this reaction as a means of virus detection in isolation, titration, and identification of influenza A, mumps, and CA (croup-associated) viruses. Prototype strains of 2 other myxoviruses (Sendai and NDV) and vaccinia produced hemadsorption in appropriate cell cultures. Prototype strains of common nonhemagglutinating viruses (herpes, Coxsackie, ECHO, adeno- and polio-viruses) gave negative results. Under some conditions hemagglutinating simian agents, which may be encountered in monkey kidney cell cultures, caused adherence of erythrocytes to the tissue cell sheet, but the characteristic patterns seen with myxoviruses were not observed. We found hemadsorption to be a simple, direct, and reliable test offering a new approach to diagnosis of some viral infections. It may also provide an investigative tool for research on virus-cell relationships.

The authors wish to thank Mr. Robert R. Padgett of the Photographic Section for his valuable assistance.

1. Vogel, J., Shelokov, A., *Science*, 1957, v126, 358.
2. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
3. Takemoto, K. K., Lerner, A. M., *ibid.*, 1957, v94, 179.
4. Eagle, H., Oyama, V. I., Levy, M., *Science*, 1956, v123, 845.
5. Salk, J., *J. Immun.*, 1944, v49, 87.
6. Utz, J. P., Kasel, J. A., Cramblett, H. G., Szwed, C F., Parrott, R. H., *New England J. Med.*, 1957, v257, 497.
7. Hull, R. N., Minner, J. R., Smith, J. W., *Am. J. Hyg.*, 1956, v63, 204.

Received January 17, 1958. P.S.E.B.M., 1958, v97.

Influence of Female Sex Hormones in Experimental Teratogenesis.* (23885)

GENTARO NISHIHARA† (Introduced by J. F. Prudden)
Department of Surgery, Columbia University, College of Physicians and Surgeons, and Surgical Services of Presbyterian Hospital, N. Y. City

Studies of congenital malformations deserve increased emphasis because of their importance as a significant health problem. Many investigators have demonstrated experimental production of mammalian congenital defects by alteration of maternal environment. The methods have included: nutritional deficiences(1-4), vitamin deficiencies (5-7), radiation(8-10), hypoxia(11-12), and more recently, cortisone(13-15). Present data indicate that under certain conditions estrogens are also teratogenic.

Materials and methods. Virgin female mice of the Webster-Swiss strain, weighing around 25 g, were employed. They were kept at least 2 weeks after shipment, and used after reaching the indicated weight. They were fed Rockland mouse diet in pellet form.‡ We employed 2 series: (A) Estrone ("Theelin") in peanut oil (1 mg/cc); and (B) Estradiol Benzoate in sesame oil (1 mg/cc). As a control for group (1), nothing was given to a comparable number of mice; and for group (2), a similar volume of sesame oil was administered. Injections were given between 10:00 and 11:00 a.m., except those on eleventh day of gestation, done at 5:00 p.m. During administration No. 22 gauge needles were inserted subcutaneously from median part of back into subcutaneous layer of left lateral breast region. This eliminated any spillage of injected material through the puncture wound. Preliminary experiments were performed upon a large number of mice to decide upon a proper dose of estrone and estradiol benzoate. 0.2 mg daily was the dosage level at which the incidence of cleft palate was highest, and the

* This work was aided by grants from Mead-Johnson Co., Evansville, Ind., and Nat. Inst. of Arthritis and Metabolic Diseases.

† Fellow of Japan Society (1957-1958) and Fulbright Fellow (1954-1957); Home address: Department of Obstetrics and Gynecology, Osaka City University Medical School, Abeno-ken, Osaka, Japan.

‡ Rockland Farms, New City, N. Y.

TABLE I. Comparison of Effect by Estrone and Estradiol Benzoate.

