with perchlorate from those without. The authors admit no knowledge of the effect of potassium perchlorate on the uptake of ^{99m}Tc by tumors but believed that, if the mechanism of technetium uptake was passive diffusion, then it would not likely have an effect.

Miller and Simmons examined four patients with and without the oral administration of 200 mg potassium perchlorate 90 min before the scintillation camera scan (*11*). In these four patients, perchlorate was associated with a lesser decline in brain radioactivity during the first minute and lower brain-to-blood ratios as measured with external counting techniques. When apparent before, the choroid plexus was rendered invisible on the scan after the administration of perchlorate.

It is not wise to extrapolate results of experimental animal studies to the clinical situation, but we believe that our study does contribute valuable information for those people using pertechnetate and perchlorate for scanning brain tumors. The advantages of our study over previous investigations are (1) we have evaluated tumor uptake in animals using implantation of tumor directly into the brain whereas most other authors have used tumors planted subcutaneously; (2) we have combined our tissue counts (tumor-to-brain and blood-to-tumor) with external scintillation camera detection to substantiate the *in vivo* validity of our tissue findings; and (3) we have attempted to evaluate the pharmacodynamics for several hours by comparing tumor uptake with and without prior administration of perchlorate. This, we believe, makes our results more analogous to the clinical situation than those previously published.

One of the advantages of having the tumor implanted into the brain is that pressure and vasculature are more analogous to that of the clinical condition than when a tumor is implanted subcutaneously. Our tumor-to-brain ratios differ from those of other authors. The average range in our experiments was 6:1 without perchlorate whereas with another sarcoma implanted subcutaneously, the ratios were around 12:1 (*5*). In a subcutaneous ependymoma, ratios averaged 22:1 (*4*). We also emphasize the importance of a ligature to prevent redistribution of blood and isotope after death and have shown that, without a noose, our tumor-to-brain ratios were almost double those obtained with it.

The administration of an agent such as perchlorate to block thyroid, gastric and salivary gland secretion of the isotope cannot be expected to leave the tumor, brain and blood concentrations and ratios unchanged. By blocking several secretion routes for pertechnetate, tissue concentrations in blood, tumor and brain

are generally increased. These levels decline more slowly, and this slow decline leads to a prolonged high tissue concentration. Whether this concentration will be advantageous or disadvantageous depends on the tissue ratios and the type of tumor being imaged. In the particular tumor we studied, tumor concentration was increased by perchlorate and declined less rapidly than did concentration in the brain so that the tumor-to-brain ratio was actually enhanced. Blood-to-tumor ratios were also slightly lower, an additional advantage in tumor visualization. If this experimental model is analogous to brain tumors in man, then the administration of perchlorate would appear to enhance detection of the tumor by pertechnetate scanning.

SUMMARY AND CONCLUSIONS

Concentration of ^{99m}Tc-pertechnetate in a mouse brain tumor (sarcoma) has been evaluated with and without the prior administration of perchlorate in an amount equivalent to dose in man.

Results show an average tumor-to-brain ratio of between 5:1 and 7:1 without perchlorate. With perchlorate, tumor-to-brain ratios average from 8:1 to 10:1. This difference was significant at the 4-hr time interval ($p < 0.05$). Equally important, the concentration of radionuclide per milligram of tumor was increased significantly by perchlorate, and the blood-to-tumor ratio was lower. Images of the tumor made with the gamma camera using the pinhole collimator show that tumor scans made with perchlorate were superior to those obtained without administration of perchlorate.

The adminisration of perchlorate before ^{99m}Tc-pertechnetate in this experimental situation significantly increased the tumor concentration and tumor-to-brain ratios. If brain tumors in man concentrate ^{99m}Tc similarly, then the use of perchlorate in the clinical brain scanning situation should enhance results by reducing salivary and choroid plexus background activity, enhancing tumor concentration of technetium and increasing the tumor-to-brain concentration ratio.

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