Functional Development of the Central Emetic Mechanism in the Puppy Dog[1] (35369)

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Several studies have been published recently on physiologic responses of postnatal animals. These reports indicated that the functional maturation of some organs is not yet completed at birth. A certain time period is needed for the functional development. Beremzhanova and Sokolov (1) and Hutchinson *et al.* (6) reported that carotid baroceptors of young kittens and puppies did not respond as those of adults to changes of arterial perfusion pressure until the fourth postnatal day or even later. The thalamocortical interneuronal relationship in the dog could not be demonstrated by electrical stimulation during the early postnatal developmental period (5). Schweiler (8) also reported that the gamma innervation of intercostal muscles was not established in the first 2 to 3 postnatal weeks. Brizzee and Vitale (2) studied the functional development of emetic apparatus in the kitten and suggested that the critical period appeared to be between 150 g (about 2 days of age) and 250 g (about 10 days of age) of body weight and various components of the emetic mechanism in the cat matured almost simultaneously. Since dogs are very sensitive to apomorphine which acts solely at chemoceptive emetic trigger zone (CTZ), and cats are sensitive only to a lesser degree (7), puppies seem to be more suitable than kittens for studying the functional development of two parts of the central emetic mechanism. Two different emetic stimuli were used to detect functional maturation of the CTZ and the vomiting center: intravenously injected apomorphine to test the reactivity of CTZ and orally administered copper sulfate to test that of the vomiting center and its afferent connections.

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Materials and Methods. Ninety puppies from 26 litters of both sexes were used. Pregnant bitches were bought from animal dealers and the birthday of each litter was exactly recorded. The day of birth was designated as day 1. Their emetic responses were tested on days 2, 5, 10, 20, 30, and 60. The animals were grouped by their age instead of body weight, which was quite variable due to breed variation. Ten ml of milk was given by stomach tube immediately before a test dose of apomorphine hydrochloride by intravenous injection. For testing the emetic response to copper sulfate, the animal was fasted overnight and a known amount of copper sulfate (calculated without including the weight of water) dissolved in 5 ml of distilled water was given intragastrically. Animals were observed for 6 hr after drug administration and the cage was examined next morning to see whether there had been vomiting during the night. The experimental data were analyzed by probit method (4) and ED_{50} with its 95% confidence limit was calculated.

Results. Copper sulfate emesis. The incidence of emetic responses to orally applied copper sulfate and ED_{50} with 95% confidence limit are shown in Table I. Some 2-day-old puppies vomited after 20 or 40 mg of copper sulfate. Latency was 3 to 12 min and their vomitus was watery fluid with or without pieces of mucus. The ED_{50} (mg/animal) decreased remarkably at day 5 and thereafter remained almost unchanged up to day 30. In day 60 group, the ED_{50} was elevated. When calculated on a body weight basis, the ED_{50} remained almost unchanged from day 5 through day 60. Latency of the emetic response of different groups ranged from 3 to 48 min. The vomiting acts of lick-

TABLE I. Emetic Responses to Oral Copper Sulfate in Puppies.

Dose (mg/animal) (day):	Age 2	5	10	20	30	60
1		4/10[a]	0/9	1/7	1/7	
2		4/8	4/8	5/8	3/6	0/5
5		7/7	7/8	8/8	6/6	1/6
8						3/4
10						6/7
20	2/9					
40	3/6					
ED_{50} with 95% confidence limit						
(mg/animal)	40.00	1.62 (0.94–2.54)	2.35 (1.66–3.72)	1.67 (1.36–2.50)	1.83 (1.15–3.22)	6.78 (3.88–9.34)
(mg/kg)	164.3	4.21 (2.70–5.23)	4.04 (2.01–7.74)	2.95 (2.30–3.60)	3.11 (1.91–4.89)	4.01 (1.61–5.33)
Av body wt (g)	241	306	396	559	767	1660

[a] Denominator indicates number of animals tested and numerator number of animals vomited.

ing, retching, and forcible respiratory movements were quite weak in 2- and 5-day-old puppies.