	No. of mothers sectioned	No. intact fetuses obtained	No. of cleft palate				% of cleft palate	No. of absorbed fetuses	Absorbed fetuses / Intact fetuses
			Total	(L)	(M)	(S)			
							%		%
Estrone	18	81	10	3	4	3	12.4	69	85.2
Estradiol benzoate	13	81	11	4	4	3	13.6	29	35.8
Sesame oil inj. cont.	20	143	1		1		.7	23	16.1
Untreated control	14	115	0				0	11	9.6
Unsectioned, untreated control	13	95	1	1			1.1	unknown	

L = large cleft palate; M = medium cleft palate; S = small cleft palate (see Fig.).

number of abortions lowest. Day of appearance of vaginal copulation plug was counted as first day of gestation. Mice with plugs were separated, and the animals with bonafide pregnancies sorted by palpation on eleventh day following initial isolation. Eleventh through sixteenth days of gestation were chosen for injections since it is known that the mouse palate closes between the fourteenth and fifteenth day. To prevent loss of offspring through cannibalism, uterine contents were removed by section on eighteenth day.

Results. In Table I, the comparative effects of estrone and estradiol are shown, with 0.2 mg of each given on twelfth and fifteenth day, and 0.1 mg on thirteenth, fourteenth and sixteenth day. It is evident that there was no difference in rate of cleft palate formation induced by estrone and estradiol benzoate. It is possible that there is a correlation between fetal absorption and cleft palate which could alter this relationship (see discussion of Table II below) if a dosage change resulted in more intact fetuses.

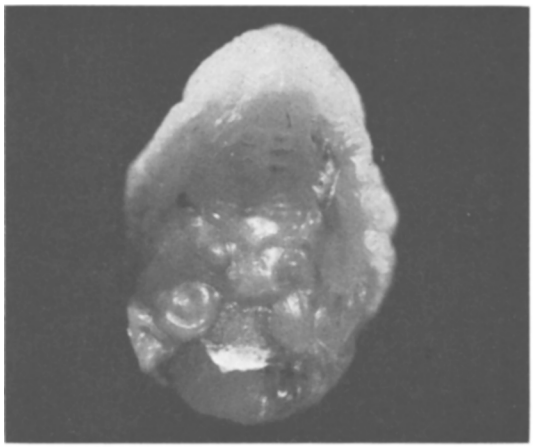

FIG. 1. A normal palate.

It should be noted that doubtful clefts appeared in both groups in which the palates were in various stage of closure. These were counted as normal (Fig. 3). Further attempts (with estrone) were made to increase the estrogen effect upon production of congenital cleft palate. In these experiments, Group A was given 0.2 mg on the 12th and 15th day, 0.1 mg on 13th, 14th and 16th day, and additional 0.05 mg on 17th day. This additional injection was given empirically, because premature deliveries were occasionally noted at the usual time of section on 18th day. The additional dose apparently prevented this difficulty. To Group D, 0.2 mg was administered on the 11th and 14th day, and 0.1 mg on 13th, 15th and 16th days, with additional 0.005 mg on 17th. In Group C and D, nothing was given on the 12th day, but injections were begun on 11th day, since the estrone was in an oil base, and it was therefore considered wise to begin one day earlier. Finally, in Group E, 0.2 mg estrone was given once a day, starting from the 11th. The results are summarized in Table II.

If, under these experimental circumstances, there were an increase in the cleft palate rate in the group receiving most estrogen, one could reasonably conclude that there is a relationship between estrogen level and propensity to produce defective offspring.

