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Effects of Dieldrin, Picrotoxin and Telodrin on the Metabolism of Ammonia in Brain

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1. Increases in the concentrations of lactic acid and pyruvic acid in rat brain during acute dieldrin poisoning are associated with hyperactivity of the brain, whereas an increase in the cerebral alanine concentration occurs before the convulsions. Throughout the dieldrin-induced seizure pattern, fluctuations in the concentration of brain ammonia are out of phase with the actual convulsions. 2. Increases in the concentrations of alanine, ammonia and lactic acid in rat brain accompany picrotoxin-induced seizures; there is no increase in the concentration of glutamine. These changes are consistent with the inhibition of glutamine synthesis. 3. In addition to previously reported changes in the concentrations of intermediary metabolites of the brain after the administration of Telodrin (Hathway & Mallinson, 1964), increases have now been found in the alanine and lactic acid concentrations. Since increases in the alanine and glutamine concentrations occur before the convulsions, liberation of ammonia also occurs before the onset of convulsions and throughout their course. Ammonia-binding mechanisms later become inadequate and free ammonia accumulates in cerebral tissues. 4. An increase in the pyruvic acid concentration of the brain after the intraperitoneal injection of either dieldrin or Telodrin is endogenous in origin. 5. The parenteral administration of a small dose of glutamine increases the cerebral concentrations of alanine and glutamic acid. Some animals previously treated with glutamine resisted Telodrin convulsions. 6. Mechanisms for the disposal of ammonia liberated in brain are discussed.

In previous work (Hathway & Mallinson, 1964) the conclusion was drawn that the action of Telodrin causes the liberation of ammonia in the brain, and that this occurs before the onset of convulsions and throughout their course. Glutamic acid, glutamine and α-oxoglutaric acid are utilized in an ammonia-binding mechanism that later becomes overwhelmed, and free ammonia accumulates in the cerebral tissues. A parallel investigation of the action of a structurally related insecticide, e.g. dieldrin, and of a totally unrelated convulsant drug, e.g. picrotoxin, was required to put the effects due to Telodrin into perspective.

We have examined the action of dieldrin and picrotoxin on some intermediary metabolites of rat brain *in vivo*, and have extended previous work on the action of Telodrin on the brain. We have found that relatively large increases in the concentrations of alanine, ammonia and lactic acid are associated with picrotoxin-induced seizures, and that acute dieldrin poisoning is accompanied by increases in the concentrations of alanine, lactic acid and pyruvic acid, and a fluctuation in ammonia concentration throughout the course of seizures. The present paper describes the results obtained and their possible interpretation.

MATERIALS AND METHODS

Dieldrin, Telodrin and picrotoxin. Dieldrin (1,2,3,4,10,-10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene) and Telodrin (1,3,4,-5,6,7,8,8-octachloro-1,3,3*a*,4,7,7*a*-hexahydro-4,7-methano-isobenzofuran) were crystallized from acetone–methanol and acetone–hexane to constant melting point (dieldrin, m.p. 177°; Telodrin, m.p. 123°). The purified dieldrin and Telodrin migrated as single substances in reversed-phase paper chromatograms developed with two solvent systems (Mitchell, 1958); phenoxyethanol–$AgNO_3$ spray was used for locating the substances on the paper (Mitchell, 1958). Picrotoxin, the bimolecular compound of picrotoxinin (the physiologically active component) and picrotin, crystallizes from water, forming prisms, m.p. 203–204°, $[\alpha]^{20}_{D}$ $-30°$ (*c* 1·0 in water). The structure of picrotoxinin (Fig. 1) and of its rearrangement products is now firmly established (Burkhill, Holker, Robertson & Taylor, 1957; Conroy, 1951, 1957*a*,*b*; Hathway, 1957; Holker *et al.* 1957; Holker, Robertson, Taylor, Holker & Williamson, 1958). The three-dimensional cage structure for picrotoxinin (Fig. 1) would be expected to be stable to light, whereas photo-

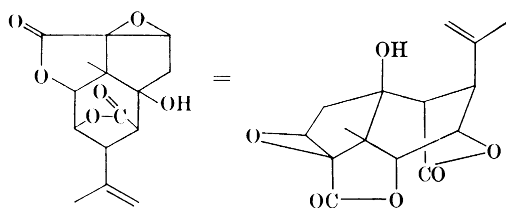

Fig. 1. Structure of picrotoxinin.

sensitivity is often cited as the cause of the deterioration of aqueous solutions (British Pharmacopoeia, 1958; Pharmacopoea Internationalis, 1951; Martin & Cook, 1956; United States Dispensatory, 1955). The deterioration of picrotoxin solutions stored in glassware that has not been washed with acid is due to their becoming alkaline, and this causes rearrangement of picrotoxinin to physiologically inactive products. In the present work, aqueous solutions were prepared daily for administration to animals.

Amino acids. L(+)-Alanine, γ-aminobutyric acid, L(+)-aspartic acid, L(+)-glutamic acid and L(+)-glutamine (Fluka A.-G., Buchs, Switzerland) were used as marker substances, and as analytical standards for quantitative estimations. Aqueous glutamine soln. (5 mg./ml.) was used for injection.

Reagents and solvents. All reagents and solvents (supplied by Hopkin and Williams Ltd., Chadwell Heath, Essex) were of AnalaR grade or, if this grade was unavailable, laboratory-reagent grade. Dimethyl sulphoxide (laboratory-reagent grade) was redistilled and the fraction b.p. 66–69°/10mm. Hg collected for use.

Experiments with animals. (*a*) Treatment of animals. Young rats (3–4 weeks old) were used (albino, Carworth Farm strain, maintained for 3 years as a closed colony in this Laboratory). A random selection of non-starved animals (100–125 g.) was made for each experiment; any animal that objected to normal handling was rejected. The general procedure was to adminster the toxicant, and after various known times to kill the animal, remove the brain and make the chemical determinations.

