The Behavioral and Neuropathologic Sequelae of Intoxication by Trimethyltin Compounds in the Rat

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Trimethyltin, when given by gavage to rats, has an LD_{50} of 12.6 mg/kg. Signs of poisoning include tremors, hyperexcitability, aggressive behavior, weight loss, and convulsions. After single (10 mg/kg) or repeated weekly doses (a maximum of four) of 4 mg/kg, rats, up to a survival time of 70 days, were perfusion-fixed for light microscopy. Trimethyltin was assayed in brain and blood in rats after similar treatments. Trimethyltin is cumulative and persistent and binds with high affinity to hemoglobin. Trimethyltin, unlike triethyltin, does not produce white matter edema in rats but does cause bilateral and symmetrical neuronal alterations involving the hippocampus (largely sparing the Sommer sector), pyriform cortex, amygdaloid nucleus, and neocortex. The earliest alteration was loss or dispersal of Nissl substance, then clumping of nuclear chromatin, followed by shrinkage and fragmentation of the nucleus within shrunken eosinophilic cytoplasm. These changes were associated with approximately 1.4 μg trimethyltin/g wet weight in brain tissue 1 day after the second dose of 4 mg/kg or 2 days after a single dose of 10 mg/kg. Signs of poisoning gradually disappeared, and 4 rats surviving 70 days appeared normal, although their brains had severe damage with cell loss in the hippocampi and each pyriform cortex. Treatment of rats with trimethyltin, therefore, provides a chronic preparation with consistent lesions in the hippocampus of use in other behavioral and neuroanatomic studies. (Am J Pathol 97:59-82, 1979)

THE SIGNS OF POISONING by trimethyltin compounds [1] and tetramethyltin [2-4] are very similar. This similarity is due to the fact that the lower molecular weight tetraalkyltin and lead compounds are rapidly converted to the corresponding trialkylmetals.[5-7]

The main signs of intoxication by trimethyltin compounds in animals are tremors and hyperexcitability. Rats become aggressive and stand up in pairs, facing each other as if sparring.[8,9] Death is always delayed and can be as late as 8 days after a single dose.[9]

Two cases of poisoning by trimethyltin chloride in man have been recently reported.[10] After exposure to a mixture of dimethyl and trimethyltin chlorides, two chemists complained of headache, episodes of violent pain in various organs, as well as memory defects, loss of vigilance, insomnia, anorexia, and disorientation. They then rapidly developed mental confusion with generalized epileptic seizures. Both subjects apparently

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recovered completely after their removal from exposure. A previously unpublished case brought to our notice was that of a chemist exposed to a trimethyltin compound, with symptoms that were described as follows: "For the first few days the subject was prone to hyperactivity and insomnia; this was followed by a few days of alternating hyperactivity and absentmindedness. The symptoms then disappeared and the subject recovered completely."

In the present paper the pathologic characteristics of the brain after both single and repeated doses of trimethyltin chloride are described. To our knowledge the only other neuropathologic report is that of Meynier,[4] who described tigrolysis of the Purkinje cells of the cerebellum after tetramethyltin administration in mice. In the present study the distribution of the compound has also been determined. A preliminary account of this work has already been published.[11]

Materials and Methods

Animals

Adult male (230–270 g) and female (180–190 g) white Wistar rats (Porton strain) were employed in the three experimental groups A, B, and C. For acute dosing (Table 1) and other experiments where trimethyltin chloride and methacrylate were administered at different intervals of time, female rats in the weight range of 180–280 g were used. Animals were allowed water and food *ad libitum*.

Determination of LD_{50}

Trimethyltin chloride (6.3, 10, 15.8, 25, and 40 mg/kg) dissolved in arachis oil was administered by gavage to five groups of 4 male rats. The LD_{50} was calculated by the method of Weil.[12]

Animal Experimental Protocol

Group A

Twenty-eight male rats received by gavage doses (a maximum of four) of trimethyltin chloride (4 mg/kg) in arachis oil at weekly intervals. Twenty-four hours after each dose (Text-figure 1) 2 rats were killed by whole-body perfusion fixation with formaldehyde, glacial acetic acid, and absolute methanol (FAM), 1:1:8. Four more animals were perfusion-fixed after 29 (2 rats), 32, and 35 days (Text-figure 1). Lumbar ganglia, sciatic nerves, and body organs were removed immediately, but the eyes, brain, cervical spinal cord, and optic nerves were left *in situ* in a damp atmosphere for at least 1 hour before their removal and storage in fixative at 4 C overnight. We cut the forebrain in the coronal plane, passing through the pituitary stalk; and blocks (3–4 mm thick) anterior and posterior to this plane and mid and parasagittal slices of the hindbrain, together with all other tissue samples, were embedded in paraffin wax.

More pairs of animals killed at the same intervals were used for the assay of trimethyltin in brain and blood.

Group B

Thirty-two male rats received by gavage doses (a maximum of four) of trimethyltin chloride (first three doses, 4 mg/kg; fourth dose, 2 mg/kg) in arachis oil at weekly intervals.

Pairs of rats were perfusion-fixed with FAM on 7 successive days (Text-figure 1) following the second dose and 1 day (2 rats) and 7 days (1 rat) following the third dose. Three rats were allowed to survive for 70 days after the first dose. Because only the brain and cervical spinal cord were examined in this group, clamping the thoracic aorta produced only brachiocephalic perfusion fixation.

Animals were taken for assay of trimethyltin in blood and brain at three times (see Table 2).

Group C

Twelve female rats received by gavage a single dose (10 mg/kg) of trimethyltin chloride in arachis oil.

Perfusion fixation was carried out as in Group B after survivals of 1–4 days (2 rats each day), 10 days (1 rat), 21 days (2 rats) and 70 days (1 rat).

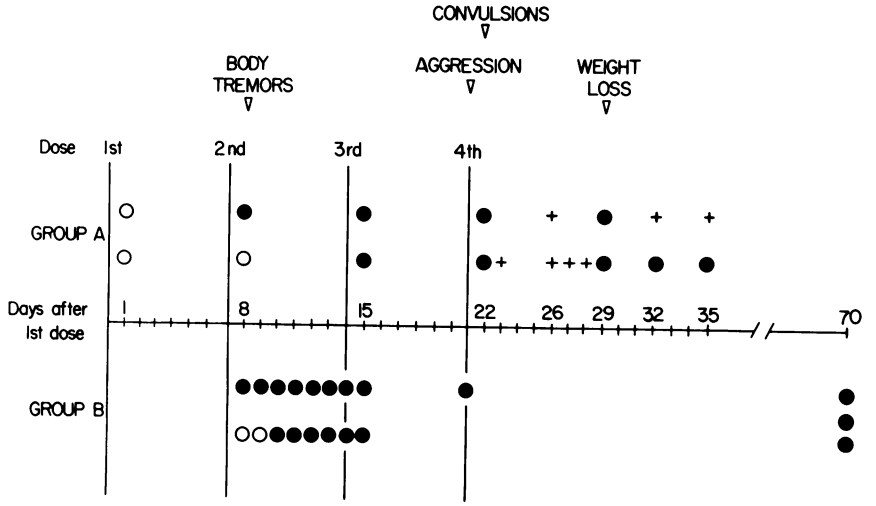
Histologic Techniques

Paraffin sections (7 μ and 12 μ) of nervous tissue were stained with cresyl fast violet, Luxol fast blue, and cresyl fast violet (LFB and CFV); hematoxylin and eosin (H&E); phosphotungstic acid hematoxylin (PTAH); and Marsland, Glees, and Erikson's silver stain. Sections (5 μ) of body organs from Group A were stained with H&E.