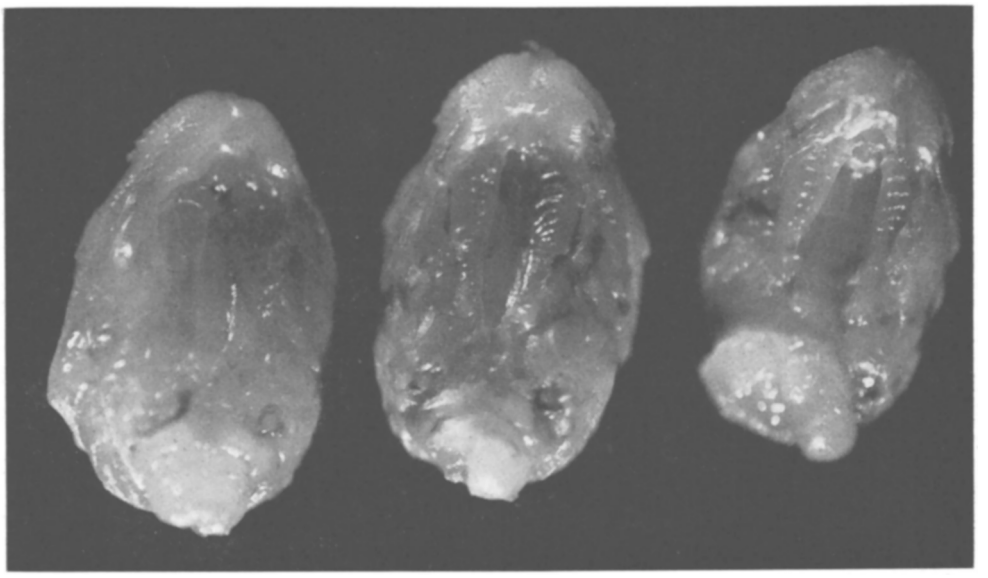

FIG. 2. Estrogen-induced cleft palates. From left to right: small, medium, and large defects.

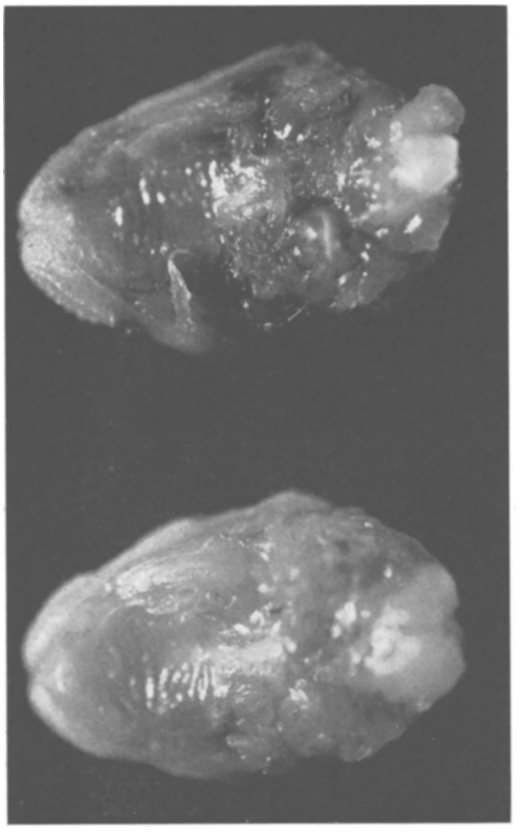

FIG. 3. Two examples of doubtful clefts. These exhibited a thin palate at the line of closure and a depression along the symphysis. Recorded as normal in results.

It should be noted, however, that even in Group D, 2 mothers out of 15 did not produce cleft palate offspring. In Group E, 6 pregnancies out of 10 ended in absorption of fetuses. Since it appeared difficult to obtain sufficient data for valid comparison with other groups, this constant dose experiment was not carried further. However, this group suggests that if estrogen is maintained at a plateau level, the fetuses are apt to be absorbed rather than coming to term with cleft palates.

Discussion. These data demonstrate that parenteral estrogen injection has teratogenic effects on developing mice embryos when administered to mothers under the stated experimental conditions. These effects are not so frequent as those demonstrated by Fraser, whose technic of cortisone administration§ has resulted in a 90% incidence of cleft palate formation in our hands. They are definite, however; and they indicate that an additional emphasis is needed on the role of estrogen secretory fluctuations in the pathogenesis of human cleft palates.

Conclusion. Under the conditions of this experiment, estrogen acted as a teratogenic agent.