(*b*) Seizure pattern for acute poisoning. Rats weighing 100 g. were given a single intraperitoneal injection of 1·5 ml. of an aqueous solution containing 1·26 mg. of picrotoxin/ml., equivalent to a dose of 19 mg./kg. body wt. The onset of convulsions occurred 5·5–7·0 min. after dosage, and the duration of continuous convulsions was 10–15 min. This dosage was 100% lethal: all animals died within 20 min., and there was no difference due to sex or size in the response to poisoning.

Rats weighing 100 g. were given a single intraperitoneal injection of 1 ml. of a soln. (10 mg./ml.) of dieldrin in dimethyl sulphoxide. The onset of seizures, which occurred 14–22 min. after dosage, was followed by two or three major convulsions, coma and death; death occurred 1 hr. after dosage. This dosage was 100% lethal, and there was no difference due to sex or size in the response to poisoning. Control rats were injected with the same volume of solvent as was used for the test animals, and they were killed at the same time.

The seizure pattern when Telodrin was administered as a 7·5 mg./kg. dose has been described by Hathway & Mallinson (1964).

(*c*) Fixation of the brain *in situ*. Conscious animals were killed by holding them head first in liquid N_2, which froze them solid in a few seconds and effected a rapid fixation of cerebral metabolites. The frozen brains were rapidly dissected out without allowing them to thaw. Except where otherwise stated, experiments were made with pairs of whole brains (2·5–3·0 g. of tissue), which were crushed in a porcelain mortar under liquid N_2.

Preparation of homogenates and haemolysates. Whole brain (2·5–3·0 g.) was dropped into the weighed tube of a tissue homogenizer (Aldridge, Emery & Street, 1960) containing 10 ml. of 12% (w/v) trichloroacetic acid at 0°. After vigorous shaking, the tube was weighed and the tissue was rapidly brought into a fine suspension by mechanical rotation of the fitting pestle. The suspension was centrifuged at 35000 *g* at 0° in the superspeed head of an MSE Major refrigerated centrifuge for 15 min., to separate phospholipids completely from the trichloroacetic acid extract (Heald, 1956). Portions of the supernatant were used for the chemical determinations; an allowance was made for the water content of brain, taken as 80% of the weight of tissue.

Blood samples were taken under ether anaesthesia. The blood was collected in syringes from the right ventricle, care being taken to exclude air bubbles, and transferred immediately to stoppered tubes each containing 5 ml. of ice-cold 10% (w/v) trichloroacetic acid with which it was well mixed and weighed. Cellular debris was sedimented by centrifuging, and portions of the supernatant were used for the estimation of pyruvic acid.

Chemical determinations. Ammonia was determined by micro-diffusion analysis on portions (1 ml.) of the 12% trichloroacetic acid extract from brain (Hathway & Mallinson, 1964), and glutamine was also determined by micro-diffusion analysis after hydrolysis of other portions (1 ml.) (Hathway & Mallinson, 1965; Harris, 1943); an allowance was made in the calculation for cerebral ammonia. Results were compared with estimations on standard solutions of either $(NH_4)_2SO_4$ or glutamine, as appropriate. α-Oxoglutaric acid and pyruvic acid were determined spectrophotometrically after separation as their 2,4-dinitrophenylhydrazones (Friedemann, 1957). For each determination, 3·0 ml. of the 12% trichloroacetic acid extract (representing approx. 600 mg. of original fresh brain tissue) was used. Lactic acid was determined on 1·0 ml. portions of the brain extract by the method of Barker & Summerson (1941). Pyruvic acid was also determined in the 10% trichloroacetic acid extract from blood by the method of Friedemann (1957). Duplicate determinations were made on each brain or blood sample.

Separation from rat brain of an amino acid fraction suitable for electrophoresis. After high-speed centrifugation of the brain homogenate in trichloroacetic acid, the sediment was re-extracted with 20 ml. of 12% trichloroacetic acid in the tissue homogenizer, and the combined supernatants were extracted with ether (4 × 30 ml.). The resulting solution (33 ml.) was evaporated (at below 38°/10 mm. Hg) to dryness in a rotating high-vacuum type evaporator (Rinco Instrument Co. Inc., Greenville, Ill., U.S.A.). The residue, which was stored over P_2O_5 at 0°/0·01 mm. Hg, was quantitatively transferred with aq. 10% (v/v) propan-2-ol to a 1 ml. standard flask, and the resulting solution was used for the estimation of amino acids.

Electrolyte solutions. Pyridine–acetic acid–water, pH 6·1,

was prepared by diluting a mixture of redistilled pyridine (100 ml.) and 99·6% acetic acid (8·0 ml.) with water to 2·5 l. Formic acid–acetic acid–water, pH 2·0, was prepared by diluting a mixture of 98–100% formic acid (78 ml.) and 99·6% acetic acid (148 ml.) with water to 2·5 l.

High-voltage electrophoresis. Cerebral amino acids were separated by electrophoresis on Whatman 3MM filter paper (60 cm. × 13·5 cm.) below 10°, 10 or 50 μl. portions of the prepared brain extract being used. Aspartic acid, γ-aminobutyric acid and glutamic acid were separated by electrophoresis in pyridine–acetate buffer, pH 6·1, for 40 min. at 100 v/cm., and alanine was resolved in formic acid–acetic acid, pH 2·0, in 1 hr. at 100 v/cm. After electrophoresis, the paper strips were infrared-heated in an air stream for 10 min. to remove most of the liquid, and were subsequently dried at 80° for 15 min. (Atfield & Morris, 1961). The cadmium acetate–ninhydrin reagent (Heilmann, Barollier & Watzke, 1957) was used for staining amino acids on the filter-paper strips, and bright-red zones, which had been developed after storage for 20 hr. over conc. H_2SO_4 to maintain an NH_3-free atmosphere, were eluted with methanol. The extinction was measured at 500 mμ for the amino acids. Duplicate determinations were made on each brain sample, and results were compared with similar estimations on standard solutions of amino acids. The recoveries of γ-aminobutyric acid, aspartic acid and glutamic acid were 95% of the theoretical, but the recovery of alanine was only 80%. The measured amino acids were eluted from zones that were free from overlapping amino acids.