Neuronal alterations were assessed quantitatively on an arbitrary three-point scale: 1 = occasional cells, 2 = several cells, and 3 = a majority of the cells involved.

Determination of Trimethyltin

^{113}Sn-trimethyltin chloride was purchased from the Radiochemical Centre, Amersham, Bucks, United Kingdom, and stored as a solution in ethanol. The counting of radioactivity was carried out with the precautions defined by Rose and Aldridge.[13]



TEXT-FIGURE 1—Dosing and perfusion fixation schedule for Groups A and B. All doses 4 mg/kg body weight except the fourth dose in Group B, which was 2 mg/kg body weight. ○ = no brain damage; ● = damaged brains; + = found dead.

Trimethyltin chloride was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Trimethyltin methacrylate was a gift from Amalgamated Dental Company, Weybridge, Surrey, United Kingdom, and 3-hydroxyflavone from Eastman Kodak Company, Rochester, New York.

Trimethyltin was determined in blood and brain tissue by a development (Aldridge and Street, manuscript in preparation) of the methods using dithizone [14] and 3-hydroxyflavone.[15,16] The rats were anesthetized with ether, as much blood as possible was removed by cardiac puncture into a heparinized syringe, and the brain was then removed.

For the description of acutely dosed animals see Table 1.

Determination of Blood in Brain

Rat erythrocytes were radiolabeled by ^{51}Cr-sodium chromate (Radiochemical Centre, Amersham, Bucks, UK). The blood was then reconstituted with plasma and injected intravenously into the tail veins of 6 control and 6 trimethyltin-treated rats. From the ^{51}Cr content of blood and brain, the blood content was calculated.[17,18]

Results

The oral LD_{50} of trimethyltin chloride in arachis oil was 12.6 mg/kg. During the course of the LD_{50} determinations, the first signs of poisoning were observed 4 hours after dosing, the rats being limp and inactive, with staring coats. By 26 hours fine tremors were first observed in the 40 mg/kg group. At 48 hours three groups (15.8, 25, and 40 mg/kg) showed varying degrees of tremor and prostration. Fatalities were observed 48–120 hours after treatment, those receiving the larger doses dying earlier. Over this period those receiving 10 mg/kg became extremely aggressive and at 72 hours required separation into individual cages. At 7 days survivors were gaining weight, and it was possible to return the isolated animals to the communal cage without signs of aggression.

Distribution of Trimethyltin in Rats

In Tables 1 and 2 are measurements of the trimethyltin content of blood and brain after a variety of dosing schedules. Like tryethyltin, trimethyltin [13] binds with highly affinity to rat hemoglobin [19] and distributes in blood very much in favor of the erythrocytes. Calculation from the results in Table 1 indicates a ratio of erythrocytes to plasma of around 120. It is clear that trimethyltin, even though it is a rather water-soluble compound, is very cumulative and persistent. The trimethyltin content of brain listed in Tables 1 and 2 is uncorrected for the erythrocyte content of brain tissue. The blood content of brain has been determined using ^{51}Cr-labeled erythrocytes and found to be for control rats (0.1 ml arachis oil/100 g body weight) and trimethyltin rats (10 mg trimethyltin chloride in 0.1 ml arachis oil per kilogram body weight) 11.1 ± 0.6 (6) and 12.5 ± 0.5

Table 1—Trimethyltin in Blood and Brain of Rats after a Single Dose

Group	Time after injection (hr)	μg Trimethyltin/g wet weight or ml (mean ± SE)		
		Blood	Plasma	Brain
1*				
	0.17	61.0 ± 1.8	0.92 ± 0.04	1.38 ± 0.06
	1	57.2 ± 0.9	1.39 ± 0.11	1.58 ± 0.05
	2	65.8 ± 1.0	1.09 ± 0.04	1.80 ± 0.11
	4	58.7 ± 2.4	0.91 ± 0.06	1.61 ± 0.03
	8	63.4 ± 4.0	0.79 ± 0.01	1.77 ± 0.10
	24	51.6 ± 2.8	1.12 ± 0.12	1.44 ± 0.07
	48	59.3 ± 2.4	0.92 ± 0.05	1.70 ± 0.04
2†				
	3	—	—	3.28 ± 0.13
3‡				
	24	136.1 ± 4.4	—	3.44 ± 0.23
4§				
	5 days	107.9 ± 2.0	—	2.89 ± 0.07

* Three rats in each group given 5 mg/kg intravenously (^{113}Sn-trimethyltin chloride).
† Four rats given 10 mg/kg intravenously (Dithizone method).
‡ Four rats given 12.5 mg/kg orally (3-hydroxyflavone method).
§ Six rats given 10 mg/kg orally (3-hydroxyflavone method).

(6) μl/g wet weight, respectively. Calculation of the trimethyltin content of brain tissue due to contamination by erythrocytes indicates that it is 46.6 ± 1.1% (6) of the total. The minimal dose to produce neuronal damage in the hippocampus is two doses of 4 mg/kg 1 week apart (Table 3). Of the 2.8 μg/g wet weight overall content of trimethyltin in the brain of such animals 1.4 μg trimethyltin is present in the brain tissue.

The 3-hydroxyflavone technique (Aldridge and Street, manuscript in preparation) is able to differentiate between trimethyltin and dimethyltin. Any dimethyltin present is probably less than 5% of the trimethyltin concentration.

Table 2—Trimethyltin Content of Blood and Brain of Rats After Multiple Dosing

Doses* (time of determination after dose)	Group†	μg Trimethyltin/g wet weight or ml (mean ± SE)	
		Blood	Brain
1 (1 day)	A	53.7 (2)‡	1.80 (2)‡
2 (1 day)	A, B	109.3 ± 1.8 (6)	2.75 ± 0.18 (6)
2 (4 days)	B	83.3 ± 10.5 (4)	1.74 ± 0.09 (4)
3 (1 day)	A, B	116.8 ± 6.6 (6)	3.08 ± 3.3 (6)
4 (1 day)	A	166.9 (2)	4.82 (2)
4 (8 days)	A	131.3 (1)	4.25 (1)

* Dosing by gavage of trimethyltin chloride (4 mg/kg body weight) in arachis oil once every 7 days. Trimethyltin determined by the 3-hydroxyflavone technique.
† For description of animals in Groups A and B, see Methods.
‡ Number of animals.

