in both basins; moreover, meltwaters may have been too abundant to allow the brackish waters to even reach the northern part of the Lake St. John basin. However, GSC-313 does not invalidate La Rocque's idea.

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References and Notes

1. P. Lasalle, "Preliminary Rept. 546" (Quebec Dept. of Natural Resources, Quebec City, Canada), in press.
2. N. R. Gadd, personal communication (1965).
3. Sample GSC-313 dated by W. Dyck, Geological Survey of Canada. Two more datings have since been made for the Lake St. John area: (i) GSC-375, marine shells, 9340 ± 160 years; elevation, 120 m; source, 48°26′N, 71°51′W; and (ii) Y-1557 (Dr. Stuiver, Yale Geochronol. Lab.), brown organic mud from bottom of a kettle hole, 7430 ± 120 years; elevation, about 237 m; source, 48°23′30″N, 72°01′W. These two sites are south of Lake St. John and west of the source of GSC-313. These results seem to confirm my interpretation.
4. Measured with an engineer's level.
5. A. S. Pearse and G. Gunter, in *Treatise on Marine Ecology and Paleoecology*, J. W. Hedgpeth, Ed. (Geol. Soc. Amer., New York, 1957), p. 129; G. Gunter, *ibid.*, p. 159.
6. N. R. Gadd, *Bull. Geol. Soc. Amer.* **75**, 1249 (1964).
7. J. A. Elson, New England Int. Geol. Conf., McGill Univ., Montreal (1962); personal communication.
8. J. Terasmae, *Bull. Geol. Surv. Can.* **56**, 19 (1960).
9. H. A. Lee, *Science* **131**, 1609 (1960).
10. A. La Rocque, *Bull. Geol. Soc. Amer.* **60**, 363 (1949).
11. I thank the Geological Survey of Canada for the carbon-14 dating and P. P. David, J. A. Elson, and N. R. Gadd for helpful suggestions.
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Cleft Palate Produced in Mice by Human-Equivalent Dosage with Triamcinolone

Abstract. *Triamcinolone produced cleft palates in mouse embryos at a dosage proportionate, by body weight, to common therapeutic dosage for humans. Thus, it showed much greater teratogenicity than other glucocorticoids tested on mice. Widely ranging doses of desoxycorticosterone did not produce cleft palates.*

Triamcinolone is used to treat diseases usually treated with glucocorticoids, especially dermatologic conditions. Hefley *et al.* (*1*) used triamcinolone for chronic allergic disorders such as asthma and chronic rhinitis in both male and female patients, 110 of whom were between the ages of 21 and 40. The effect of an intramuscular dose of 40 to 100 mg lasted for 40 days on average so that treatment of a mother before she knew she was pregnant could continue to exert an effect through the period of embryonic palate closure (33 days after the first missed menstrual period). The teratogenic effects of this drug should therefore be determined in relation to common human dosage. Fraser (*2*) reviewed the ratios of teratogenic dosage of various drugs, as applied to experimental animals, with therapeutic human dosage, on the basis of body weight; ratios ranged from 1 for tetracycline in rats to 400 for cortisone in mice. The figure of 400 may lead one to believe that there is a large margin of safety between teratogenic and therapeutic doses of glucocorticoids. This ratio is lower with some of the newer glucocorticoids, even allowing for their greater therapeutic effect per milligram in human beings (*3*, *4*). In this report we show that triamcinolone departs radically from the ratio of teratogenic to therapeutic dosage found with other corticosteroids.

Vaginal plugs were used as a criterion for timing pregnancies in 118 mice from matings within five inbred strains. From 11 to 14 days after conception, the pregnant mice were treated as follows: desoxycorticosterone acetate or desoxycorticosterone trimethylacetate was injected intramuscularly, triamcinolone diacetate was given by stomach tube, or triamcinolone acetonide was injected intramuscularly or subcutaneously. One or four daily injections were given at the times and dosages shown in Table 1. Uteri were removed at day 18 and fixed in Bouin's fluid. Fetuses were then removed and their palates were studied with a dissecting microscope after removal of the lower jaw. Average palate stage was calculated by assigning values of 0.25, 0.50, 0.75, and 1.00 to palate stages 1 (both shelves vertical), 3 (one shelf horizontal), 4 (both shelves horizontal), and 7 (normal palate), respectively (*5*).