Apomorphine emesis. Emetic responses could not be elicited by intravenous injection of apomorphine as high as 1 mg/kg of body weight in seven 2-day-old puppies, all of which survived. In the day 5 group, puppies vomited to graded doses from 0.1 to 0.3 mg/kg of apomorphine. The ED_{50} decreased remarkably up to day 10 and thereafter kept decreasing with a much slower rate up to day 30. The ED_{50} at days 30 and 60 was very close to that of adult dogs (6–12 kg). The ED_{50} and its 95% confidence limit at various ages are given in Table II. Vomiting occurred within 0.3 to 6.5 min after the injection and there was no significant differences in latency among groups.

Discussion. Copper sulfate given orally stimulates receptors on the inner surface of the upper gastrointestinal tract to elicit reflex emetic responses (9). Some of the 2-day-old puppies vomited in response to copper sul-

TABLE II. Emetic Responses of Puppies to Intravenous Injection of Apomorphine.

Dose (μg/kg)	Age Day 2	Day 5	Day 10	Day 20	Day 30	Day 60	Adult
10				0/3[a]	1/6	1/7	7/18
15			0/4	1/7	6/9	7/10	18/19
20			3/9	4/8	9/11	3/3	
30			5/10	4/4			
50			6/9				
100		1/10	5/6				
200		5/8					
300		8/9					
1000	0/7						
ED_{50} with 95% confidence limit (μg/kg)	>1000	171.3 (133.0–217.9)	32.6 (21.01–100.2)	19.6 (16.4–24.7)	13.9 (9.3–16.4)	13.1 (10.0–15.7)	10.6 (9.0–11.8)
Av body wt (g)	258	340	521	999	1529	2960	7850

[a] Denominator indicates number of animals tested and numerator number of animals vomited.

fate, the reactivity of copper sulfate increased remarkably up to day 5, and thereafter it remained almost unchanged. The results with copper sulfate indicate that afferent reflex pathways from visceral organs to the vomiting center, the vomiting center, and efferent pathways must be established within 2 days after birth in some puppies and their maturation was almost completed at day 5. Although the ED_{50} of copper sulfate (mg/animal) was elevated in the day 60 group, the ED_{50} calculated on a body weight basis showed no significant difference among groups. This is interpreted as the result of an increase of inner surface area of the upper gastrointestinal tract along with body growth between days 30 and 60 instead of changes in the sensitivity of vomiting mechanism.

It is known that apomorphine intravenously injected acts only at a single site, the CTZ (9). Two-day-old puppies did not vomit even to a dose of 1 mg/kg of apomorphine which is usually used to check complete ablation of CTZ. The reactivity to apomorphine appeared at day 5, its sensitivity increased remarkably up to day 10 and thereafter further increased with a much slower rate up to day 30, when the ED_{50} was very close to that of adults. Since performance of vomiting induced by apomorphine depends on the CTZ, the vomiting center, and efferent pathways of emetic acts, these results, together with those of copper sulfate emesis, suggest that function of the CTZ and/or neural connections between the CTZ and the vomiting center develops during the early postnatal period between days 2 and 5. Progressive maturation of the CTZ continues and is almost complete at about day 30. It seems that function of the vomiting mechanism responding to apomorphine and to copper sulfate did not develop at the same rate.

Brizzee and Marshall (3) observed that kittens (100 g body wt) did not vomit in response to oral copper sulfate but that 25% of the animals did respond to tartar emetic. According to their report, kittens weighing 85 to 150 g are about 1 to 3 days old. Therefore the earliest age of a functioning vomiting center seems to be similar in kittens and puppies. However, the rate of maturation of vomiting center cannot be compared between these two species because only two dose levels were tested in kittens and the ED_{50} cannot be calculated.

Summary. Ninety dogs at different ages (from days 2 to 60) were tested for their emetic responses to graded doses of apomorphine injected intravenously and to copper sulfate administered orally. None of the 2-day-old puppies vomited in response to apomorphine, up to a dose of 1 mg/kg. However, some did vomit when given 20 or 40 mg of copper sulfate. The reactivity to copper sulfate increased remarkably from days 2 to 5, and thereafter it remained almost unchanged up to day 60. The reactivity to apomorphine first appeared at day 5 and increased remarkably up to day 10. Thereafter, it continued to increase at a much slower rate up to day 30, at which time the ED_{50} was very close to that of adults. These results suggest that the emetic response mediated by the chemoceptive emetic trigger zone (CTZ) did not function at the second day after birth, but that the vomiting center could be excited by impulses from the gastrointestinal tract, and that the vomiting center and CTZ matured at different rates in puppies.

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