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Concentration of Intrathecal ^{3}H-Labeled Metrizamide in Normal Dog Brain: Age-Related Differences

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Responses to intrathecal metrizamide in dogs were found to be age-related: Adult dogs had seizures; 7-week-old dogs appeared stuporous; 6-day-old dogs were clinically unaffected. The brain metrizamide concentrations 4, 6, and 20 hr after intrathecal injection correlated directly with the occurrence and severity of neurotoxic symptoms. Age-related differences in brain metrizamide concentration may be explained by two factors. The first is the failure of current clinical guidelines to adjust the recommended dosage of metrizamide to reflect differences among age groups in brain weight rather than body weight. This error resulted in lower doses/gram of brain weight being given to the puppies. However, the large differences in brain metrizamide concentrations among the three groups of dogs could not be explained solely by differences in the dose. A second factor, physiologic age-related differences in brain penetration, is believed to be operative. The precise nature of these differences is unclear.

Metrizamide is the only water-soluble contrast agent currently approved by the United States Food and Drug Administration for injection into the cerebrospinal fluid (CSF). When this substance collects in excessive concentration in the cerebral cisterns and enters the brain it can cause a psychoorganic syndrome and/or seizures [1]. These neurotoxic side effects are less serious and occur less frequently in infants and children than in adults [2]. We investigated this significant age-related difference experimentally by quantitating the total amount, "bound" fraction, and respective rates of clearance of ^{3}H-labeled metrizamide in 6-day-old, 7-week-old, and adult dog brains at 4, 6, and 20 hr after intrathecal injection.

Materials and Methods

Tritiated metrizamide with an activity of 575 μCi (21.3 MBq)/mg was obtained from Research Chemicals, Inc. (Baton Rouge, LA). This agent had a chemical purity of greater than 98% as determined by thin-layer chromatography. (See Appendix for synthetic preparation and chemical/radiochemical analysis.)

The ^{3}H-labeled metrizamide was injected into CSF of 10 hydrated animals divided into three groups. Group 1 comprised three 6-day-old dogs, group 2 comprised four 7-week-old dogs, and group 3 comprised three adult dogs.

In order to assess blood-brain barrier integrity in the 6-day-old dogs, two litter-mates received an intravenous bolus of 0.3 ml and 0.68 ml of 2% Evans blue, respectively. They were sacrificed at 1 hr after dye injection by an intracardiac dose of euthanasia agent T61 (American Hoechsy Co., Summerville, NJ). The brains were immediately removed and examined for blue staining.

Group 1: 6-Day-Old Dogs

Three 6-day-old (0.3–0.4 kg) mongrel puppies from the same litter were anesthetized with 3%–5% Halothane (Ayerst, New York, NY). A 25 gauge butterfly needle was introduced

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into the cisterna magna and a small amount of CSF was withdrawn to document needle position. Next, 150 mg (86 mCi [3.2 GBq]) of tritium-labeled metrizamide diluted with commercial diluent (Winthrop, New York, NY) to 170 mg I/ml was injected slowly. The puppies were inverted for 1 min after injection, then allowed to recover from anesthesia. The animals were observed while alive at 0.5, 1, 2, 4, 6, 8, 12, 16 and 20 hr after injection for neurotoxic side effects. One animal was sacrificed by intracardiac euthanasia with T61 at 4 hr, one at 6 hr, and one at 20 hr after the metrizamide injection. Immediately after each animal's death the brain was removed, and representative samples of the frontal lobes, occipital lobes, and cerebellum and brainstem were obtained and stored at −8°C.

For processing, a part of each sample was allowed to thaw, homogenized, and washed twice with ice-cold (0–4°C) 10% trichloroacetic acid (TCA). The supernatants of the cold TCA wash from each of the three regions were pooled for each dog brain. The radioactivity was counted for at least 5,000 counts in a liquid scintillation spectrometer after addition of 10 ml of scintillation fluid (15 g PPO, 15 mg POPOP, 240 g naphthalene, 1 L toluene, 1 L dioxane and 1 L 95% ethanol). This radioactivity represented the "unbound" metrizamide present in each brain. The sediment remaining after the cold TCA washings was then heated twice to 90°C for 15 min with 5% TCA. The radioactivity in the pooled supernatant thus obtained was counted as described above. The radioactivity in this heated supernatant represented the "bound" portion of the metrizamide in each brain.