Table 3—Distribution, Grade, and Frequency of Neuronal Damage in Groups A and B After Multiple Dosing

Animal no. and group	Survival (days)	Doses	Neocortex	Hippocampus				Pyriform cortex	Amygdaloid nucleus	Cerebellum Purkinje	Spinal cord
				h 1	h 2	h 3–5	Fd				
1 A	1	1	0	0	0	0	0	0	0	0	0
2 A	1	1	0	0	0	0	0	0	0	0	0
3 A	8	2	0	0	1	1	0	0	0	0	0
4 A	8	2	0	0	0	0	0	0	0	0	0
21 B	8	2	0	0	1	0	0	0	0	0	0
22 B	8	2	0	0	0	0	0	0	0	0	0
23 B	9	2	0	0	1	1	0	0	0	0	0
24 B	9	2	0	0	0	0	0	0	0	0	0
25 B	10	2	0	0	0	1	0	0	0	0	0
26 B	10	2	0	1	1	1	0	1	1	0	0
27 B	11	2	0	1	1	1	0	1	1	0	0
28 B	11	2	0	1	1	2	0	1	1	0	0
29 B	12	2	0	1	1	1	0	2	1	0	0
30 B	12	2	0	1	1	1	0	1	1	0	0
31 B	13	2	0	1	1	1	0	2	1	0	0
32 B	13	2	0	2	1	2	0	2	1	0	0
33 B	14	2	0	1	1	2	0	2	1	0	0
34 B	14	2	0	1	1	2	0	1	1	0	0
5 A	15	3	0	1	1	2	0	1	1	0	0
6 A	15	3	0	1	1	2	0	1	1	0	0
35 B	15	3	0	1	1	2	0	2	1	0	0
36 B	15	3	0	1	2	2	0	1	1	0	0
103 B	21	3	1	2	1	2	1	3	2	0	0
7 A	22	4	1	2	2	3	1	2	2	0	1
8 A	22	4	1	1	1	2	0	2	1	0	1
9 A	29	4	1	2	2	3	2	3	2	0	1
10 A	29	4	2	3	2	3	2	3	2	1	1
11 A	32	4	1	2	1	2	2	3	2	1	1
12 A	35	4	1	3	3	3	2	3	2	1	0
104 B	70	4	2	3	3	3	2	3	2	0	0
105 B	70	4	1	2	3	3	1	3	2	0	0
106 B	70	4	2	2	3	3	1	3	2	0	0
Frequency (out of 32)			10	23	26	26	9	23	23	3	5

Fd = Fascia dentata; 0 = no damage, 1 = occasional cells damaged, 2 = several cells damaged, 3 = majority of cells damaged. All doses 4 mg/kg body weight except the fourth dose in Group B, which was 2 mg/kg body weight.

Behavioral Changes After Single and Multiple Dosing

Tremors of head and neck muscles were first observed in 1 rat of Group B 24 hours after the second dose. Other rats of this group gradually developed similar tremors, and they occurred in the majority of animals of both Groups A and B after the third dose. One animal in Group B developed generalized body tremors 2 days after the fourth dose. In Group C 1 rat developed tremors of head and neck within 24 hours after the single dose, and a second rat within 48 hours. After 4 days the survivors of Group C developed generalized body tremors. One showed considerable respiratory distress at 9 days and was perfusion-fixed the following day.

Animals in Groups A and B became very irritable and hyperexcitable, with staring coats, 7 days after the third dose (Text-figure 1), and cage mates frequently engaged in sparring activity, standing up in pairs, facing each other. Convulsions of varying severity, first observed the day after the fourth dose, occurred very occasionally, and sometimes these seemed to be precipitated by the sparring activity or sudden noise or movement near the cage. Finally, the aggressive activity became so violent that the animals had to be placed in separate cages. In Group C similar aggressive behavior, sparring activity, and convulsions were observed after 3 days of survival. However, after 18 days it was possible to return the 3 remaining rats of this group to a single cage, where after 2 hours of considerable agitation and slight aggression they settled down quietly. The condition of the rats in Group A rapidly deteriorated after the fourth dose. They lost weight, and 7 died 48 hours or more after this last dose (Text-figure 1). Two rats killed after 29 days were moribund at the time of perfusion. Body tremors gradually subsided in the 4 animals of Groups B and C that were allowed to survive for 70 days, and they appeared normal at the time of perfusion.

On a 14-day schedule trimethyltin chloride and trimethyltin methacrylate were approximately equitoxic.

Pathologic Characteristics

General

Histologic examination of the body organs of the perfusion-fixed animals in Group A provided no cause of death outside the central nervous system in the 7 rats found dead. In the first pair of animals killed after 24 hours several mitoses were seen in the liver. Hydronephrosis was present in 6 rats and vacuolar degeneration of renal tubules in the 2 animals killed

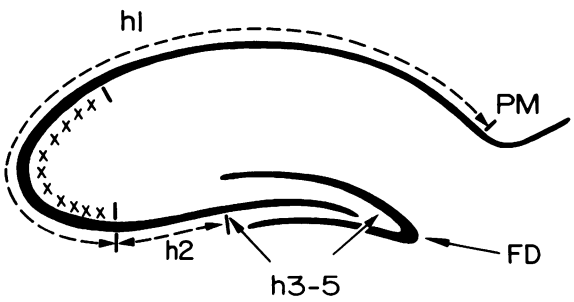
after 32 and 35 days of survival (Text-figure 1). There was slight atrophy of the spleen in one of the animals surviving 29 days.

Neuropathologic

In Group A macroscopic examination showed minimal brain swelling, indicated by herniation of the inferior cerebellar vermis in 4 of the 12 perfusion-fixed animals, in all 7 rats found dead, and in the majority of animals of Groups B and C. On slicing the fixed brains the hippocampi were seen to be shrunken and the lateral ventricles enlarged in the 2 rats of Group A surviving 32 and 35 days and in all 4 rats of Groups B and C surviving 70 days.

Microscopic examination of the retinas, optic nerves, lumbar spinal ganglia, and sciatic nerves revealed no abnormalities. In all three groups, while there was no evidence of white matter edema, there was unequivocal evidence of neuronal damage, which was invariably bilateral and symmetrical. In the forebrain this damage eventually involved, in longer-surviving animals, the neocortex, the pyriform cortex, the amygdaloid nucleus, and the whole of the hippocampus but with the frequent exception of the Sommer sector of h1 (Text-figure 2). In the neocortex damaged neurons were, except in 2 animals, rare and diffusely scattered in layers II, III, and V of the paramedian and lateral regions.

