Summary. Administration of 50% deuterium oxide to rabbits as drinking water has been found to result in potent inhibition of DNA synthesis in the bone marrow and thymus, and to a lesser extent, in the liver. A decreased number of circulating erythrocytes was observed under these conditions, but no significant change in erythrocyte or bone marrow morphology was noted. DNA preparations from deuterated animals appear to be slightly denatured in comparison to DNA preparations from control animals.

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Emetic Responses of Monkeys to Apomorphine, Hydergine, Deslanoside and Protoveratrine.* (27468)

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The discovery of the chemoceptive emetic trigger zone in the area postrema led to reinvestigation of the sites of action of many emetic agents(1). It has been reported that apomorphine(2), morphine and Hydergine (3), and intravenous copper sulfate(4) caused vomiting by acting on the trigger zone; intravenous digitalis glucosides act mainly on the trigger zone but also on other areas(1); and oral copper sulfate acts peripherally on the gastrointestinal tract(4). It has also been found that X-radiation(5,6) and motion sickness(7) induce vomiting by acting on the trigger zone. Most of these experiments have been done in dogs and some in cats(8) and it has been shown that there are a number of differences in emetic mechanism between these 2 species(9,10,11). It is interesting to study, therefore, whether the emetic mechanism in monkeys differs from that in the other 2 animals. Brizzee *et al.*(12) reported that the trigger zone of monkeys is virtually unresponsive to drugs, but the action of various emetic agents in these animals has been investigated far less thoroughly than it has been in dogs and cats.

We chose 3 groups of drugs: first, drugs which cause vomiting by acting solely or mainly on the trigger zone, such as apomorphine(2) and Hydergine(3) and deslanoside (1,8); second, drugs which act on the gastrointestinal tract, such as oral copper sulfate (4); third, drugs which cause emesis by acting on areas other than the trigger zone, such as protoveratrine A(13). Our results indicate that while monkeys are less sensitive to all emetics, both those acting on the trigger zone and those acting on peripheral sites, the trigger zone of these animals is indeed responsive to drugs.

Materials and methods. Fifty-two monkeys (*Macacus cyclopis*) of either sex weighing 2.3 to 5.2 kg were used. Animals receiving emetic agents intravenously such as apomorphine, Hydergine, deslanoside and protoveratrine A† were fed about 20 minutes before injection. Those challenged with copper sulfate were fasted for at least 18 hours before the compound, calculated dry and dissolved in 40 ml of water, was given *via* a stomach tube. Gut denervated monkeys were

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† Hydergine, deslanoside and protoveratrine A were kindly supplied by Sandoz Pharmaceuticals, Hanover, N. J.

fasted more than 24 hours before being tested. Protoveratrine A was dissolved in 0.25% acetic acid. Monkeys were observed for 7 hours after the drug administration, and the cage was examined next morning to determine whether there had been vomiting during the night. No animal was tested more than once every 3 days. Deslanoside was injected at intervals of more than 1 week.

The chemoceptive emetic trigger zone was surgically removed according to the technic of Wang and Borison(2). Gut denervation was carried out in 2 or 3 stages and included trans-thoracic vagotomy and lower thoracic and abdominal sympathectomy(4). Cardiac denervation was carried out through a midline thoracic incision, and both vagotomy and upper thoracic sympathectomy (first 5-6 thoracic ganglia) were performed in a one-stage operation which all tolerated well.

Post-operative tests with emetic agents were carried out 1½ months after the trigger zone ablation, 2 weeks after gut denervation, and 1½ months after cardiac denervation. Brains of the trigger zone ablated monkeys were fixed with 10% formalin and processed for histologic study. Peripheral nerve sections were verified by gross post-mortem dissection.

Results. Apomorphine and Hydergine. 0.015 to 0.3 mg/kg of apomorphine or Hydergine was injected intravenously in 5 monkeys, but none showed any effect and no vomiting was observed.