The amount of DNA in each brain sample was estimated by using diphenylamine reagent. Using D-2-deoxyribose as a standard [3], the concentration or amount of bound and unbound metrizamide was expressed in counts/min/100 μm of D-2-deoxyribose on a per kilogram body weight and per gram brain weight basis. The percentage of bound metrizamide was calculated for each of the three brains, and the rates of clearance of the bound and total amounts of metrizamide, respectively, were determined by plotting the counts/min against the time elapsed (4, 6, or 20 hr) between injection and sampling.

Group 2: 7-Week-Old Dogs

Four 7-week-old (3 kg) mongrel puppies from the same litter were sedated with 10–15 mg/kg of intravenous Nembutal (Parke-Davis, Morris Plains, NJ). One dog was sacrificed by intravenous euthanasia agent T61 1 min before metrizamide injection. A 22 gauge needle was introduced into the cisterna magna, 0.6 ml of CSF was aspirated, and 250 mg (144 mCi [5.3 GBq]) of tritiated metrizamide diluted with commercial diluent to 170 mg I/ml was injected slowly. The dogs were inverted for 1.5 min after injection to maximize intracranial entry. The other three dogs were sacrificed 4, 6, and 20 hr after metrizamide injection, respectively. The three animals sacrificed after injection were observed for neurotoxic side effects at intervals as stated for group 1. Their brains were removed immediately after sacrifice. The brain of the animal that was sacrificed before metrizamide injection was not removed until 1 hr after injection to determine the degree of brain penetration of metrizamide occurring after death. All four brains were analyzed as described for group 1.

Group 3: Adult Dogs

Three adult (20–30 kg) mongrel dogs were sedated with 25–30 mg of intravenous ketamine hydrochloride (Parke-Davis) and 4 mg of diazepam (Valium, Roche Labs., Nutley, NJ). A 22 gauge needle was introduced into the cisterna magna and 3–5 ml of CSF was aspirated. The dogs were then placed in a 45° head-down position. An intrathecal injection of 1.8 g of commercial metrizamide (Amipaque, Winthrop) combined with 500 mg (288 mCi [10.65 GBq]) of tritium-labeled metrizamide diluted with commercial diluent to a concentration of 170 mg I/ml was administered slowly. Proper needle placement was verified immediately after injection by a lateral radiograph in one dog and by computed tomographic (CT) scans in the other two. After the injection all animals were observed for neurotoxic side effects at intervals as stated for group 1. Seizures were treated by intravenous administration of diazepam. The three dogs were sacrificed at 4, 6, and 20 hr after metrizamide injection, respectively. The brains were removed immediately after death and analyzed as described for group 1.

Results

No symptoms were seen in the 6-day-old (group 1) dogs. The 7-week-old (group 2) dogs appeared stuporous at the serial examinations carried out before they were sacrificed, although these were conducted long after the effects of prestudy sedation had subsided. The 7-week-old animal sacrificed at 20 hr had weakness of the hind limbs, which may have been the result of a traumatic cisternal puncture rather than an effect of the metrizamide. All of the adult (group 3) dogs had seizures within 2 hr after injection. These animals were treated with diazepam, and no additional seizures occurred.

The total, bound, and unbound amounts of labeled metrizamide per 100 μg of D-2-deoxyribose per kg of body weight and per gram of brain weight and the percentage of bound agent within the brains of all three groups are given in table 1. In group 3 each value was multiplied by 4.6, since only 22% of the metrizamide given to these animals was radioactive. The corrected values for each group are shown graphically in figure 1.