The neuronal alterations which in the longer-surviving animals culminated in naked nuclei, cell "ghosts" and eventual cell loss were characterized by condensation and clumping of nuclear chromatin, giving a fragmented appearance to the shrunken nucleus with occasional karyorrhexis (Figure 1). The cytoplasm appeared shrunken and eosinophilic. In a less frequent variation, probably the precursor of the above alteration, the condensation and clumping of nuclear chromatin occurred within a nucleus of normal size, while the eosinophilic cytoplasm showed only minor shrinkage (Figure 2). In some cells the nuclei were not fragmented and appeared as shrunken, variably stained, oval or triangular masses (Figures 1



TEXT-FIGURE 2—Schematic diagram of the hippocampus in a coronal plane showing regions h 1–5 and the Sommer sector (× × × ×) of h 1. PM = paramedian zone; FD = fascia dentata.

and 2). Among these damaged cells, with their conspicuous nuclear changes, were other neurons in which the nucleus was normal but the unshrunken cytoplasm showed loss or dispersal of Nissl substance and moderate eosinophilia (Figure 3).

A second and quite distinct type of neuronal alteration observed in a minority of animals of the three groups was selective chromatolysis (SC). In this alteration, recently described by Brown and co-workers [20,21] and Brierley and co-workers,[22] the nucleus becomes eccentric and usually kidney-shaped. The Nissl substance is lost in the central brightly eosinophilic cytoplasm (Figure 4).

Groups A and B

The brains of 5 animals were normal (Text-figure 1; Table 3). The distribution and grade of the altered neurons showing nuclear changes (but excluding SC) in the remaining 27 rats are shown in Table 3.

In 3 rats killed 1 and 2 days after the second dose, damage was minimal and confined to h 2 and h 3–5 (Figure 5) of the hippocampus. Occasional neurons in these regions showed loss or dispersal of Nissl material (Figure 5) but were otherwise normal.

As survival increased after the second dose, the damage additionally involved h 1, the pyriform cortex (Figure 1), and the amygdaloid nucleus. An increasing number of neurons in the vulnerable regions (Figure 3) showed a loss of Nissl substance but were otherwise normal. Reactive astrocytes (with mitoses) and microglia (Figure 1), first observed 3 days after the second dose, became more numerous in the damaged regions; and a few examples of neuronophagia were seen.

In 4 rats killed 1 day after the third dose, damaged neurons occurred in the vulnerable sites (Figure 6) described above; but in a fifth rat killed 7 days after the third dose, Grade 1 damage also involved the fascia dentata of the hippocampus and scattered cells in the lateral neocortex. In all 5 rats there was neuronophagia (Figure 7) and a gliomesodermal reaction.

In the 6 animals of Group A killed after the fourth dose, the most severe damage, with some cell loss and marked gliomesodermal reaction (Figure 8), was seen in the hippocampus (sparing the Sommer sector and fascia dentata) and pyriform cortex. In the usually vulnerable sites many of the otherwise unaltered neurons showed a loss of Nissl substance. In the 2 rats surviving 32 and 35 days there was a shrinkage of the hippocampi and an enlargement of the ventricles, as already seen macroscopically (Figure 9).

In the 4 animals surviving 29, 32, and 35 days, damage in the cerebellum included white matter damage in folia with retraction balls demonstrable with silver stain and also small foci of vacuolation in the outer half

of the molecular layer (Figure 10). In 3 of these animals occasional damaged Purkinje cells with shrunken nuclei and brightly eosinophilic cytoplasm (Figure 10) showed thickenings (torpedoes) of their axons. Several chromatolytic neurons were present in the brain stems of all 4 animals. Occasional cells showing central chromatolysis occurred in the ventral horns of the cervical spinal cords of the 5 rats surviving 22–32 days.

In all 3 animals of Group B killed after 70 days, there was severe damage, including cell loss and a corresponding gliomesodermal reaction in the hippocampus and pyriform cortex. In all 3 rats there was Grade 1 damage in the ventrolateral portions of the Sommer sectors. The hippocampi were shrunken, each pyriform cortex was thinned, and the ventricles were enlarged (Figure 11).

In both groups SC (with one exception all Grade 1) was seen in decreasing order of frequency in the pyriform cortex, amygdaloid nucleus, thalamus, neocortex, and h 3–5 in 8 animals (Animals 5, 6, 7, 9, 10, 29, 35, 36; Table 3).

Group C

The brains of 2 rats killed 1 day after the single dose were normal. The distribution and grade of the altered neurons were normal. The distribution and grade of the altered neurons showing nuclear changes (excluding SC) in the remaining 10 damaged brains are shown in Table 4.

In 2 animals killed after 2 days, Grade 1 damage was confined to h 2, h 3–5, and the fascia dentata of the hippocampus of one and the fascia dentata alone in the other. In addition to neurons showing the typical nuclear alterations, several in the above regions and in h 1 (but sparing the Sommer sector), the pyriform cortex and amygdaloid nucleus only showed a loss of Nissl substance and increased eosinophilia of the cytoplasm.

In the 4 rats killed after 3 and 4 days, damage additionally involved h 1, the neocortex, the pyriform cortex, and the amygdaloid nucleus (3 rats only). The fascia dentata (Figure 2) was damaged in all 4. The left Sommer sector of 1 rat had 1 or 2 damaged cells. The majority of neurons in the hippocampus and pyriform cortex and a much smaller proportion in the Sommer sector of h 1 and the amygdaloid nucleus showed a loss of Nissl substance with eosinophilia of the cytoplasm. In addition, SC was seen in the pyriform cortex (Grade 2 in 3) of all 4 rats. It also occurred in h 3–5 (Grade 1) of 3 and in the amygdaloid nucleus (Grade 2 in one) of 3. In both animals killed at 3 days, there were small foci of vacuolation in the molecular layer of the cerebellum, while the Purkinje cells were normal.

Table 4—Distribution, Grade, and Frequency of Neuronal Damage in Group C After a Single Dose*

Animal no.	Survival (days)	Neocortex	Hippocampus				Pyriform cortex	Amygdaloid nucleus	Cerebellum Purkinje
			h 1	h 2	h 3–5	Fd			
13	1	0	0	0	0	0	0	0	0
14	1	0	0	0	0	0	0	0	0
15	2	0	0	1	1	1	0	0	0
16	2	0	0	0	0	1	0	0	0
17	3	1	1	1	1	2	2	1	0
18	3	1	1	0	0	1	1	0	0
19	4	1	1	0	1	2	2	1	0
20	4	1	1	1	1	1	1	1	0
100	10	1	2	1	1	1	3	2	0
101	21	1	2	3	3	1	3	3	0
102	21	2	3	3	3	2	3	3	1
107	70	1	3	3	3	2	2	2	0
Frequency (out of 12)		8	8	7	8	10	8	7	1

* Single dose = 10 mg/kg body weight.
Fd = Fascia dentata; 0 = no damage, 1 = occasional cells damaged, 2 = several cells damaged, 3 = majority of cells damaged.

The pyriform cortex was the most severely damage region in the one animal killed after 10 days. Many large reactive astrocytes and rod cells were seen here, also in the damaged hippocampus, and to a much lesser extent in the lateral neocortex. Neuronophagia was seen in the pyriform cortex.

Both animals killed at 21 days had Grade 3 damage in the pyriform cortex, h 2, h 3–5, and the amygdaloid nucleus, accompanied by striking gliomesodermal reactions (Figure 12). In both rats Grade 2 damage occurred in the Sommer sector. Occasional damaged Purkinje cells were seen in the cerebellum of one.

