after sublethal X-irradiation. Total mortality was four-fold less among guinea pigs vaccinated by the sc route when the same treatment intervals were compared.

Summary. Sublethal X-irradiation of guinea pigs 12 days before to 3 days after sc vaccination with viable *P. tularensis* LVS resulted in a maximum mortality of 7.5% at 2 intervals. Surviving animals, however, displayed an agglutinin response and resistance to respiratory challenge similar to that of nonirradiated vaccinated animals.

We wish to acknowledge the collaboration of Lt. Colonel Joshua Henderson in the X-irradiation procedures.

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Teratogenic Compounds of *Veratrum californicum* (Durand). III Malformations of the Veratramine-Induced Type from Ingestion of Plant Or Roots. (32474)

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We have previously reported that the alkaloid veratramine, a parent alkamine of the steroidal class of veratrum alkaloids, produced congenital abnormalities in lambs born to ewes that ingested the alkaloid on the 14th day of gestation(1). The malformation was characterized by slight lateral or medial bowing of the front legs, slight to marked flexure of the knee joint, looseness (hypermobility) of the hock or stifle joints, or a

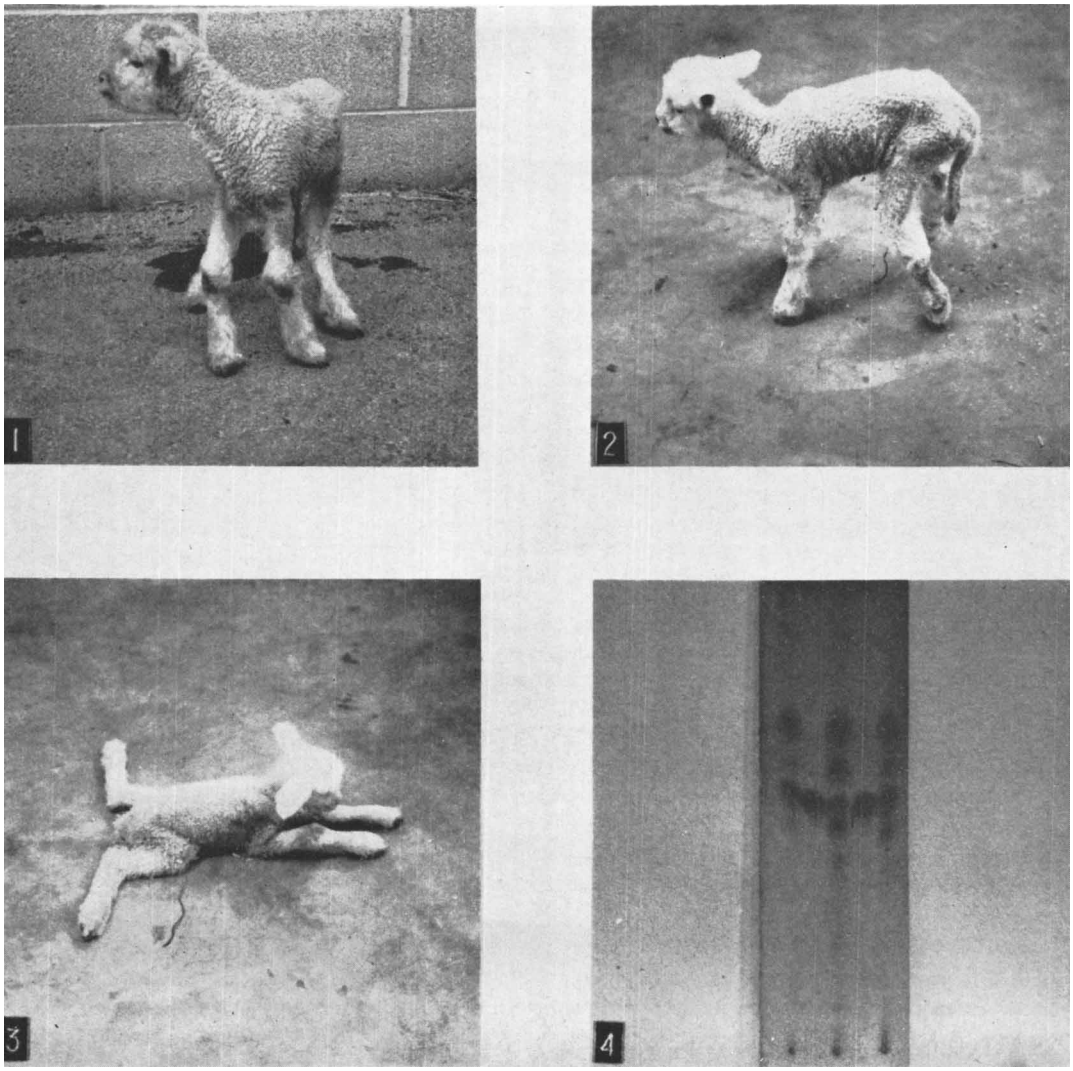
TABLE I. Non-Cyclopian Teratogenic Effects in Offspring from Ewes Dosed with *V. californicum* on the 14th Day of Gestation.