A cursory examination of the data obtained in this study suggested that the clearance of metrizamide from the brain was exponential. To confirm this observation, the following statistical analysis was performed: Since sequential CT scanning data in adult dogs after cisternal injection of metrizamide have shown that brain metrizamide concentrations peak 2–4 hr after injection (Hayman LA, unpublished data), it was assumed that postpeak clearance of metrizamide occurred at a constant rate within each of the three groups. The counts/min, W, at a given time t (where baseline t = 4 hr after metrizamide injection) can be expressed as $W_{t,i} = A_i \exp(-\lambda_i t)$, in which $t \geq 0$ (equation A). In this equation, i = 1, 2, 3, corresponds to the three groups of dogs; A_i represents the amount of metrizamide present at 4 hr; and λ_i is the rate of metrizamide clearance from the brain. To estimate A_i and λ_i for i = 1, 2, 3, equation A may be transformed into a linear equation by taking the logarithm of both sides: $Z_{t,i} = \ln(W_{t,i}) = \ln A_i - t\lambda_i = B_i - t\lambda_i$ (equation B). Using standard regression techniques [4], B_i (which is the natural logarithm of A_i) and λ_i can be estimated for i = 1, 2, 3, and linear hypotheses involving these parameters can be tested.

The results for clearance of total and bound metrizamide, respectively, for all three groups are given in table 2. As

TABLE 1: **Clearance of Radioactive (^{3}H-Labeled) Metrizamide from Normal Dog Brain: Age-Related Differences**

Group (Age): Time of Sacrifice	Brain Concentration of ^{3}H-Labeled Metrizamide			
	Bound	Unbound	Total	% Bound
1 (6 days)				
4 hr postinjection:				
/kg body wt	15,998	375,998	391,995	
/g brain wt	400	9,400	9,800	4
6 hr postinjection:				
/kg body wt	11,647	301,442	313,088	
/g brain wt	291	7,536	7,827	4
20 hr postinjection:				
/kg body wt	6,586	107,272	113,858	
/g body wt	165	2,682	2,846	6
2 (7 weeks):				
1 hr preinjection:				
/kg body wt	357,900	1,628,713	1,986,613	
/g brain wt	8,382	38,143	46,525	18
4 hr postinjection:				
/kg body wt	761,000	2,303,225	3,064,225	
/g brain wt	17,822	53,940	71,762	25
6 hr postinjection:				
/kg body wt	537,638	1,644,338	2,181,975	
/g brain wt	12,591	38,509	51,100	25
20 hr postinjection:				
/kg body wt	76,788	284,938	361,725	
/g brain wt	1,798	6,673	8,471	21
3 (adult):*				
4 hr postinjection:				
/kg body wt	4,806,367	12,482,733	17,289,100	
/g brain wt	961,273	2,496,547	3,457,820	28
6 hr postinjection:				
/kg body wt	2,859,015	11,888,585	14,737,250	
/g brain wt	571,803	2,377,717	2,947,450	19
20 hr postinjection:				
/kg body wt	1,111,763	4,770,718	5,882,480	
/g brain wt	222,353	954,144	1,176,496	19

Note.—Numbers (except percentages in last column) represent counts/min/100 μg of D-2-deoxyribose/g of tritiated metrizamide injected/kg of body weight and per gram (/g) of brain weight.

* Actual values for group 3 were multiplied × 4.6, since only 22% of the metrizamide administered to these dogs was radioactive.

shown by the plots in figure 1 and the large R_2 adjusted values in table 2, the exponential model, equation A, fits very well, particularly for total metrizamide. This indicates that the clearance of both total and bound metrizamide from the brain in all three groups is exponential.

For both total and bound metrizamide, the clearance rate for 7-week-old dogs was significantly higher than the rate for either 6-day-old or adult dogs (table 2). Clearance rates for total metrizamide were not statistically different between the 6-day-old and the adult dogs ($0.1 < p < 0.2$). The clearance rate of bound metrizamide in 6-day-old pups was significantly lower than that for adult dogs ($p < 0.005$).

As shown in table 2, the total brain metrizamide concentration between the groups differed significantly. At 4 hr the 6-day-old had less than the 7-week-old dogs ($p < 0.01$) and the 7-week-old had less than the adult dogs ($p < 0.001$). The bound metrizamide followed the same pattern, with the 6-day-old dogs having less at 4 hr than the 7-week-old dogs ($p < 0.005$) and the 7-week-old dogs less than the adult dogs ($p < 0.025$).