§ 2.5 mg of cortisone acetate/day for 4 days starting on 12th day of gestation.

TABLE II. Comparison of Estrone Effect by Groups.

	No. of mothers sectioned	No. intact fetuses obtained	No. of cleft palate				% of cleft palate	No. of absorbed fetuses	Absorbed fetuses / Intact fetuses
			Total	(L)	(M)	(S)			
							%		%
Group A	18	81	10	3	4	3	12.3	69	85.2
B	15	88	13	3	8	2	14.8	36	40.9
C	19	63	17	5	7	5	27.0	72	114.3
D	15	71	25	10	6	9	35.2	43	60.6
E	10	14	3			3	21.5	62	442.9

This work was done in the laboratory of Dr. John F. Prudden. His advice and encouragement are gratefully acknowledged.

1. Warkany, J., Nelson, R. C., *Science,* 1940, v92, 383.

2. Warkany, J., *J. Pediat.,* 1944, v25, 476.

3. Nelson, M. M., Evans, H. M., *J. Nutrition,* 1949, v38, 11.

4. Nelson, M. M., Asling, C. W., Evans, H. M., *ibid.,* 1952, v48, 61.

5. Hale, F., *Texas State J. Med.,* 1937, v33, 228.

6. Warkany, J., Schraffenberger, E., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 92.

7. Wilson, J. G., Roth, C. B., Warkany, J., *Am. J. Anat.,* 1953, v92, 189.

8. Wilson, J. G., *J. Cell. and Comp. Physiol.,* 1954, v43, Suppl. 1.

9. Hicks, S. P., *Am. J. Roentgenol.,* 1953, v69, 272.

10. Russell, L. B., Russell, W. L., *J. Cell. and Comp. Physiol.,* 1954, v43, Suppl. 1.

11. Ingalls, T. H., Avis, F. R., Curley, F. J., Temin, H., *J. Hered.,* 1953, v44, 185.

12. Habegger, H., Philbrook, F. R., Curley, F. J., Ingalls, T. H., *Am. J. Ophth.,* 1956, v42, 377.

13. Fraser, F. C., Fainstat, T. D., *Pediatrics,* 1951, v8, 527.

14. Fraser, F. C., Kalter, H., Walker, B. E., Fainstat, T. D., *J. Cell. and Comp. Physiol.,* 1954, v43, (Suppl. 1) 237.

15. Ingalls, T. H., Curley, F. J., *New Eng. J. Med.,* 1957, v256, 1036.

Received January 21, 1958. P.S.E.B.M., 1958, v97.

Desoxyribonuclease (DNA'ase) Activity of Rat Mammary Gland During Pregnancy and Lactation.*† (23886)

DAVID R. GRIFFITH AND CHARLES W. TURNER
(With technical assistance of Mary E. Powell)
Department of Dairy Husbandry, University of Missouri, Columbia

In previous publications(1,2), it was reported the content of desoxyribonucleic acid (DNA) of rat mammary gland epithelial cells decreased following parturition(1); and DNA-phosphorous of mammary gland also declined at day of parturition when determined on dry tissue(2). An explanation of these unusual findings was sought in determining DNA'ase activity of mammary gland during pregnancy and lactation to see if there was correlation between DNA and DNA'ase activity.

Methods. Data were obtained from primiparous albino rats during late pregnancy (18-20 days), day of parturition and during advanced lactation (20-21 day). The animals were killed, the 6 posterior mammary glands rapidly removed. These were placed in ice-cold sucrose or saline solution, diluted to 50 mg/ml, homogenized for 2½ minutes in a Waring Blendor, then centrifuged for 10 minutes at 2600 rpm. The supernatant was saved for the following procedure. Six individual samples were prepared by addition of one ml

*Contribution from the Mo. Agr. Exp. Sta., Journal Series No. 1834. Approved by Director.

† This investigation was supported in part by grant from Am. Cancer Soc.