Cadmium acetate–ninhydrin reagent. Fresh reagent was prepared daily by mixing a soln. of cadmium acetate dihydrate (50 mg.) in aq. 16⅔% (v/v) acetic acid (6 ml.) and a soln. of ninhydrin (Fluka A.-G.) (0·5 g.) in acetone (50 ml.). No colour is produced by this reagent with the paper alone.

RESULTS

In the general plan of the work, four groups of young rats were given respectively dieldrin, picrotoxin, Telodrin or glutamine. These substances were administered at zero time, the animals were killed at various times thereafter and the concentrations of some normal metabolites in the brain were determined. The normal undisturbed concentrations of these metabolites in the brain, expressed as the means ± S.E.M. in terms of μmoles/100 g. wet. wt., with numbers of determinations in parentheses, were: alanine, 27·6 ± 1·4 (6); ammonia, 57 ± 3·3 (8); glutamic acid 651 ± 22 (3); glutamine, 462 ± 17·3 (8); lactic acid, 245 ± 15·2 (3); α-oxoglutaric acid, 4·9 ± 0·26 (4); pyruvic acid, 12·6 ± 0·7 (4). This concentration of cerebral alanine is comparable with that (28 μmoles/100 g. wet wt.) found by De Ropp & Snedeker (1960) for Wistar rats (weighing 250 g.), but, since the recovery of alanine in our hands was only 80% of the theoretical, absolute values for the cerebral alanine concentration in young rats are considerably higher than the values that we report in the present paper. The concentrations of cerebral lactic acid and pyruvic acid are comparable with the concentrations (262 ± 19·0 and 11·2 ± 0·7 μmoles/100 g. wet wt.) reported by Ridge (1963) for more mature animals. The concentrations of most of the other cerebral metabolites are comparable with corresponding concentrations found for somewhat heavier animals by Hathway & Mallinson (1964); an exception is glutamic acid, where an even lower value (486 μmoles/100 g. wet wt.) had previously been found in the brain of slightly older rats. In the present work, control values for the concentration of glutamic acid in the brain of adult rats were 1070 ± 28 (3) μmoles/100 g. wet wt.

Effects of dieldrin on the concentrations of brain constituents in vivo. After a latent period, animals that have received a toxic dose of dieldrin show muscular spasms, and clonic–tonic convulsions, which are mainly clonic, end in a tonic extensor spasm. Each convulsion is followed by coma from which the animal can recover, but in animals that have suffered two or three convulsions the post-convulsive coma becomes longer and eventually ends in death.

The effect of the intraperitoneal administration of dieldrin dissolved in dimethyl sulphoxide on the concentrations of some normal metabolites of rat brain was initially investigated just after the first convulsion, 18 min. after dosage. Dimethyl sulphoxide produced no significant changes in the concentrations of alanine, ammonia, glutamic acid, glutamine, lactic acid and pyruvic acid at the same time-interval (18 min.) after the administration of the solvent. For cerebral α-oxoglutaric acid, the concentration [10·4 ± 2·2 (3) μmoles/100 g. wet wt.] in the rats treated with dimethyl sulphoxide is probably significantly different ($P < 0.05$) from that in normal animals, whereas 18 min. after dosage the dieldrin-treated animals with cerebral content 11·6 ± 1·0 (7) μmoles/100 g. wet wt. are not significantly different ($P > 0.05$) from the dimethyl sulphoxide-treated rats. Because of the interference of the solvent in this way, no acceptable conclusion can be reached about the effect of dieldrin on the cerebral α-oxoglutaric acid concentration. The respective concentrations of cerebral ammonia [52 ± 2·3 (7) μmoles/100 g.] and glutamine [473 ± 8·3 (7) μmoles/100 g.] 18 min. after the administration of dieldrin are not significantly different ($P > 0.5$ and $P > 0.05$ respectively) from those in normal animals. Since the concentrations of cerebral alanine, lactic acid and pyruvic acid 18 min. after the administration of dieldrin were each significantly different from those in normal animals (Table 1), the time-course for the changes in the concentrations of the metabolites after the administration of dieldrin was investigated (Table 1).

Though the concentration of each of these cere-

bral metabolites undergoes a significant elevation ($P < 0{\cdot}001$) during the dieldrin-induced seizure pattern, the principal point of interest is the significant change in the concentration of alanine ($P < 0{\cdot}001$) that occurs before convulsions, 12 min. after dosage (Table 1). A significant elevation in the concentration of cerebral alanine has therefore been demonstrated before the onset of dieldrin-induced seizures. Increased variability in the determinations of alanine concentration after convulsions in comparison with preconvulsive values is reflected in the S.E.M. values (Table 1), and this suggests that, after convulsions, changes due to convulsive activity complicate the significant change in the concentration of cerebral alanine that occurs before convulsions. The results show that the change in the cerebral alanine concentration is significantly related to the action of dieldrin. The cerebral content of lactic acid does not change significantly until after the onset of seizures (Table 1), when the relatively large increase is characteristic of the state of convulsive activity, and is also associated with seizures brought about by other agents (McIlwain, 1959*a*). The time-course for the change in pyruvic acid content of rat brain after the administration of dieldrin shows that the pyruvic acid concentration rose to near maximum only after convulsions had occurred, but the concentration fell below the normal value (Table 1) in the period of coma and less violent seizures that follows the tonic extensor seizure. This suggests that the increase in cerebral pyruvic acid concentration is associated with hyperactivity of the brain. Although an increase occurred before convulsions, 10 min. after dosage, this was not significant ($P > 0{\cdot}05$).