	14th day dose		
Ewe No.	Amt (g)	Doses (No.)	Effect on offspring
			Root
528	45	2	(Normal)
220	45	2	(Abnormal) single male lamb with looseness (hypermobility) in the hock and stifle joints.
561	45	2	(Normal)
522	55	a.m. only	(Abnormal) twins—male very weak; female no muscular control in rear limbs.
662	55	a.m. only	(Normal)
376	45	2	(Normal)
777	45	2	(Abnormal) twins—female with looseness (hypermobility) in hock and stifle joints; female dead at birth.
293	45 25	a.m. p.m.	(Abnormal) single female lamb with right front leg twisted.
			Tops
515	225	1	(Normal)
743	225	1	(Normal)
641	200	1	(Abnormal) single female lamb with one front leg bowed laterally, the other medially.
813	200	1	(Abnormal) single female lamb with rear limbs somewhat twisted and poor muscular control in rear limbs.

complete lack of skeletal muscular control (inability to rise). The alkaloid veratramine has been isolated from various members of the Veratrum genus(2) but no reports exist on its isolation from *Veratrum californicum*.

We report here production of typical veratramine-induced type malformations in lambs born to ewes that ingested *V. californicum* plant or roots on the 14th day of gestation, and a preliminary identification of veratramine in *V. californicum* by thin layer chromatography.

Materials and methods. Twelve cross bred ewes were given *V. californicum* roots or tops by stomach tube on the 14th day of gestation. The dose levels are identified in Table I. These are dose levels commonly given to induce cyclopia and do so at an incidence of over 50% in some experiments. The congenital defects which are the subject of this



FIGS. 1-3. Photographs of selected lambs born to ewes given *Veratrum californicum* on 14th day after breeding. (1) Lamb from ewe 641 with one front leg bowed laterally and one medially; (2) Lamb from ewe 777 with a marked looseness of hock and stifle joints; (3) Lamb from ewe 522 with no muscular control in rear limbs. Fig. 4. Thin layer chromatogram of one step crystallization preparations from benzene extracts of 3 separate collections of *Veratrum californicum*. The two single spots between the 3 extracts are authentic veratramine. Each of the 3 extracts has from 7-9 spots, one of which possess the Rf value of veratramine.

report occurred among lambs not affected with cyclopia.

Thin layer chromatography of crystalline preparation from benzene extracts(3) of *V. californicum* roots was done on alumina. The mixed crystalline material was obtained by evaporation of nearly all the benzene from the extract *in vacuo* whereupon crystallization occurred. Benzene methanol (6:1 v,v) was used as developer and bromphenol blue-acetone (20 mg/100 ml) followed by 0.5 M phosphate-citrate buffer pH 3.95 as spray reagents to visualize the spots on the thin layer plates. The plates were photographed immediately after spraying, prior to fading.

Results and discussion. The production of the veratramine-induced type malformations by the plant *V. californicum* is described in Table I and Fig. 1-3. The results were in every way similar to those obtained with pure veratramine including the marked improvement or recovery of afflicted animals within a few days to weeks after birth(1).

These results suggested the presence of veratramine in the plant. We had previously isolated veratrosine, the glucoside of veratramine, from this plant(3) further suggesting the likelihood of the presence of the latter in at least precursor concentrations. We sought, therefore, to identify veratramine in mixed crystalline preparations from benzene extracts of the plant roots by thin layer chromatography. Fig. 4 shows 3 such benzene extracts chromatographed with authentic veratramine. All 3 possess spots of identical R*f* with veratramine. It thus appears probable that the veratramine-induced type malformations caused by *C. californicum* root or top may be due to the presence of that alkaloid in the plant.

Summary. Congenital malformations of the type produced by maternal ingestion of the alkaloid veratramine occurred from maternal ingestion of the plant *Veratrum californicum.* A preliminary identification of veratramine in benzene extracts from the plant by thin layer chromatography was achieved.

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Altered Distribution of Lesions After Repeated Passive Transfers of Allergic Encephalomyelitis.* (32475)

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Several investigators have studied the protection against active induction of experimental allergic encephalomyelitis (EAE) afforded by a variety of treatments (reviewed in (1)). It is likely, though not proven, that such treatments act by inhibition of the process of immunization against the neural tissue antigen-adjuvant mixture. There are few studies on the influence of one attack of EAE upon a subsequent attack(1,2). Such studies must take into account effects of the first attack on subsequent active immunization as well as effects on the target organ, the central nervous system itself. The production of EAE by passive transfer of living immunized lymphoid cells(1,3) simplifies this problem. The recipient animal can be subjected to repeated attacks of EAE induced by waves of actively immunized, encephalitogenic cells, each wave derived from fresh and immunologically naive donors. In this way, effects of previous attacks on active

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