The one animal allowed to survive for 70 days had Grade 3 damage (with cell loss; Figure 13) and an intense gliomesodermal reaction throughout the shrunken hippocampi, with the exception of the dorsolateral portions of the Sommer sectors, which were preserved, and the fascia dentata (Grade 2). Lesser damage involved the other vulnerable sites. The ventricles were enlarged, as in Group B.

Discussion

The present investigation has shown that either single or repeated doses of trimethyltin, unlike triethyltin and other higher homologs, does not produce edema of the central nervous system.[9,23] The finding of unequivocal bilaterally symmetrical neuronal damage in clearly defined sites in optimally perfusion-fixed brains contrasts with the finding of minimal neuronal damage following triethyltin intoxication [23] and provides a further example of the remarkable selective vulnerability of the brain to noxious agents.

In the repeated-dose series (Table 3) the most vulnerable regions were the large pyramidal cell band h 1–5 of the hippocampus (largely sparing the Sommer sector), the pyriform cortex, and the amygdaloid nucleus. In the single-dose series (Table 4) there was a comparable pattern of vulnerability, except for the early and consistent involvement of the fascia dentata. This contrasts with its late involvement in the repeated-dose groups and may represent a rapid buildup in concentration at this site following a single large dose. However, in all three groups Grade 3 damage was never attained in this region of the hippocampus or in the neocortex. A remarkably constant and inexplicable feature was the relative sparing of the Sommer sector of h 1, particularly its dorsolateral region, although there was increasing involvement of the ventrolateral region in the longer-surviving animals.

The sequence of early changes that eventually leads to the death and

disappearance of neurons apparently begins with a loss or dispersal of Nissl substance within unshrunken cytoplasm, while the nucleus remains normal. This is followed by condensation and clumping of nuclear chromatin within the unshrunken nucleus, while the cytoplasm shows slight shrinkage. This stage, as judged by its relative infrequency, does not persist long. The nucleus then shrinks and is often fragmented, while the cytoplasm becomes more shrunken and brightly eosinophilic. This latter stage resembles the later stage of ischemic cell change, ie, homogenizing cell change.[24,25] However, unlike homogenizing cell change, it is not preceded by microvacuolation, ie, swelling of mitochondria.[26,27] The presence of all stages in this sequence at any time up to 35 days in Group A suggests a continuous recruitment of damaged neurons with an increasing concentration of the neurotoxin. In all three groups, it appears, dissolution of Nissl material in cells is followed by their eventual destruction and removal by phagocytosis; this early loss of Nissl material may persist for varying time periods before the nucleus becomes involved.

While it might be premature to speculate on the significance of the early dissolution of Nissl material in the absence of ultrastructural studies, a probable disruption of the polysomal clusters of ribosomes to form free single ribosomes would indicate a cessation of RNA and protein synthesis,[28] while progressive depletion of all components of the granular endoplasmic reticulum could be regarded as a change leading to nerve-cell death.[29] Further, it has been shown that dense chromatin is not active in RNA synthesis.[30] Thus, the clumping of nuclear chromatin, one of the first signs of cell death, has been interpreted as nuclear inactivation with a termination of transcription.[31,32]

The distinctive neuronal alteration, selective chromatolysis, seen in all three groups of the present study was first described by Ito and co-workers [33] in the gerbil brain following unilateral carotid occlusion. However, they termed it "reactive change" and attributed it solely to ischemia. More recently Brown and co-workers [20,21] and Brierley and co-workers [22] demonstrated in the gerbil that this neuronal alteration, which they termed "SC" because of its cytologic features and restriction to h 1 and the deeper layers of the neocortex, was entirely distinct from classical ischemic cell change. Unlike ischemic cell change it was not recognisable before 3–4 hours in paraffin sections, it appeared to have a slow time course, and its frequency was not related to the duration of ischemia but probably to the epileptic seizures (overt and subclinical) that follow carotid occlusion in this species.

In the rat SC was observed in 3 of a series of 150 Levine [34] preparations

surviving 1 and 2 days after exposure to nitrogen,[21] where it was confined to the paramedian region of the hippocampus in 2 and the thalamus in 1. All 3 rats had severe convulsions not only after exposure to nitrogen but during the few hours before perfusion fixation, suggesting an association between SC and epileptic seizures in this species also. There may be a similar association in the present series. The one animal in Group A with the most severe and widespread distribution of SC was moribund at the time of perfusion, possibly as a consequence of seizure activity. The presence of brain swelling in all the animals of Group A found dead suggests that they may have died as a result of seizure, since examination of the body organs showed no lesions that might cause death.

In searching for an explanation for the selective distribution of the brain damage, vascular factors are probably not involved. The hippocampus might be expected to differ with respect to its blood flow from the pyriform cortex, due to the length and winding course of the artery supplying the Sommer sector [35] and the rake-like arrangements of the branches of the main artery.[36,37] In the present study both regions exhibited similar orders of vulnerability. Further, within the hippocampus itself the sparing of the Sommer sector of h 1 could not be explained on a vascular basis.

Authors of the concept of pathoclisis [38,39] proposed that specific physiochemical properties within the vulnerable regions or topistic units are responsible for the varying susceptibility to noxious agents. In the light of present knowledge, it is clear that in the hippocampus metabolic differentiation is present. Maske [40] has demonstrated a high concentration of zinc in h 2 and h 3 and a low concentration in h 1 of animals, findings that have been confirmed in man by Friede. [41] McLardy [42] showed that the zinc is concentrated in the terminations of the mossy fibers coming from the dentate gyrus. Friede [41] found a high level of lactic dehydrogenase in h 2 and h 3. The finding of Coggeshall and MacLean [43] that a single dose of 3-acetylpyridine, an analog of nicotinic acid, produces partial or complete loss of neurons in h 2 and h 3 of the hippocampi of adult mice provides further evidence of metabolic differentiation within that region. However, no such evidence can explain the severe damage in h 2–5 and the sparing of the Sommer sector in the present series. Perhaps other biochemical factors as yet unknown but common to the hippocampus, pyriform cortex, amygdaloid nucleus, and the neocortex may explain the selective neurotoxicity of the compound.

Trimethyltin in the rat is very cumulative and persitent. Calculation from the results in Table 1 and the use of the blood volume determined by

the ^{51}Cr method show that approximately 70% of the injected trimethyltin is present in the blood. It is not known whether the accumulation and persistence of trimethyltin is determined by the depot produced by its binding to rat hemoglobin. The affinity of trimethyltin for rat hemoglobin is 3.8×10^5 M^{-1} but for many other hemoglobins is much less.[13,19] Whether trimethyltin is cumulative in other species would be informative.

The minimum overall concentration in brain associated with neuronal damage is approximately 1.4 μg/g wet weight. Further work will be required to establish whether the selective damage to neurons in the hippocampus is due to a higher concentration of trimethyltin in these cells or to a difference in their intrinsic sensitivity to this compound; the latter would presumably reflect some metabolic differentiation in the affected cells.