Deslanoside. Deslanoside was injected intravenously in 47 normal monkeys. The results are shown in Table I. 94.5% of monkeys vomited to 0.1 mg/kg, with a latency ranging from 4 minutes to overnight. Smaller doses produced a lower incidence of vomiting and also a longer latency. Even with 0.1 mg/kg dose, the latency was still considerably longer than in dogs and cats. The monkeys vomited several times and the emetic action lasted for a long time. One monkey (#40) vomited 20 times, from 11 minutes to 23 hours and 24 minutes after injection. In 15 animals the emetic action lasted overnight.

Seventeen trigger zone ablated monkeys were tested. After the operation only 4 of these animals vomited to deslanoside: 1 to 0.1 mg/kg; 2 to 0.15 mg/kg; and 1 to 0.2 mg/kg. We were unable to differentiate "early emesis" in normal and "late emesis" in trigger zone ablated animals, as is possible in dogs and cats.

Four cardiac denervated monkeys were tested with 0.1 mg/kg of deslanoside. All vomited with an average latency of 2 hours and 8 minutes. Although 4 trigger zone ablated monkeys vomited to deslanoside, 3 of them died at the second test with this agent. Thus it was possible to test deslanoside only in one monkey (#61) with cardiac denervation plus trigger zone ablation. This monkey vomited to 0.1 mg/kg of deslanoside with a latency of 1 hour and 7 minutes before the trigger zone ablation, and to 0.1 mg/kg with a latency of 2 hours after this operation plus cardiac denervation. However, there is some doubt as to the completeness of trigger zone ablation in this monkey.

Copper sulfate. Various doses of copper sulfate were given orally to 18 monkeys. The emetic responses occurred usually within 2 hours. 63.6% of the animals vomited to 160 mg of copper sulfate with an average latency of 37.6 minutes, but 2 did not vomit even to 360 mg of copper sulfate (Table II). The vomiting came in one or 2 bouts.

Before gut denervation in 3 monkeys, the threshold emetic dose was 120, 160, and 360 mg, respectively. After the operation 2 animals did not vomit to 360 mg, and 1 in which the threshold before the operation was 120 mg, vomited to 360 mg with a latency of approximately 5 hours (Table III).

Protoveratrine A. Among 21 monkeys tested on protoveratrine A, 83.3% vomited to 15 μg/kg with an average latency of 5.5 minutes (Table IV). The vomiting, which came in several bouts, started within 15 minutes and ceased within 1 hour.

Four cardiac denervated monkeys and 4 trigger zone ablated plus cardiac denervated animals were also tested. Neither the threshold emetic dose nor the latency seemed to be altered in these animals (Table V).

Discussion. According to our results, both the emetic action and the toxicity of deslanoside are different from those reported by Brizzee *et al.*(12), who used *Macaca mulatta* and

TABLE I. Comparison of Effective Emetic Dose of Intravenously Injected Deslanoside in Normal, Trigger Zone Ablated, and Cardiac Denervated Monkeys.

Dose (mg/kg)	Normal monkeys				Trigger zone ablated monkeys			Cardiac denervated monkeys			
			Latency						Latency		
	Tested	Vomited	Avg	Range	Tested	Vomited	Latency	Tested	Vomited	Avg	Range
.07	3	1	3 hr 49 min.								
.08	3	1	2 " 15 "								
.09	3	2	1 " 36 "	1 hr 14 min.-1 hr 58 min.							
.10	36	34	1 " 41 "	4 min.-overnight	16	1†	1 hr 7 min.	4	4	2 hr 8 min.	28 min.-5 hr 54 min.
.12	2*	2	6 " 15 "	6 hr 9 min.-6 hr 20 min.	9	0					
.15					7‡	2	49 min., overnight				
.18					2	0					
.20					1	1§	22 min.				