The mean fraction of bound metrizamide was statistically lower in 6-day-old (group 1) than in identical brain samples analyzed in 7-week-old (groups 2) dogs (paired t test $t_2 = 10.6$; $p < 0.01$). The mean fraction bound in group 1 also was lower than the mean value obtained from random brain samples examined in the adult dogs (group 3) ($t_2 = 5.5$; $p < 0.05$). The mean fractions of bound metrizamide in the adult (group 3) and 7-week-old (group 2) dogs were not statistically different ($p > 0.5$).

Figure 1 also shows the total and bound amounts of metrizamide present in the brain of the 7-week-old dog who received the agent before death. Labeled metrizamide, when allowed to equilibrate with the dead brain for 1 hr, produced levels of brain "binding" and penetration in this animal comparable to those seen in its litter-mates.

There was no leakage of Evans blue dye into the brains of the two 6-day-old dogs injected with this substance. This suggests that the differences noted between this group and the adult animals were not attributable to an immature blood-brain barrier.

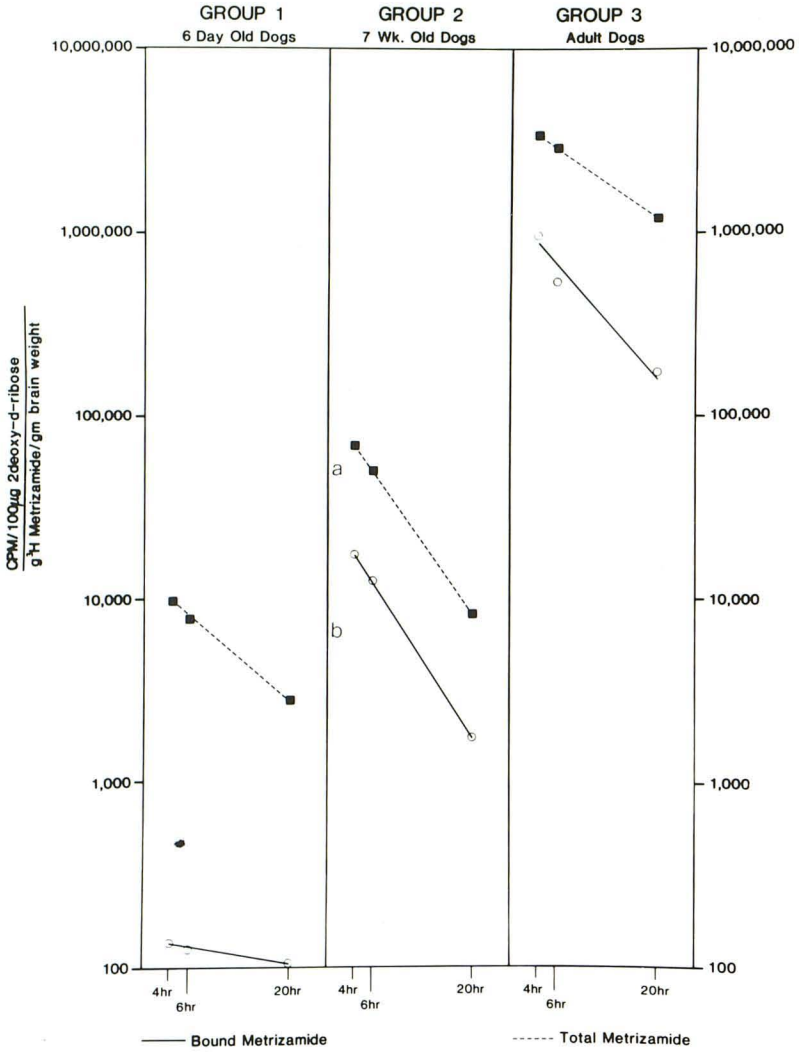

Fig. 1.—Semilog plot of mean counts/min/100 μg of D-2-deoxyribose per gram of ^{3}H-labeled metrizamide per gram of brain weight in 6-day-old, 7-week-old, and adult dogs at 4, 6, and 20 hr after intrathecal injection of tritiated metrizamide. Total and bound amounts of brain metrizamide are significantly higher in adult dogs as compared with 7-week-old dogs, and significantly lower in 6-day-old dogs than in the other two groups of dogs. Entry of intrathecal metrizamide into dead brain of one animal in 7-week-old group indicated by a (= total amount of metrizamide) and b (= bound fraction). Group 3 values used in data analysis were corrected to reflect counts/min that would have resulted if all the metrizamide administered to these dogs had been labeled with tritium (see text).

Discussion

The diminished neurotoxicity of intrathecal metrizamide in infants and children has been documented [2] (Sleven D, personal communication). To investigate this phenomenon, we designed the present study to correlate neurotoxic symptoms with age and with brain metrizamide concentration, binding, and clearance in dogs after injection into the CSF of the cisterna magna.

Brain metrizamide concentration correlates with the presence and severity of neurotoxic symptoms. The oldest dogs (group 3) with the highest brain metrizamide concentrations had the most severe neurotoxic symptoms (seizures), while the youngest dogs (group 1) with the lowest brain metrizamide concentrations had no neurotoxic symptoms. The group 2 dogs had brain concentrations intermediate between the other two groups and developed less severe neurotoxic symptoms (stupor) than the group 3 dogs. This tendency of animals to experience lethargy and recover slowly from anesthesia as a manifestation of neurotoxicity after intrathecal administration of metrizamide has been noted in other studies [5].