The relationship between the striking behavioral changes and the selective pattern of brain damage produced by trimethyltin is of considerable interest. The hippocampus, the amygdaloid nucleus, and the pyriform cortex are all part of the so-called limbic system, which is regarded as being concerned with the behavioral expression of emotions. There is evidence that the amygdaloid complex mediates in the rage–fear response[44]; and the hippocampus, involved in learning and recent memory processes, may provide the delicate dynamic equilibrium of the entire system. It is not surprising, therefore, that the extremely aggressive behavior found in the present study is manifested by animals with lesions in these regions.

Convulsions were first observed in Groups A and B on the day following the first signs of aggression and may have been related to the moderate to severe damage with a gliomesodermal reaction in the hippocampi and amygdala. Damage to the fascia dentata first occurred around this time. In Group C there was a similar close correlation between aggression and convulsions, both occurring on the third day following the single dose. Damage at this time was minimal, with no glial reaction in the hippocampi and amygdala; but the fascia dentata was already involved.

It is possible that the onset of aggressive behavior, and possibly the convulsions, is due to the presence of trimethyltin in the vulnerable sites rather than the eventual cell death. In Group A the highest concentration in the brain was attained on the day following the fourth dose and just after the onset of overt aggression. A similar effect may account for the finding that aggressive behavior died out in 3 survivors of Group C when they were placed together again after 18 days survival; yet the brains of 2 of these showed severe damage in the vulnerable sites when they were killed at 21 days. It is noteworthy that in spite of extensive bilateral damage, in-

cluding shrinkage of the hippocampi with cell loss, the animals surviving for 70 days looked normal. Kimble [45] reported no apparent sensory or motor deficit in rats where aspiration techniques were used to produce large bilateral hippocampal lesions with virtually complete removal of the dorsal and dorsolateral portions of the hippocampus, including the posterior dorsal fornix and portions of the subiculum. It was not until specific measurements were made that any behavioral changes could be identified. In the gerbil, clinically normal long-term survivors of right common carotid occlusion with extensive damage in the corresponding hemisphere have been reported.[24]

The present findings provide a chronic preparation with consistent lesions in the hippocampus that could be used for other behavioral and neuroanatomic studies.

It has been suggested that in man intoxication by trimethyltin is less severe than that caused by triethyltin.[10] In the rat our experiments have shown that trimethyltin produces severe and permanent damage in the central nervous system. In contrast, triethyltin in the rat and man produces a different lesion, which is reversible.[23,46]

In view of the remarkable differences in behavior and neuropathologic characteristics in rats after intoxication by trimethyltin and triethyltin, an extensive study of the structure–activity relationships for the triorgano-tin and -lead compounds is in progress.

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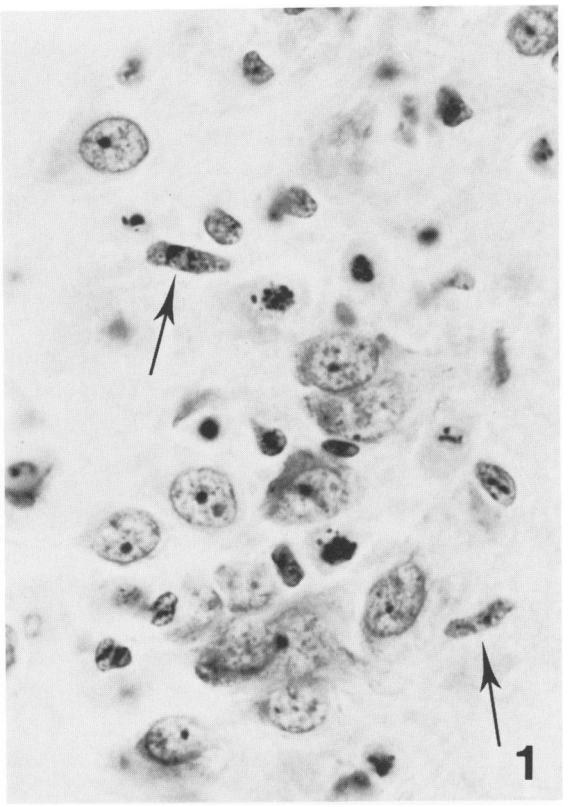
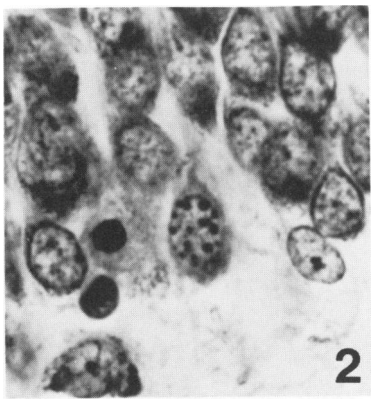
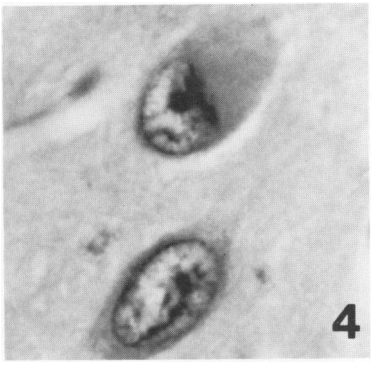