* These 2 monkeys did not vomit to 0.1 mg/kg. † This monkey (#61) also vomited to 0.1 mg/kg with the latency of 2 hr after trigger zone ablation plus cardiac denervation. ‡ Five monkeys died. § Vomited once in 2 trials. This monkey died at the second test.

cynomolgus. One reason for the discrepancy may be sub-species differences. Deslanoside-induced emesis in *Macaca cyclopis* has a long latency (average over one hour, and often 6 to 7 hours), while in dogs and cats the latency is short, only several minutes. On dogs and cats, Wang(1) and Borison(8) observed "early and late emesis" in normal animals, and only "late emesis" in the trigger zone ablated animals after intravenous injection of deslanoside. On *Macacus cyclopsis*, however, we could not differentiate the 2 phases in either normal or trigger zone ablated animals, because the latency in both ranges widely from a few minutes to overnight. As in dogs and cats, a few trigger zone ablated monkeys vomited to large doses of deslanoside. Since only 4 out of 17 operated animals vomited to large doses of deslanoside, the possibility remains that the trigger zone has not been completely ablated. In this respect, histological examination of the lesions did not yield any positive finding. The long delayed emetic responses, on the other hand, make it difficult to ascertain whether the emetic responses to large doses of deslanoside in the operated monkeys are due to incomplete ablation of the trigger zone, or to the existence of a second, alternate site of action, as in the case of the dog.

TABLE II. Effective Emetic Doses of Orally Administered Copper Sulfate in Normal Monkeys.

Dose (mg)	Monkeys tested	Monkeys vomited	Latency (min.) Avg	Latency (min.) Range
80	5	1	69	
120	9	5	45.8	13–101
160	11	7	37.6	7– 88
240	4*	1	83	
360	4*	2	73	56– 90

* These 4 monkeys did not vomit to 160 mg.

Among the drugs which act on the trigger zone, only deslanoside can induce vomiting in monkeys. They are refractory to large doses of apomorphine or Hydergine. Brand *et al.*(9) used a much larger dose of apomorphine (25 mg/kg s.c.) to elicit vomiting in the cat. Although the emetic action of the digitalis glycosides is not restricted to the trigger zone, it can be used to check the com-

TABLE III. Comparison of Emetic Responses of Orally Administered Copper Sulfate in Monkeys before and after Gut Denervation.

Monkey No.	Body wt (kg)	Before denervation: Threshold emetic dose (mg)	Before denervation: Latency (min.)	After denervation: Threshold emetic dose (mg)	After denervation: Latency (min.)
43	3.6	360	56	neg.*	
50	2.6	120	101	360	300
85	3.9	160	88	neg.	

* neg. indicates no vomiting even to the dose of 360 mg. Monkey #43 and #50 died after the test of 360 mg of oral copper sulfate with severe diarrhea.

pleteness of the trigger zone in monkeys.

Monkeys are less sensitive to oral copper sulfate than dogs and cats, but their mechanism of vomiting to oral copper sulfate seems to be essentially similar(4,8).

There is a remarkable species difference in the response to the drugs which act on the trigger zone. Dogs vomit to apomorphine, morphine, Hydergine, intravenous copper sulfate, and digitalis glucosides. Cats vomit to digitalis glucosides, very high doses of apomorphine, lethal doses of intravenous copper sulfate, but not to Hydergine(9). Monkeys vomit to digitalis glucosides, but not to apomorphine and Hydergine(12). Chloropromazine has selective protective action against various emetic drugs in dogs(14), but is not as effective in cats(9). The early vomiting following X-radiation can be prevented by trigger zone ablation in dogs(5,6), but not in cats(11). Trigger zone ablation protected dogs but not cats from the emetic action of nitrogen mustard, nicotine, and lobeline(10, 15). When taken together with the present study, these results indicate that the receptors in the emetic trigger zone seem to be of more than one type, and that the functional organization of the emetic receptors also appears to differ among dogs, cats, and monkeys.