The differences in brain metrizamide concentration among the three groups were caused by two factors. First, when the 7-week-old (group 2) dogs were given the same dose of metrizamide (80 mg/kg body weight) as the adult dogs, age-related differences in the brain:body weight ratio were not taken into account. Maximum brain weight for age expressed as a percentage of body weight is 3.0% ± 1% for 6-day-old dogs, 3.02% ± 1.7% for 7-week-old dogs, and 0.4% ± 0.1% for adult dogs [6]. Thus, on a *brain weight* basis the 7-week-old dogs received a metrizamide dose of 2 g/g brain weight, whereas the adult dogs received a metrizamide dose of 16 g/g brain weight. This eightfold higher dose given to the adult dogs partly explains the 50-fold higher metrizamide concentration found in the adult brains at 4, 6, and 20 hr after injection. The failure to compute the intrathecal contrast-medium dose for infants and children on a brain-weight basis results in a similar situation in the clinical setting. Current recommendations assign children approximately 50% of the adult dose of metrizamide on the basis of body weight (table 3) [7]. This reduced dose/kg of brain weight partly explains the reduced neurotoxic side effects noted clinically in the younger

TABLE 2: **Clearance of Radioactive (^{3}H-Labeled) Metrizamide from Normal Dog Brain: Predicted and Observed Logarithms of Counts/Min Related to Age and Time of Sacrifice**

Logarithm	Group: Age		
	1: 6 days (i = 1)	2: 7 weeks (i = 2)	3: Adult (i = 3)
Bound metrizamide:*			
A_i	8.8362	11.0167	12.8597
$\bar{B}_i \pm SD$	8.7348 ± 0.0882	10.9868 ± 0.0260	12.6946 ± 0.1437
$\bar{\lambda}_i \pm SD$	0.0500 ± 0.0126	0.1418 ± 0.0037	0.0826 ± 0.0204
$Z_{0,i}$ predicted (observed)	8.7348 (8.8362)	10.9868 (11.0167)	12.6946 (12.8597)
$Z_{2,i}$ predicted (observed)	8.6347 (8.5188)	10.7033 (10.6692)	12.5294 (12.3403)
$Z_{16,i}$ predicted (observed)	7.9342 (7.9487)	8.7188 (8.7231)	11.3730 (11.3957)
R^2 adjusted (%)	88.2	99.9	88.5
Total metrizamide†			
A_i	12.0350	12.4096	14.1399
$\bar{B}_i \pm SD$	12.0005 ± 0.0300	12.3740 ± 0.0310	14.1276 ± 0.0107
$\bar{\lambda}_i \pm SD$	0.0745 ± 0.0043	0.1316 ± 0.0044	0.0667 ± 0.0015
$Z_{0,i}$ predicted (observed)	12.0005 (12.0350)	12.3740 (12.4096)	14.1276 (14.1399)
$Z_{2,i}$ predicted (observed)	11.8497 (11.8103)	12.1107 (12.0700)	13.9942 (13.9802)
$Z_{16,i}$ predicted (observed)	10.7938 (10.7987)	10.2678 (10.2729)	13.0600 (13.0618)
R^2 adjusted (%)	99.4	99.8	99.9

