mixture was then reinjected into the leg vein. The quantity of drug was calculated to produce a maximum possible concentration in the plasma of about 65 μg/ml. In one experiment 125 mg of CB 1414 was injected into a 10 kg dog and this killed the dog in 60 min. When only the acid form of CB 1414 was available, a weighed amount was dissolved in 1 N sodium or potassium hydroxide. The pH was adjusted to 7·5 with strong dihydrogen phosphate solution prior to mixing with the dog's blood.

The concentration of CB 1414 in the circulating plasma was determined from 3-ml samples of heparinized blood which were withdrawn from the other fore-leg of the dog. Between the injection and the withdrawal of the first blood sample 3 minutes were allowed to elapse to permit mixing. The amounts recovered are listed in Table 1.

TABLE 1. RECOVERY OF CB 1414 FROM THE CIRCULATING PLASMA OF DOGS

Injected quantity 2·5 mg/kg (65 μg/ml of plasma)		Injected quantity 12·5 mg/kg (325 μg/ml of plasma)	
Time (min)	conc. in plasma (μg/ml)	Time (min)	conc. in plasma (μg/ml)
3	1·72	3	19
10	1·53	10	7·5
		20	6·5
30	0·5	30	5
60	0·3	60	died

Using a pump-oxygenator system, perfusion of an isolated dog liver *in vivo* with CB 1414 was carried out by Dr. W. Zingg of the Department of Surgery. 55 mg. of the drug in solution in 10 ml of whole blood was added to a reservoir directly connected to the hepatic artery. Half-a-dozen blood samples were withdrawn from the hepatic vein at 5 min intervals, in which no trace of CB 1414 was detected. The amount of drug added was calculated to produce a concentration of approximately 40 μg/ml of circulating plasma.

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Tetanus toxin activity and ganglioside content of rat brain

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THE ISOLATION of a receptor substance for the exotoxin of *Clostridium tetani* has been recently reported by van Heyningen and his associates.[1-5] This receptor substance consists of gangliosides.[2, 3] The criteria for the identification of gangliosides as the tetanus toxin receptor substance resides in the observation that gangliosides and tetanus toxin associate to form a complex. The complex formation

was reported to be highly specific for gangliosides, however, more recent reports suggest that ganglioside can complex with other proteins such as Staphylococcus toxin, *Shigella shigae* neurotoxin, and blood serum.[6, 7] In addition (a) the ganglioside-tetanus complex is easily dissociable;[1] (b) complex formation with purified gangliosides does not reduce the toxicity of the toxin except at a high glycolipid-to-toxin ratio[1]; and (c) although the N-acetyl neuraminic acid moiety of the ganglioside is necessary for complex formation,[4] tetanus toxin in excess does not inhibit the removal of neuraminic acid from gangliosides by neuraminidase prepared from *Clostridium perfringens*.[8] In view of the large amount of ganglioside in the central nervous system and the very small amount of tetanus toxin required to be lethal (a million-fold difference at least) and the ready dissociation of the ganglioside-toxin complex, it was decided to investigate further the toxicity of tetanus toxin *in vivo*.

A preliminary experiment employed male white rats (Holtzman variety) 4, 8, 12, 18, and 30 days old. The minimal lethal dose of the tetanus toxin was determined for each age group with titration of a 50 per cent serial dilution of the toxin. It was found that the minimal lethal dose was the same for all groups of rats. Age appeared not be a factor. Table 1 presents data from another experiment

TABLE 1. GANGLIOSIDE CONTENT OF BRAIN AND THE TOXICITY OF TETANUS TOXIN

Holtzman male white rats of the appropriate age were injected subcutaneously (left hind leg) with serially diluted tetanus toxin (Wyeth, lot 1728, MLD 1-350,000) at the equivalent of 0·5% body weight. Eight rats were used at each dilution of the toxin. Death due to the toxin occurred in 2–3 days. All animals receiving nonlethal doses of the toxin were observed for 6 weeks. The ganglioside content of brain was determined with groups of 10 animals of each age.[8, 9] Protein was estimated by the procedure of Warburg and Christian.[10]