Effects of picrotoxin on the concentrations of brain constituents in vivo. The intraperitoneal injection of rats with a lethal dose of picrotoxin produces clonic convulsions after a short latent period. These convulsive movements, which are continuous and increasingly violent, end in a tonic extensor seizure, in which the four limbs show a prolonged and intense extensor spasm. After the extensor spasm, the animal lies in a flaccid state for a variable period, until a second phase begins of much less violent clonic seizures of rapidly diminishing severity; finally, convulsive activity ceases. In rats treated with picrotoxin, the tonic extensor seizure is associated with chromodacryorrhea (the secretion of so-called 'bloody tears' from the Harderian glands). Picrotoxin seizures exhibit prolonged spike activity in the electroencephalographic recording; there are no alternating periods of quiescence and activity that occur in records taken during dieldrin- and Telodrin-induced convulsions.

After the onset of convulsions, 8 min. after dosage, the concentrations in the brain, expressed

Table 1. *Effects of dieldrin on the alanine, lactic acid and pyruvic acid concentrations in the brain of young rats*

The administration of 1 ml. of a solution (10 mg./ml.) of dieldrin in dimethyl sulphoxide/100 g. body wt. by intraperitoneal injection caused onset of seizures 14–22 min. after dosage. Dieldrin was administered at zero time and the animals were killed at various times thereafter. The concentrations of the cerebral metabolites are expressed as the means ±S.E.M., with the numbers of determinations in parentheses. Probabilities (P) refer to the differences of the results for dieldrin-treated animals from those of the untreated animals.

Time after dosage (min.) ...	0	6	10	12	15	18	25	30	40	50
Cerebral metabolite	Concn. of metabolite (μmoles/100 g. wet wt.)									
Alanine	27·6±1·4 (6)	36±2·0 (3) ($P < 0{\cdot}01$)	—	43±2·0 (3) ($P < 0{\cdot}001$)	—	70±8·5 (3) ($P < 0{\cdot}01$)	—	76±8·5 (3) ($P < 0{\cdot}01$)	101±8·5 (3) ($P < 0{\cdot}001$)	—
Lactic acid	245±15·2 (3)	—	223±15·2 (3) ($P > 0{\cdot}05$)	—	399±95 (3) ($P > 0{\cdot}05$)	648±95 (3) ($P < 0{\cdot}01$)	662±95 (3) ($P < 0{\cdot}01$)	726±95 (3) ($P < 0{\cdot}001$)	814±95 (3) ($P < 0{\cdot}001$)	—
Pyruvic acid	12·6±0·7 (4)	—	15·2±0·8 (3) ($P > 0{\cdot}05$)	—	—	19·0±1·9 (6) ($P < 0{\cdot}05$)	—	24·0±1·1 (3) ($P < 0{\cdot}001$)	17·9±1·1 (3) ($P < 0{\cdot}01$)	8·2±1·1 (3) ($P < 0{\cdot}05$)

Table 2. *Effects of picrotoxin on the ammonia, alanine and lactic acid concentrations in the brain of young rats*

The administration of 1·5 ml. of an aqueous solution (1·26 mg./ml.) of picrotoxin/100 g. body wt. by intraperitoneal injection caused onset of convulsions 5·5–7·0 min. after dosage. Picrotoxin was administered at zero time and the animals were killed at various times thereafter. The concentrations of the cerebral metabolites are expressed as the means ± S.E.M., with the numbers of determinations in parentheses. Probabilities (P) refer to the differences of the results for picrotoxin-treated animals from those of the untreated animals.

	Conc. of metabolite (μmoles/100 g. wet wt.)				
Cerebral metabolite / Time of dosage (min.) ...	0	4	8	12	16
Ammonia	57±3·3 (8)	54±5·3 (3) ($P > 0·05$)	90±4·2 (9) ($P < 0·001$)	100±7·2 (3) ($P < 0·001$)	110±7·2 (3) ($P < 0·001$)
Alanine	27·6±1·4 (6)	37±2·0 (3) ($P < 0·01$)	40±3·9 (3) ($P < 0·05$)	62±3·9 (3) ($P < 0·001$)	72±3·9 (3) ($P < 0·001$)
Lactic acid	245±15·2 (3)	245±15·2 (3)	569±15·8 (3) ($P < 0·001$)	624±15·8 (3) ($P < 0·001$)	820±15·8 (3) ($P < 0·001$)

in terms of μmoles/100 g. wet wt., with the numbers of determinations in parentheses, were: glutamine, 444±14·8 (9); α-oxoglutaric acid, 5·7±0·64 (8); pyruvic acid, 12·2±1·1 (9). These are not significantly different ($P > 0·5$) from those in normal animals. The cerebral contents of alanine, free ammonia and lactic acid 8 min. after the administration of picrotoxin were significantly different from those in normal animals (Table 2), and the time-courses for the changes in the concentrations of these metabolites after the administration of picrotoxin were therefore investigated (Table 2).

The main point of interest is the significant change in the cerebral content of alanine ($P < 0·01$) that occurs before convulsions, 4 min. after dosage (Table 2). The present work confirms the increase observed by De Ropp & Snedeker (1961) in the concentration of cerebral alanine ($P < 0·001$) in mature rats that were killed during picrotoxin-induced convulsions. Our results show that the change in alanine concentration is significantly related to the action of picrotoxin. The concentrations of cerebral ammonia and lactic acid do not change significantly until after the onset of convulsions (Table 2), and significant changes ($P < 0·001$) occur in the seizure pattern, 16 min. after convulsions.