TABLE IV. Effective Emetic Doses of Intravenously Injected Protoveratrine A in Normal Monkeys.

Dose (μg/kg)	Monkeys tested	Monkeys vomited	Latency (min.) Avg	Latency (min.) Range
5	5	2	7.5	2 –13
7	4	3	9.3	6 –13
10	2	2	3	2 – 4
15	6	5	5.5	2.5–10
20	1*	1	12	

* This monkey did not vomit to 15 μg/kg.

Summary. Apomorphine, Hydergine, deslanoside, copper sulfate, and protoveratrine A were tested for their emetic action in monkeys. Apomorphine or Hydergine, even in doses as high as 0.3 mg/kg, i.v., could not provoke vomiting in monkeys. 0.1 mg/kg i.v., of deslanoside induced vomiting lasting several hours and with a long latency in 94.5% of the monkeys. After trigger zone ablation, most of the monkeys did not vomit to deslanoside and only a few vomited to high doses of this agent.

Oral copper sulfate in a dose of 160 mg provoked vomiting in 63.6% of monkeys. After gut denervation these animals did not vomit even to 360 mg, except for one which vomited to this dose after a long latency. 83.3% of the monkeys vomited to 15 μg/kg of intravenous protoveratrine A. Cardiac denervation or trigger zone ablation plus cardiac denervation influenced neither the threshold

TABLE V. Comparison of Threshold Emetic Dose of Intravenously Injected Protoveratrine A among Normal, Cardiac Denervated, and Trigger Zone Ablated plus Cardiac Denervated Monkeys.

Condition	No. of monkeys	Avg threshold emetic dose (μg/kg)	Latency (min.) Avg	Latency (min.) Range
Normal	11	11	8	2–13
Cardiac denervation	4	9.3	4	1–10
Trigger zone ablation + cardiac denervation	4	10.5	6.8	1–15

emetic dose nor the latency of this agent.

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Silicon in Biological Material. I. Determinations Eliminating Silicon as a Contaminant. (27469)

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Silicon has been found to be present in all biological material(1-7). Since the content of silicon is small, a micro-method is required for the determination and extreme care must be taken to avoid contamination from outside sources of silicon, such as glassware and dust of the air. King(1) has shown that blood samples stored in glass tubes have a higher silicon content than those stored in cellophane tubes. Therefore, previously reported values for silicon in biological materials, obtained with the use of glassware, are probably too high. Inasmuch as nearly all laboratory wares are now available in materials other than glass, contamination of biological samples by extraneous silicon can be eliminated.

The present study is concerned with 1) determination of silicon content in biological material by a modification of King's method with the use of the least amount of glassware; and 2) absorption and excretion of silicon in the rat.

Equipment and reagents. All laboratory wares were made of either polyethylene or polypropylene plastics or stainless steel. They were washed separately from laboratory glassware in plastic dish pans with haemosol, rinsed with tap water, then distilled water and drained and re-rinsed with redistilled water, and dried in a 50° oven prior to use.

All water used was redistilled in a pyrex distilling set; this was the only place in the entire procedure that glass containers were employed. Such water was promptly stored in large polyethylene bottles. For ashing and subsequent fusion of the samples with Na_2CO_3, 8 ml platinum crucibles were used. Measurements of absorbancy were made in a Beckman DU spectrophotometer. Except for items 1 to 4 below, all reagents are as described in the original method of King *et al.*(2). All chemicals used were "reagent grade".

1. Silicon Standard Solution—a stock solution was made from sodium meta-silicate, $Na_2SiO_3 \cdot 9H_2O$ (10.15057 g/liter), representing an equivalent of 1 g of silicon/liter. A working standard solution containing 0.5 μg Si/ml was prepared from the stock solution. The $Na_2SiO_3 \cdot 9H_2O$ used is completely soluble. 2. Phenolphthalein Indicator—1% in ethanol. 3. Activated Carbon