Age	Brain ganglioside	Total brain ganglioside	Ratio: ganglioside	Tetanus toxin	
				Minimal lethal	Maximal nonlethal
(days)	(μM/g)*	(μM)	body weight (μM/g body wt.)	dose tested (mμg toxin protein/g body wt.)	dose tested
7	1·27±0·46	0·78	0·034	0·93	0·67
31	3·90±0·74	5·89	0·068	0·93	0·67

* Molarity of ganglioside is based on the amount of N-acetyl neuraminic acid liberated by neuraminidase (*C. perfringens*) or mild acid hydrolysis (1 hr at 80 °C in 0·1 N H_2SO_4), calculated on the assumption of 1 mole N-acetyl neuraminic acid per mole ganglioside.

comparing the toxicity of tetanus toxin on rats 7 days and 31 days old with the ganglioside content of brains. It is apparent that, even though the total ganglioside content of the central nervous system varied over a sixfold range and the total brain ganglioside per gram of body weight of the rats for the two age groups increased twofold, there was no change in the minimal lethal dose of the tetanus toxin. Experimental controls (not reported) showed that the animals could be protected against the tetanus toxin by prior administration of tetanus antitoxin.

The experimental results obtained indicate *no* direct relationship between the lethal action of tetanus toxin and total ganglioside content; it is therefore possible that gangliosides are *not* the receptor substance for tetanus toxin. Of course, an alternative explanation is that gangliosides have multiple functions, and that only a small fraction of the gangliosides, at specific loci, is involved in the essential functions inhibited by the tetanus toxin protein.

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Free amino acids in brain of mice treated with L-glutamic acid-γ-hydrazide

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DIFFERENT carbonyl trapping agents can elevate or depress the levels of γ-aminobutyric acid (GABA)* in the brain of adult rodents. Hydroxylamine and aminooxyacetic acid injected into rats increase GABA in the brain,[1, 2] whereas thiosemicarbazide lowers the levels of this amino acid and decreases GAD activity.[3] To the authors' knowledge, the effects of GAH on the free amino acid pattern of the brain have not been investigated, and it was considered of interest to carry out experiments in this direction. Theoretically, GAH might block PyP-dependent enzymes such as GABA-T and GAD it could be an antimetabolite of glutamic acid and glutamine, and it could induce changes in their cerebral concentrations or in those of their metabolically related amino acids.

Adult, unfasted mice (local strain), previously fed with a commercial diet (Purina Laboratory Chow), were used. Different groups of animals were injected intraperitoneally with GAH (hemihydrate, A grade; California Corp. for Biochemical Research; 160 mg/kg body weight). The animals were killed by decapitation after 3·5, 6·5 and 24 hr, respectively. The controls in each case were injected with saline solution. In the first experiments the maximum changes in the GABA and alanine levels were obtained more than 6·5 hr after the injection of the drug. Other groups of mice were injected with different doses of GAH (40, 80, 360, 720 and 1440 mg/kg, respectively) and sacrificed after 6·5 hr. After decapitation the brains were rapidly removed and immersed in liquid air for 1·5 to 2 min. The brains were weighed while frozen and homogenized in Potter–Elvehjem homogenizers with 15 vol of 80% ethyl alcohol. The resulting suspensions, after treatment by the method of Awapara,[4] furnished extracts free of lipids and protein. These extracts were dried with the aid of an infrared lamp and the dried residues were dissolved in enough distilled water to give a 10- to 20-fold concentration The paper chromatographic methods used for the free amino acid analysis of the extracts have been described elsewhere.[5] The bidimensional descending technique was used (80% phenol and acetic acid : butanol : water, 1 : 4 : 1, by vol) for running duplicate series of chromatograms (Whatman no. 1 paper). The concentration of each amino acid in the chromatograms (glutamine included) was

* The abbreviations used are: GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GAH, L-glutamic acid-γ-hydrazide; PyP, pyridoxal phosphate; GABA-T, γ-aminobutyric acid-α-keto-glutaric acid transaminase.