Effects of Telodrin on the concentrations of brain constituents in vivo. Several effects of Telodrin have already been described by Hathway & Mallinson (1964), and it has now been found that significant changes ($P < 0·001$) in the cerebral contents of alanine and lactic acid occur after the intraperitoneal administration of Telodrin dissolved in dimethyl sulphoxide (Table 3). The principal points of interest are the significant changes in the concentration of alanine ($P < 0·01$ and $P < 0·001$) that occur before convulsions, 10 and 15 min. respectively after dosage (Table 3). The results indicate that the change in cerebral alanine concentration is significantly related to the action of Telodrin. The cerebral content of lactic acid does not change significantly until after the onset of seizures (Table 3), when the relatively large increase is characteristic of convulsive activity, and is associated with seizures brought about by dieldrin, picrotoxin and other agents.

Effects of glutamine on the concentrations of brain constituents in vivo. Since Hathway & Mallinson (1964) had found that, when a convulsive dose of Telodrin was administered to rats that had been treated 40 min. previously with 50 mg. of glutamine/kg., one-third of the animals resisted convulsions and recovered, the effect of this dose of glutamine has now been explored. The changes in the cerebral concentrations of alanine, 40 min. after dosage, and of glutamic acid, 30 min. after dosage, are significant ($P < 0·001$) (Table 4). No

Table 3. *Effects of Telodrin on the alanine and lactic acid concentrations in the brain of young rats*

The administration of 0·75 ml. of a solution (1 mg./ml.) of Telodrin in dimethyl sulphoxide/100 g. body wt. by intraperitoneal injection caused onset of seizures 19 min. after dosage. Telodrin was administered at zero time and the animals were killed at various times thereafter. The concentrations of the cerebral metabolites are expressed as means ± S.E.M., with the numbers of determinations in parentheses. Probabilities (P) refer to the differences of the results for Telodrin-treated animals from those of the untreated animals.

		Concn. of metabolite (μmoles/100 g. wet. wt)						
Cerebral metabolite	Time after dosage (min.) ...	0	10	15	20	25	30	35
Alanine		27·6 ± 1·4 (6)	36·0 ± 2·0 (3) ($P < 0·01$)	51 ± 2·0 (3) ($P < 0·001$)	—	80 ± 7·4 (3) ($P < 0·01$)	—	85 ± 7·4 (3) ($P < 0·01$)
Lactic acid		245 ± 15·2 (3)	251 ± 15·2 (3) ($P > 0·05$)	224 ± 15·2 (3) ($P > 0·05$)	341 ± 47·0 (3) ($P > 0·05$)	637 ± 36·4 (5) ($P < 0·001$)	680 ± 47·0 (3) ($P < 0·001$)	761 ± 47·0 (3) ($P < 0·001$)

Table 4. *Effects of glutamine on the alanine and glutamic acid concentrations in the brain of young rats*

Glutamine was administered parenterally [1 ml. of an aqueous solution (5 mg./ml.) of glutamine/100 g. body wt. by intraperitoneal injection] at zero time and the animals were killed at various times thereafter. The concentrations of the cerebral metabolites are expressed as means ± S.E.M., with the numbers of determinations in parentheses. Probabilities (P) refer to the differences of the results for glutamine-treated animals from those of the untreated animals.

		Concn. of metabolite (μmoles/100 g. wet wt.)					
Cerebral metabolite	Time after dosage (min.) ...	0	10	20	30	40	60
Alanine		27·6 ± 1·4 (6)	—	35 ± 2·0 (6) ($P < 0·01$)	—	48 ± 2·0 (3) ($P < 0·001$)	40 ± 2·0 (3) ($P < 0·001$)
Glutamic acid		651 ± 22 (3)	691 ± 22 (3) ($P > 0·05$)	732 ± 22 (3) ($P < 0·05$)	883 ± 22 (3) ($P < 0·001$)	590 ± 22 (3) ($P > 0·05$)	—

change occurred in the concentrations of γ-aminobutyric acid, ammonia, aspartic acid and glutamine, which were measured in some of the experiments. After treatment with glutamine, which does not cause convulsions, the small spread of the S.E.M. values for alanine concentrations (Table 4), which does not alter appreciably throughout the course of the experiment, is comparable with the spread of the S.E.M. values for alanine concentrations before dieldrin-, picrotoxin- and Telodrin-induced convulsions (Tables 1, 2 and 3).

Variance comparisons. Before convulsions, (1) variance between animals of the concentrations of the cerebral metabolites did not change with time after dosage, and (2) variance of the alanine concentration was not affected by the various agents causing convulsions. Before convulsions, the estimates of S.E.M. values were therefore taken from variance estimated by pooling over preconvulsive times and over the four experiments. After convulsions it was justifiable to pool the separate estimates of variance of the alanine concentration within each experiment. After the administration of dieldrin, the difference between pooled variances after convulsions [218·7 (6 degrees of freedom)] and before them [12·35 (21 degrees of freedom)] is significant ($P < 0{\cdot}001$). Similarly, after the administration of picrotoxin the difference between pooled variances after convulsions [46·67 (6 degrees of freedom)] and before them is significant ($P < 0{\cdot}001$), and after the administration of Telodrin the difference between pooled variances after convulsions [165·3 (4 degrees of freedom)] and before them is significant ($P < 0{\cdot}001$). In all three seizure patterns, increased variability of cerebral alanine concentrations after convulsions in comparison with values before convulsions strongly suggests that, after convulsions, changes due to convulsive activity complicate the significant change in the concentration of this metabolite that occurs before convulsions with all three convulsive agents.