Note.—A_i = amount of metrizamide present in brain at 4 hr after intrathecal injection; B_i = natural logarithm of A_i; λ_i = rate of metrizamide clearance from brain; $Z_{t,i}$ (predicted) = $B_i - \bar{\lambda}_i t$ (where baseline t = 4 hr after injection). R^2 adjusted is a measure of how well the exponential model ($Z_{t,i}$ predicted) fits the data obtained ($Z_{t,i}$ observed); 100% would be a perfect fit.

* Tests of hypotheses (all t statistics have two degrees of freedom): $B_1 < B_2$: $t = 21.6$ ($p < 0.005$); $B_1 < B_3$: $t = 7.6$ ($p < 0.025$); $\lambda_1 < \lambda_2$: $t = 86.2$ ($p < 0.001$); $\lambda_3 < \lambda_2$: $t = 35.2$ ($p < 0.001$); $\lambda_1 < \lambda_3$: $t = 16.8$ ($p < 0.005$).

† Tests of hypotheses (all t statistics have two degrees of freedom): $B_1 < B_2$: $t = 10.4$ ($p < 0.01$); $B_2 < B_3$: $t = 34.4$ ($p < 0.001$); $\lambda_1 < \lambda_2$: $t = 9.1$ ($p < 0.025$); $\lambda_3 < \lambda_2$: $t = 14.1$ ($p < 0.001$); λ_2:λ_3: $t = 1.9$ ($0.1 < p < 0.2$).

TABLE 3: **Recommended Myelographic Dosage of Metrizamide According to Age, Expressed in Terms of Body Weight and Brain Weight**

Age (Years)	Maximum Dose [7]		Weight (kg)		Dose (g)/Weight (kg)	
	Vol (ml)	Conc (mg I/ml)	Body [8]	Brain [9]	Body	Brain
1	5	210	10	0.9	0.2	2.4
5	7	210	20	1.1	0.2	2.4
10	10	270	32	1.3	0.1	3.3
15–65	15	270	60–70	1.3	0.1	6.5

Note.—Vol = volume; conc = concentration.

groups. The failure to consider the intracisternal dose on the basis of brain weight was noted in rat experiments to cause variations in the neurotoxicity of contrast media. Larger rats with an adult brain weight received larger doses of contrast medium on a brain-weight basis than smaller adult rats with the same brain weight when dose was calculated on a body-weight basis. This differential resulted in increased neurotoxic effects in the heavier rats [10].

A second factor contributing to the reduced brain metrizamide concentration in younger animals appears to be an age-related difference in the brain itself. When 6-day-old (group 1) dogs were given a metrizamide dose of 11 g/g brain weight, the brain metrizamide concentration was 300 times less than that of the adult (group 3) dogs, which were given a dose of 16 g/g brain weight. We conclude that differences in the brain metrizamide concentration observed between these groups is primarily the result of physiologic age-related differences and cannot be explained solely by a difference in the dose of metrizamide administered.

The precise physiologic differences between the 6-day-old and adult dogs resulting in drastically different brain metrizamide concentrations cannot be definitely ascertained from this experiment. It is possible that there is an increased CSF clearance of metrizamide in the 6-day-old as compared with the adult dogs. This could radically reduce the cisternal concentration of metrizamide, thus reducing its brain entry. Further work in our laboratory has been undertaken to examine this theory. It is also possible that structural differences in the adult brain allow more cisternal metrizamide to enter it. It is extremely unlikely that there is reduced metrizamide clearance from adult brains, since there was no statistical difference in the current experiment between the brain metrizamide clearance rates in the 6-day-old and adult dogs between 4 and 20 hr after injection.