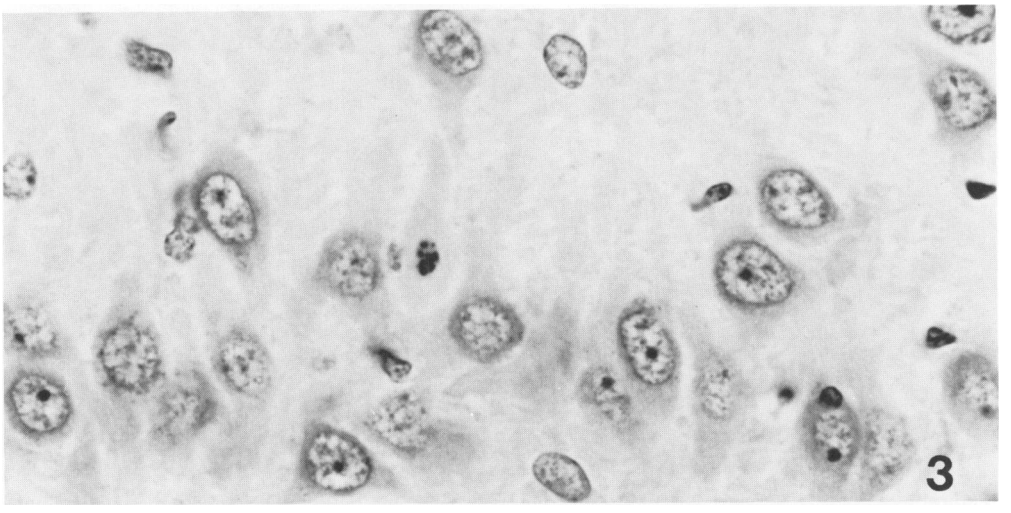
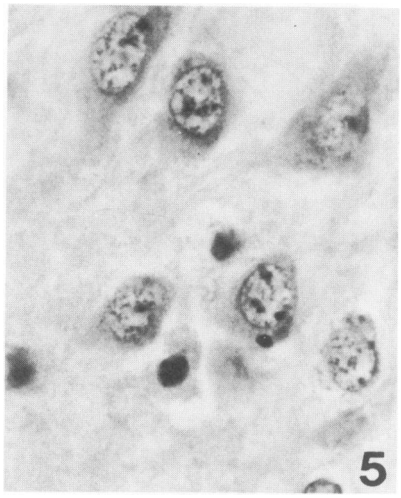
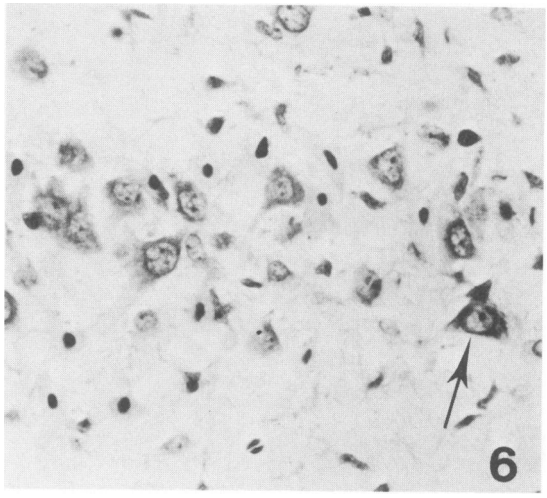
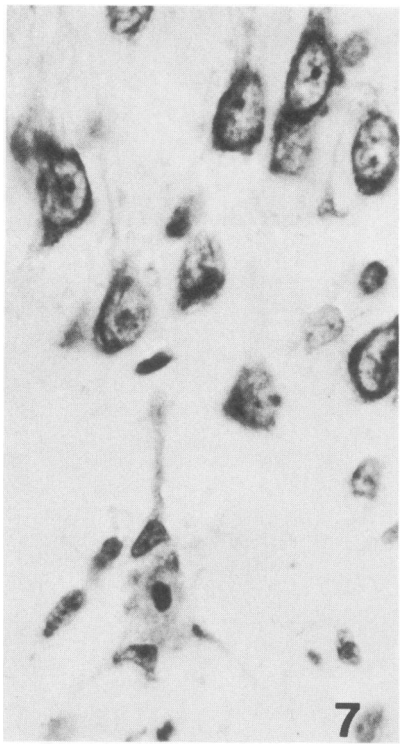


Figure 1—Group B, survival 13 days. Several damaged neurons with fragmented nuclei and eosinophilic cytoplasm in the pyriform cortex. Note the two microglial rod cells (*arrows*). (H&E, ×880) **Figure 2**—Group C, survival 3 days. A damaged neuron in the fascia dentata showing condensation and clumping of chromatin in the unshrunken nucleus. Adjacent neuron has a pyknotic nucleus. (LFB&CFV, ×1024) **Figure 3**—As in Figure 1. Hippocampus h 3–5, showing the majority of neurons with normal nuclei. Their unshrunken cytoplasm shows loss or dispersal of Nissl substance and moderate eosinophilia. A shrunken neuron (*center*) with a fragmented nucleus and eosinophilic cytoplasm (H&E, ×880) **Figure 4**—Group C, survival 4 days. A neuron (*top*) in the pyriform cortex showing selective chromatolysis. The nucleus is eccentric and the cytoplasm brightly eosinophilic. (H&E, ×1180)







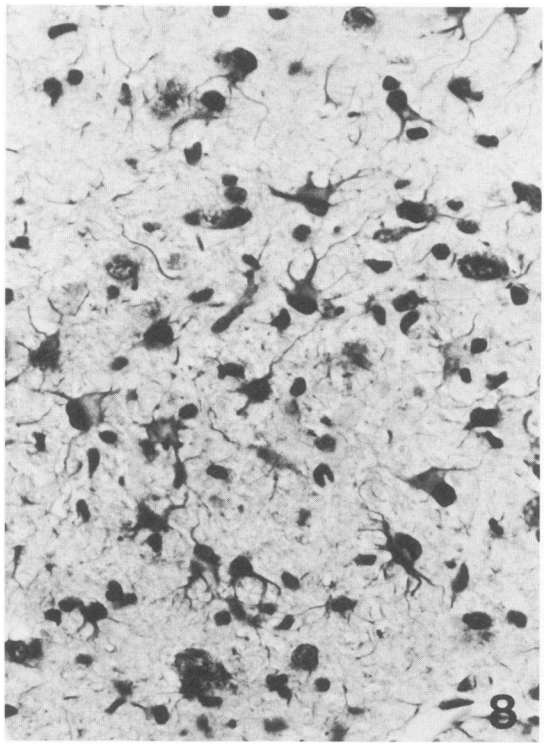
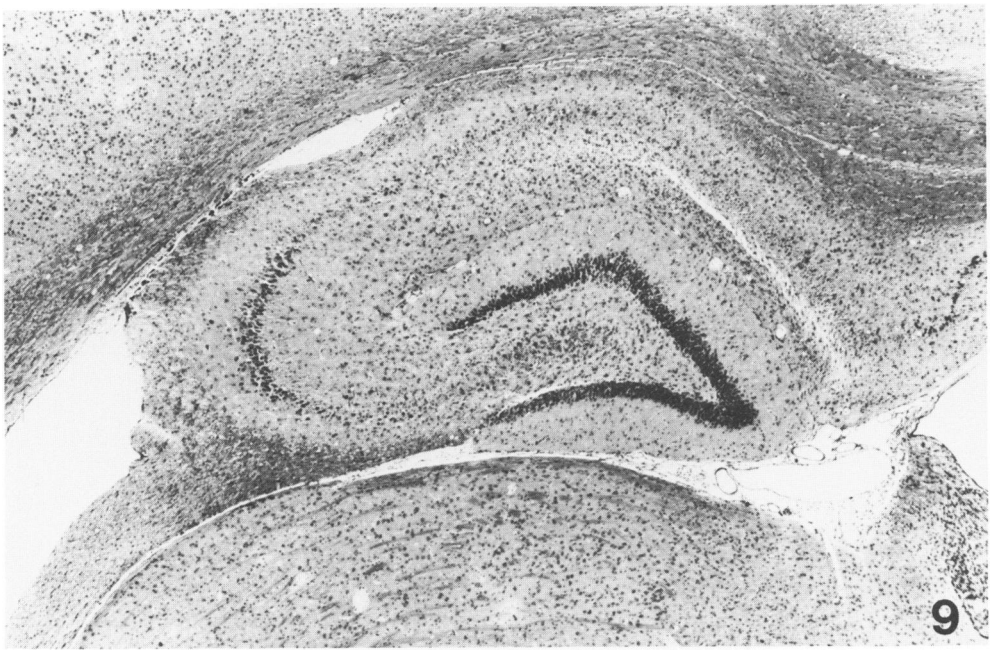