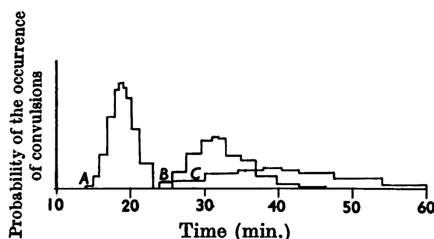

Fig. 2. Seizure pattern for acute dieldrin poisoning: *A*, first convulsion; *B*, second convulsion; *C*, third convulsion. Histograms were prepared from individual observations on 60 animals.

Effects of dieldrin on the free ammonia concentration in the brain in vivo. Because of the rather irregular seizure pattern for acute dieldrin poisoning, a time-course for the probability of the occurrence of convulsions was constructed (Fig. 2). The fact that not all the animals had a third convulsion and that the time of this convulsion was very variable accounts for the low values for the probability of the occurrence of the third convulsion.

Groups of rats were killed 8, 18, 36 or 50 min. after the intraperitoneal injection of dieldrin, and other groups were killed during the first, second and third convulsions; the ammonia content of single brains was measured. The time-course for the change in ammonia content of rat brain after the administration of dieldrin was determined (Table 5) by using the mean times for the occurrence of the convulsions (Fig. 2). The concentration of cerebral ammonia in rats, killed 8 min. after the administration of dieldrin, was taken as the value for the control animals. The ammonia concentration at this time was $40 \pm 4{\cdot}5$ (6) μmoles/100 g. wet wt.; this value is lower than that for normal animals [$57 \pm 3{\cdot}3$ (8) μmoles/100 g.], but not significantly lower ($P > 0{\cdot}05$) than that [$48 \pm 6{\cdot}6$ (4) μmoles/100 g.] for animals 18 min. after the administration of dimethyl sulphoxide. The concentration of free ammonia undergoes a significant elevation ($P < 0{\cdot}001$) during the dieldrin-induced seizure pattern, 50 min. after dosage, and this also occurred in picrotoxin- and Telodrin-induced convulsions. The principal point of interest in Table 5 is that fluctuations of brain ammonia concentration occur out of phase with the periods of intense cerebral activity that alternate with periods of quiescence in the electroencephalographic recording. When the times after dosage were small, the fluctuations (at 12 and 18 min.) were not significant in comparison with the controls and with animals in the first convulsion ($P > 0{\cdot}05$), but when the times were longer the changes at 36 and 50 min. were significantly different from values in the second and third convulsion. These findings justified our application of a fitting equation to the data in Table 5, as follows:

$$[\text{Ammonia}] = K + b_1 t + b_2 x$$

where t is the time after dosage, and where x is a variable such that $x = 1$ during convulsion and $x = 0$ otherwise. The constants can be evaluated: $K = 39{\cdot}6$, $b_1 = 0{\cdot}543$ and $b_2 = -8{\cdot}34$. The experimental results listed in Table 5 can then be calculated by substituting values for t and x in the equation:

$$[\text{Ammonia}] = 39{\cdot}6 + 0{\cdot}543t - 8{\cdot}34x.$$

When the value of t is small, the contribution of the quantity $0{\cdot}543t$ to the concentration of ammonia is

Table 5. *Effect of dieldrin on the free ammonia concentration in the brain of young rats*

Groups of rats were killed 8 (controls), 18, 36 or 50 min. after the intraperitoneal injection of 1 ml. of a solution (10 mg./ml.) of dieldrin in dimethyl sulphoxide/100 g. body wt., and other groups were killed during the first, second and third convulsions at 16, 30 and 40 min. after dosage. The results refer to the mean concentrations in single brains ± S.E.M., with the numbers of determinations in parentheses. Probabilities (P) refer to the differences of the results for the treated animals from those of the controls. The calculated values were derived from the equation: [Ammonia] = $39.6 + 0.543t - 8.34x$ (see the text for details).

Time after dosage (min.) ...	8	12	16	18	30	36	40	50
Concn. of free ammonia (μmoles/100 g. wet wt.)	(controls) 40 ± 4.5 (6)	51 ± 6.4 (3) ($P > 0.05$)	(1st convulsion) 40 ± 3.4 (11)	52 ± 4.2 (7) ($P > 0.05$)	(2nd convulsion) 42 ± 4.5 (6) ($P > 0.05$)	63 ± 5.6 (4) ($P < 0.01$)	(3rd convulsion) 51.5 ± 5.6 (4) ($P > 0.05$)	65 ± 3.2 (12) ($P < 0.001$)
Calc. values	43·9	46·0	40·0	49·4	47·6	59·1	52·9	66·7

a small one. The change in concentration of cerebral ammonia that accompanies a convulsion (-8.34 μmoles/100 g.) is significant ($P < 0.025$), and the conclusion is drawn that the fluctuating increases in the concentration of ammonia contribute to the seizures.

Effects of the intraperitoneal injection of dieldrin and Telodrin on blood and brain pyruvic acid concentrations. Since the changes in the pyruvic acid content of brain follow the hyperactivity of the animals during acute dieldrin and Telodrin poisoning, the possibility of an 'overspill' from blood to brain arises. The relative rapidity with which injected pyruvic acid is removed from the general circulation in comparison with its relatively slow penetration into cerebral cells (Klein & Olsen, 1947; Himwich, 1951) makes this rather unlikely. Elevated concentrations of blood pyruvic acid produced by the injection of either dimethyl sulphoxide or water, and by puncture of the body and abdominal walls, are therefore related to stress caused by the injection, and are comparable with the stress effect of a tourniquet on the concentration of rat blood pyruvic acid (McShan, Potter, Goldman, Shipley & Meyer, 1945–1946). The fact that dimethyl sulphoxide produces a similar rise in the pyruvic acid content of blood to that produced by dieldrin and Telodrin, but has no effect on that of the brain, makes it unlikely that the high concentration in the brain due to the insecticides is related to that in the blood. The plots of the time-courses for cerebral and blood pyruvic acid concentrations in acute dieldrin and Telodrin poisoning (Fig. 3) show that the concentration of cerebral pyruvic acid is higher than that in blood 25 min. or more after dosing, and in the absence of a mechanism for