Triamcinolone acetonide given intramuscularly to A/J strain pregnant mice on days 11 (11 days, 8 hours, assuming ovulation took place at 2 a.m.) to 14 caused excessive resorption in doses of 0.025 mg × 4 or higher (Table 1). Doses of 0.0125 to 0.001 mg/day caused cleft palates with frequencies ranging from 100 to 18 percent. The degree of inhibition of palatine shelf movement caused by triamcinolone over this dose range is reflected in the average palate stage (*5*), which increased from 0.55 to 0.96. Single doses of triamcinolone acetate also produced some clefts when injected on days 11, 12, and and 14.

The intramuscular, subcutaneous, and oral methods of administration were all effective, although triamcinolone diacetate given orally was less effective than triamcinolone acetonide given intramuscularly. Progression of the palatine shelves from a sagittal to a transverse plane was no greater with the subcutaneous than with the intramuscular method of administration, as shown by figures for average palate stage (Table 1). Cleft palate was induced by triamcinolone acetonide in strains C3H, C57BL, and DBA; strain 129/J was more resistant to the teratogenic effects of triamcinolone than strain A/J.

After administration of desoxycorticosterone, fetuses appeared normal in size and color. The three embryos with cleft lip–cleft palate do not constitute a frequency exceeding the expected spontaneous occurrence of 10 to 15 percent in the A/J strain.

Cortisone (*6*) and triamcinolone can best be compared for teratogenic potency at the highest dosage that causes cleft palates in 100 percent of offspring but does not cause a high frequency of resorption; this dosage would be 2.5 mg × 4 for cortisone (*6*) and 0.0125 mg × 4 for triamcinolone acetonide. The two teratogens produced almost identical average palate stages at such dosages (0.51 for cortisone and 0.55 for triamcinolone). Thus, 2.5 mg as opposed to 0.0125 mg represents a dosage difference of 200 times for teratogenic effect in mice, whereas these two glucocorticoids differ by a dosage factor of only about 6 times in therapeutic effect on man. Similarly, triamcinolone was 10 times more potent as a cleft-palate teratogen than dexamethasone, which was given in doses of 0.15 mg to produce cleft palate in 100 percent of the offspring of A/J strain mice (*4*). If we assume a weight difference of 2000 times between human beings and mice, the lowest dose of triamcinolone acetonide causing cleft palate in strain A/J mice, 0.001 mg × 4, would be equivalent to a dose of 2 mg/day for 4 days in human beings; this dosage is well within the recommended therapeutic range.

The relative roles of anti-inflammatory and salt-retention effects of glucocorticoids should also be investigated. There are reasons for implicating the latter effect in the report of changes in amount of amniotic fluid associated with treatment by cortisone and certain other teratogens that produce cleft palate (*7*);

Table 1. Palate morphology 18 days after conception in fetuses from mice treated with triamcinolone or desoxycorticosterone. Dosage was intramuscular except where otherwise indicated. CLCP, cleft lip and cleft palate.

Drug administration		Litters		Palate morphology			Palate stage (avg.)
Dosage (mg)	Days after conception*	No.	Resorbed	Normal	Cleft	CLCP	
Triamcinolone acetonide: strain A/J							
0.5 × 4	11 to 14	5	5				
0.2 × 4	11 to 14	3	3				
0.05 × 4	11 to 14	4	4				
0.025 × 4	11 to 14	4	3		2		0.63
0.0125 × 4	11 to 14	7			43	2	0.55
0.006 × 4	11 to 14	5		13	20		0.83
0.003 × 4	11 to 14	3		15	8	1	0.90
0.001 × 4	11 to 14	2		14	3		0.96
0.0005 × 4	11 to 14	5		38		1	1.00
0.05	11	1			7		0.71
0.0125	12	2		4	2	1	0.92
0.0125	13	2		9		1	1.00
0.0125	14	4		14	3		0.96
0.006 × 4†	11 to 14	5		9	15	2	0.76
Triamcinolone diacetate: strain A/J							
0.02 × 4‡	11 to 14	3		12	7		0.91
0.01 × 4‡	11 to 14	3		22			1.00
0.02‡	14	3		26			1.00
Triamcinolone acetonide: strain 129/J							
0.025 × 4	11 to 14	6	3	6	5		0.95
0.0125 × 4	11 to 14	9	5	13	5		0.92
0.006 × 4	11 to 14	3	1	8			1.00
0.003 × 4	11 to 14	4	1	27			1.00
0.001 × 4	11 to 14	1		5			1.00
Triamcinolone acetonide: strain C3H/HeJ							
0.0125 × 4	11 to 14	5		8§	13		0.76
Triamcinolone acetonide: strain C57BL/6J							
0.0125 × 4	11 to 14	3	2		3		0.75
Triamcinolone acetonide: strain DBA/1J							
0.0125 × 4	11 to 14	14	1	29	42		0.80
Desoxycorticosterone acetate: strain A/J							
0.10 × 4	11 to 14	2		7		1	
0.15 × 4	11 to 14	2		11			
0.50 × 4	11 to 14	4		28		1	
1.00 × 4	11 to 14	2		11			
1.25 × 4‖	11 to 14	2		12#		1	