Finally, there are two reasons why it is unlikely that immaturity of the endothelial tight junctions that form the blood-brain barrier in the 6-day-old dogs allowed the escape of metrizamide from the brain extracellular space into the blood, thus lowering the brain metrizamide concentration. The first is that the brain metrizamide clearances between adult dogs and 6-day-old dogs were not statistically different. The second is that the blood-brain barrier integrity in two dogs from the same litter as the 6-day-old dogs in group 1 was tested 6 and 7 days after birth, respectively, and did not demonstrate an immature blood-brain barrier with Evans blue testing.

Although the degree of clinical neurotoxicity observed was proportional to the brain metrizamide concentration during the study period, another factor may have been

operative in causing the neurotoxic effects. When the tritiated metrizamide was extracted from the brain samples by washing with TCA, a part of the metrizamide remained bound to the tissue. To extract this part it was necessary to denature the brain protein. This part of the brain metrizamide, referred to as the bound fraction, was significantly lower in the 6-day-old as compared with the 7-week-old and adult dogs. That is to say, a higher percentage of the metrizamide in the older dogs' brains required denaturing to extract it from the tissue. These findings may indicate that the amount of contrast medium that "binds" to brain is proportional to neurotoxicity and that metrizamide binding to brain increases with age. Since metrizamide binding also occurred in the brain of the dog sacrificed before intrathecal injection with metrizamide, it may have occurred as a postmortem phenomenon during the few minutes that elapsed between death and removal of the brains from the CSF.

Appendix: Synthesis of Tritiated Metrizamide

The following process was used in the synthesis of ^{3}H-labeled metrizamide [2-(3-acetamido-5-*N*-methylacetamido-2,4,6-triiodobenzamido)-deoxy-D-glucose]: The tritium label was introduced in the first step by acetylation of 3-amino-5-*N*-methylacetamido-2,4,6-triiodobenzoyl chloride with acetic anhydride ($[C^3H_3CO]_2O$;NEN; 50 mCi [1.85 GBq]/mmol). From this key intermediate, metrizamide was prepared in good yields by condensation with D-(+)-glucosamine HCl (Sigma) in dimethylforamide (DMF) in the presence of excess K_2CO_3. (Proprietary information provided by T. Jacobson, Nyegaard, Oslo.) Purification of this reaction mixture involved evaporation of DMF under high vacuum and resolution of an aqueous solution of the resulting material on an Amberlite MB-1 (Mallinckrodt) ion exchange column, followed by lipolization to yield an amorphous solid. Recrystallization of the product from boiling isopropanol rendered a microcrystalline white solid whose melting-point characteristics were identical to that of commercially available metrizamide (Sigma), showing a broad range from 240° to 255°C with extensive decomposition. Determination of the mixed melting point of a 50:50 mixture of labeled and commercial metrizamide showed no depression in melting point relative to the separate components.

The chemical purity of the labeled metrizamide was analyzed by mass spectroscopy and thin-layer chromatography. While absolute confirmation of compound identity was not possible by mass spectroscopy owing to low volatility, spectra of the trimethylsilyl derivatives of both labeled and commercial metrizamide were obtained by direct probe insertion (Finnigan 3200), and the two spectra were essentially identical. Thin-layer chromatography (Silica Gel 60 F_{254}; $CHCL_3$:MeOH::2:1) indicated the preponderance of metrizamide (R_f = 0.45) as well as the presence of a small amount of metrizoic acid (R_f = 0.18).

Radiochemical analysis indicated the final product used in the study had a specific activity of 575 μCi(21.3 MBq)/mg. The radiochemical purity of the compound as assayed by thin-layer chromatography indicated that greater than 98% of the tritium label was found in the band identified as metrizamide.

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