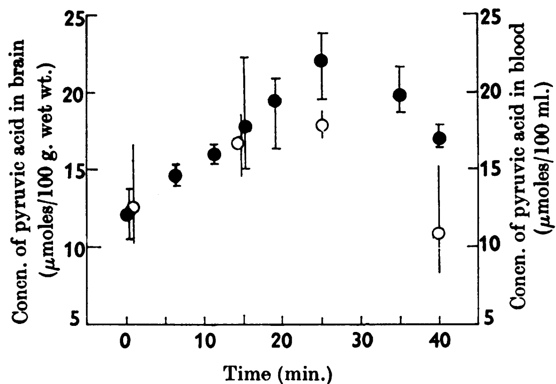

Fig. 3. Changes in the pyruvic acid concentrations in rat blood (○) and brain (●) after a single intraperitoneal injection of Telodrin (7·5 mg./kg. body wt.). Each range of values is derived from at least three pairs of animals. Death occurred 50 min. after dosage.

the active transport of pyruvic acid the origin of the rise in cerebral pyruvic acid content is probably endogenous.

DISCUSSION

The conversion of ammonia into glutamine in the brain is regarded as the principal mechanism for protection against the toxic effects of NH_4^+ ion. One molecule of ammonia combines with α-oxoglutaric acid to form glutamic acid, and a second molecule of ammonia combines with glutamic acid to form glutamine.

That glutamine synthesis can represent a significant proportion of the total cerebral metabolism in acute Telodrin poisoning is shown by comparing the maximum rate of glutamine synthesis (0·2 μmole/g. of brain tissue/min.) calculated from the concentrations (560 and 680 μmoles/100 g. wet wt.) at 6 and 12 min. respectively after dosing (Hathway & Mallinson, 1964) with the normal rate of glucose utilization (0·4 μmole/g./min.) calculated from an arteriovenous glucose difference of 13·2 mg./100 ml. (Rodnight, McIlwain & Tresize, 1959) and a flow rate of 0·6 ml./g./min. for cerebral blood. The availability of pyruvic acid would be approx. 0·8 μmole/g./min., i.e. only four times the rate of glutamine synthesis. The latter was about the same in a dozen experiments, in which the final glutamine concentration ranged from 0·6 to 0·8μ-mole/g. This mechanism appears to have been operating at its maximum capacity, and the NH_4^+ ion concentration is not rate-limiting in this range (Tews, Carter, Roa & Stone, 1963). These observations and the fact that the tricarboxylic acid cycle would have to proceed at 0·7 μmole/g./min. to account for respiration from glucose *in vitro* at 2·0 μmoles of oxygen/g./min. (Beloff-Chain, Catanzaro, Chain, Masi & Pocchiari, 1955) are consistent with the later deceleration of glutamine synthesis that has been described (Hathway & Mallinson, 1964). The decrease in glutamic acid concentration was small (0·52 μmole/g.) but significant ($P < 0{\cdot}025$) (Hathway & Mallinson, 1964), whereas the concentration of α-oxoglutaric acid was, if anything, increased; it is therefore uncertain whether the supply of α-oxoglutaric acid was in fact rate-limiting. The rate of glycolysis is profoundly affected during convulsions (McIlwain, 1959*b*; Wolfe & Elliott, 1962), but in the preconvulsive phase of Telodrin poisoning, in which the maximum rate of glutamine synthesis occurred, deviations from the normal rates of utilization of glucose and supply of pyruvic acid are unlikely to be significant.

Other methods for the utilization of ammonia in the brain should be considered. Since there is a high activity of the relevant transaminases in cerebral tissues (Awapara & Seale, 1952), transaminations involving oxaloacetic acid and pyruvic acid may contribute to the main method for ammonia disposal. Transamination involving oxaloacetic acid is not especially important to present considerations, since dieldrin, glutamine, picrotoxin and Telodrin brought about no change in the cerebral content of aspartic acid; on the other hand, transamination involving pyruvic acid seems to be of particular importance, since all four substances brought about a significant change in the concentration of alanine in the brain. We envisage that glutamic acid is the donor of the amino group in this transamination, in which case glutamic acid and α-oxoglutaric acid are involved in the conversion of ammonia into alanine in the brain.

The increases in glutamine and alanine concentrations are evidence that the action of Telodrin leads to the liberation of ammonia in the brain before the onset of convulsions and throughout their course. α-Oxoglutaric acid, glutamic acid and glutamine are utilized in a principal ammonia-binding mechanism, and α-oxoglutaric acid, glutamic acid, pyruvic acid and alanine are utilized in a subsidiary mechanism for ammonia disposal. Later in the seizure pattern these mechanisms become inadequate for ammonia-binding, and free ammonia accumulates in cerebral tissues.

After the administration of picrotoxin, although there is an increase in alanine and ammonia concentrations, there is no increase in that of glutamine. Enhanced glutaminase activity might account for the phenomenon, but this would not account for the unchanged glutamine concentration during acute picrotoxin poisoning. A more likely explanation is that picrotoxin inhibits glutamine synthesis in the brain. Since the alanine concentration increases it seems likely that the action of picrotoxin leads to the liberation of ammonia in the brain before the onset of convulsions, and that during the preconvulsive phase a mechanism involving α-oxoglutaric acid, glutamic acid, pyruvic acid and alanine disposes of this ammonia. At the onset of seizures and after, free ammonia accumulates in cerebral tissues, when presumably this method of ammonia disposal is no longer adequate. The possibility that free NH_4^+ ion and alanine synthesis are not related seems unlikely, since this would cause a fall in the cerebral content of glutamic acid, which was not observed either in the present work or in that of De Ropp & Snedeker (1961).