* Vaginal plug seen on day 0. † Administered subcutaneously. ‡ Administered orally. § Two embryos had cleft uvula. ‖ Desoxycorticosterone trimethylacetate. #One embryo had spina bifida.

mechanically reduced volume of amniotic fluid can cause cleft palate (*8*). It is clearly not the sodium-retention effects of cortisone that cause cleft palate; triamcinolone, essentially free of such effects, is a potent inducer of cleft palate, whereas desoxycorticosterone, even in doses as large as the human dose (without adjustment for weight difference), produced no cleft palates in mice.

The many variables encountered in screening drugs for teratogenic potential (*2*) make it difficult to screen any type of drug thoroughly. It now appears that, at least in mice, closely related drugs can differ so radically in teratogenic potency that evidence of safety collected for one of a group of related compounds may be quite inapplicable to others of that group. Pregnant women treated with cortisone have long been watched closely without any sign of marked increase in frequency of cleft palate. If one may extrapolate from experience with mice, each glucocorticoid must be individually evaluated.

The problem of extrapolating from results with one or two species of laboratory animals is a major one facing experimental teratologists (*2*). To bar all drugs during pregnancy is impractical, but having to wait for the frequency of infant malformation to provoke investigation of drug intake is not ideal. The testing of a drug like triamcinolone on a wide variety of mammals may provide a better estimate of its potential teratogenicity for humans (*8*).

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References and Notes

1. B. F. Hefley *et al.*, *Ann Allergy* **22**, 244 (1964).
2. F. C. Fraser, in *Second International Conference on Congenital Malformations* (International Medical Congress, New York, 1964), p. 277.
3. H. Fujino, S. Handa, T. Katsuki, *Proc. Congenital Anomal. Res. Assoc. Japan* **1**, 4 (1962).
4. L. Pinsky and A. M. DiGeorge, *Science* **147**, 402 (1965).
5. B. E. Walker and B. Crain, *Am. J. Anat.* **107**, 49 (1960).
6. F. C. Fraser and T. D. Fainstat, *Pediatrics* **8**, 527 (1951).
7. B. E. Walker, *Proc. Soc. Exp. Biol. Med.* **118**, 606 (1965).
8. ——, *Science* **130**, 981 (1959).
9. Supported by PHS grant HD-00153.

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Wyeomyia Subgroup of Arbovirus: Isolation from Man

Abstract. *An agent, serologically identical to a Wyeomyia virus obtained from mosquitoes, was isolated from a worker on the inter-American highway project in Darien Province in eastern Panama. He experienced a mild febrile illness with recovery. A significant rise in antibody titer to this virus was demonstrated in his serum during convalescence. Neutralizing antibodies to this newly isolated strain were found in 10 of 59 blood samples from inhabitants of Darien Province. The virus is designated the Darien strain.*

The arthropod-borne Wyeomyia virus was first isolated in 1940 from *Wyeomyia melanocephala* in Colombia (*1*) and has since been classified as belonging to the Bunyamwera group (*2*). For many years there were no further reports of its occurrence, but recently new strains have been isolated repeatedly from mosquitoes in Brazil (*3*), Trinidad (*4*), Colombia (*5*), and Panama (*6, 7*). Serological differences have been demonstrated among these various strains which are now regarded as forming a subgroup, called the Wyeomyia complex (*8*). We report the first isolation of a virus strain of this subgroup from a vertebrate host. It is proposed to call this strain Darien for the place where the patient worked.

During the course of a preliminary survey of diseases along the proposed Darien Province section of the inter-American highway in eastern Panama, an adult male worker of the road-surveying company was seen at the El Real field station of the Gorgas Memorial Laboratory. Physical examination showed no abnormal signs or symptoms other than a low-grade fever. Malaria parasites were not found in the blood smear; the white cell count was 3800 per cubic millimeter with 73 percent neutrophiles, 23 percent lymphocytes, 3 percent monocytes, and 1 percent eosinophiles. The red blood count was 4.12 million per cubic millimeter and the hemoglobin was 13 g per 100 ml.

Blood serum from this patient was inoculated intracerebrally into a litter of suckling mice. One mouse was found dead on each of days 10, 11, and 13 after inoculation; the fourth mouse was sick on the 11th day. Brain suspension from the sick mouse was passed to another litter of suckling mice, and all of these were either dead or sick by the