Figure 5—Group B, survival 9 days. Hippocampus h 3–5. One neuron with a pyknotic nucleus and shrunken eosinophilic cytoplasm. The remaining neurons show a loss of Nissl substance. (H&E, ×900) **Figure 6**—Group A, survival 15 days. Hippocampus h 3–5. Damaged neurons with pyknotic nuclei and shrunken, faintly staining cytoplasm accompanied by reactive astrocytes and microglia. Other neurons with normal nuclei show a loss of Nissl substance. Note the normal neuron (*arrow*). (LFB&CFV, ×430) **Figure 7**—As in Figure 6. Neuronophagia in the pyriform cortex; the damaged neuron is surrounded by microglial cells. (LFB&CFV, ×665) **Figure 8**—Group A, survival 29 days. Hippocampus h 3–5 showing severe damage with a marked gliomesodermal reaction. Many neurons are reduced to naked nuclei, and there is cell loss. Note the large gemistocytic astrocytes. (PTAH, ×450)



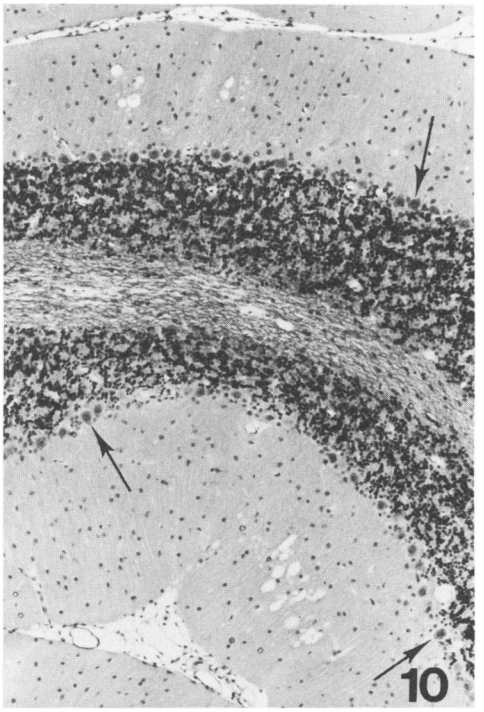
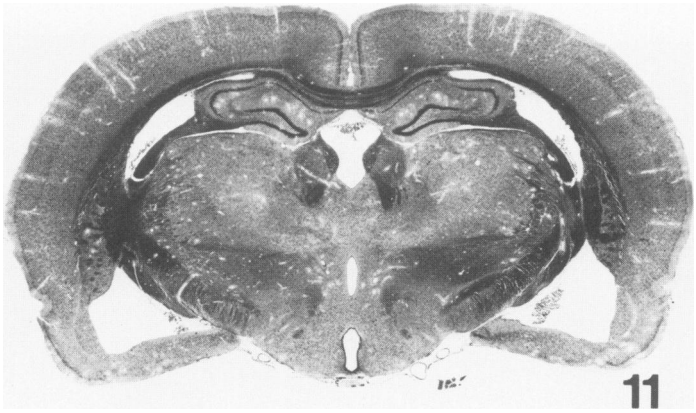


Figure 9—Group A, survival 35 days. Slight shrinkage of the hippocampus and enlargement of the ventricles. There is extensive cell loss in the pyramidal cell band (largely sparing the Sommer sector) and a marked gliomesodermal reaction. (PTAH, ×33) **Figure 10**—Group A, survival 29 days. Cerebellum showing damaged white matter in a folium, small foci of vacuolation in the molecular layer, and damaged eosinophilic Purkinje neurons (*arrows*). (H&E, ×85)



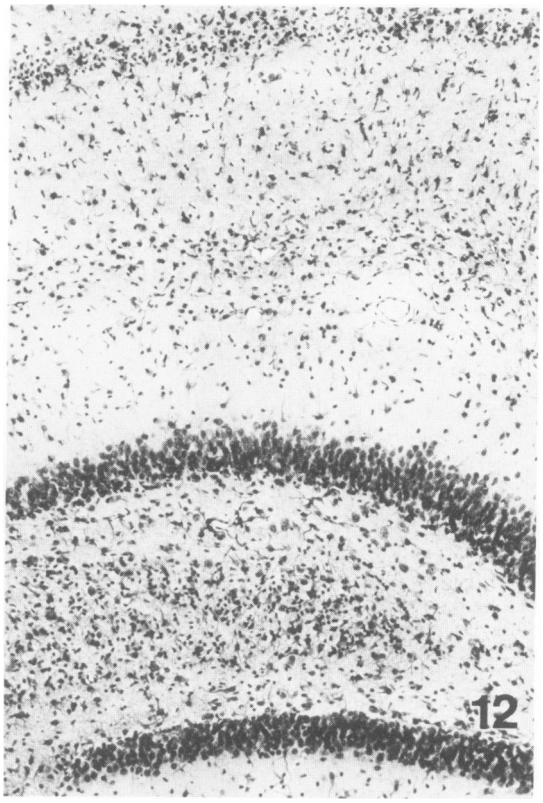


Figure 11—Group B, survival 70 days. Low-power micrograph showing bilateral shrinkage of the hippocampi and enlargement of the ventricles. (PTAH, ×7) **Figure 12**—Group C, survival 21 days. Hippocampus with severe neuronal damage in h 1 (*top*) and h 3–5 accompanied by a marked gliomesodermal reaction. (PTAH, ×96)

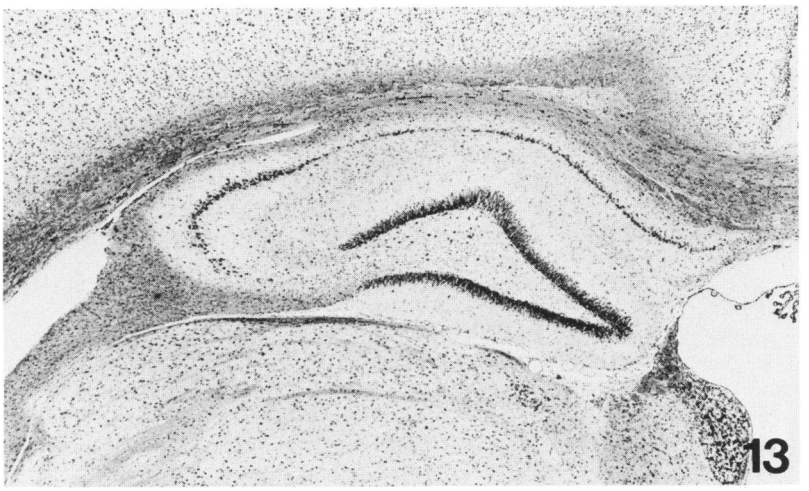


Figure 13—Group C, survival 70 days. Hippocampus showing slight shrinkage with marked cell loss in h 1, h 2, and h 3–5 but preservation of the dorsolateral portion of the Sommer sector. The ventricles are enlarged. (LFB&CFV, ×24)

[*End of Article*]