The present observations extend the limited data on the concentrations of intermediary metabolites of rat brain during picrotoxin convulsions. Our results agree with the reports that the concentration of alanine in rat brain is significantly increased by the action of picrotoxin whereas those of the other amino acids are not affected (De Ropp & Snedeker, 1961), and that the concentration of glutamine is

not changed by picrotoxin (Richter & Dawson, 1948). In this connexion, Sullivan & Strong (1955) reported that picrotoxin-induced seizures did not alter the glutamic acid content of rabbit brain. Picrotoxin produces different changes in the concentrations of cerebral metabolites in the dog from those produced in rodents. Thus Tews *et al.* (1963) found no significant rise in the cerebral ammonia concentration, and, of the amino acids and phosphoamines investigated, a decrease in only that of aspartic acid occurred. The concentration of lactic acid in dog brain increased during acute picrotoxin poisoning (Stone, Tews & Mitchell, 1960).

The fact that a fluctuating release of cerebral ammonia was detected before the onset of dieldrin-induced seizures and throughout their course suggests that the brain deals efficiently with the liberated NH_4^+ ion. The decrease in the concentration of cerebral ammonia, which accompanies a convulsion, may be related to the increased rate of circulation and oxygen consumption of the brain that has been observed in cats (Geiger & Magnes, 1947) and in monkeys (Schmidt, Kety & Pennes, 1945) during drug-induced convulsions. The action of dieldrin therefore leads to the liberation of ammonia in the brain before the onset of convulsions and throughout their course. α-Oxoglutaric acid, glutamic acid, pyruvic acid and alanine are utilized in an ammonia-binding mechanism. Later in the seizure pattern this mechanism is inadequate for ammonia-binding, and free ammonia accumulates in cerebral tissues; the glutamine concentration does not increase. Therefore dieldrin appears to inhibit glutamine synthesis in the brain, and in this connexion work by Lahiri & Quastel (1963) on the effect of fluoroacetic acid *in vitro* on the metabolism of ammonia in brain is relevant.

The intraperitoneal administration of 50 mg. of glutamine/kg. increases the cerebral content of glutamic acid, and an equivalent of ammonia, which is simultaneously liberated, is utilized in the formation of alanine by the mechanism involving α-oxoglutaric acid, glutamic acid, pyruvic acid and alanine, and not in glutamine synthesis. At first sight the present observations seem to be at variance with other work where the passage of glutamine across the blood–brain barrier was not accompanied by a change in the cerebral content of glutamic acid when mature rats were injected intravenously with 1·2–1·3 g. of glutamine/kg. (Schwerin, Bessman & Waelsch, 1950). In these experiments large amounts of glutamine were also taken up by the liver and kidneys, in which the rise in glutamine content was accompanied by the liberation of glutamic acid. Ammonia is simultaneously released in these organs and transported to the brain (e.g. Salvatore, Bocchini & Cimino, 1963; Tews *et al.* 1963), whereas glutamic acid is not taken up by the brain (Schwerin *et al.* 1950). After the administration of the unphysiological dose of glutamine (Schwerin *et al.* 1950) the increase in the concentration of cerebral ammonia appears to have been sufficient to have opposed glutaminase activity, with the result that the rise in cerebral glutamine concentration was not accompanied by a release of glutamic acid.

The fact that the parenteral administration of 50 mg. of glutamine/kg., which presumably penetrates the brain, leads to a rise in glutamic acid concentration of as much as 230 μmoles/100 g. wet wt., and that the equivalent amount of ammonia released is utilized in the formation of alanine and not in glutamine synthesis, seems to afford an explanation for the recovery of some of the animals that received a lethal dose of Telodrin after the administration of 50 mg. of glutamine/kg. (Hathway & Mallinson, 1964). An additional supply of glutamic acid thus became available for normal brain metabolism at the time when the maximum rate of glutamine synthesis due to Telodrin had begun to deplete the cerebral pool of glutamic acid, and this supply was not diminished by combination with the extra ammonia from injected glutamine since this ammonia had already been converted into alanine. This may be evidence of the 'compartmentalization' of two mechanisms for the disposal of ammonia in the brain.

In agreement with the suggested mechanism for the antidotal action of glutamine, animals killed 30 min. after the injection of glutamine and 16 min. after the injection of Telodrin have a cerebral concentration of alanine (120 μmoles/100 g. wet wt.) that approximates to the sum of the separate concentrations of alanine after the administration of glutamine and Telodrin respectively, a normal concentration of cerebral glutamic acid (670 μmoles/100 g.) and a cerebral concentration of glutamine (720 μmoles/100 g.) compatible with Telodrin poisoning. These results suffer from the limitation that there is no means of predicting whether animals that were killed before the onset of seizures would have otherwise survived Telodrin poisoning.

The present work draws attention to the role of alanine formation in the disposal of free NH_4^+ ion. De Ropp & Snedeker (1961) have reported an increase in the cerebral content of alanine during seizures brought about by electro-shock, 1,5-pentamethylenetetrazole, semicarbazide or strychnine. Present studies suggest that the effects of ammonia are not entirely dependent on the presence of high cerebral concentrations, but are related in part to the mechanism whereby ammonia enters the cerebral metabolic pathways.

After this paper had been submitted for publication, Witter & Farrior (1964) showed that dieldrin-induced seizures cause a significant increase ($P < 0.001$) in the alanine content of rat brain. These workers do not cite the concentrations of cerebral metabolites in their normal animals, and they did not investigate the preconvulsive effect on the cerebral alanine concentration.

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