strip cannot be inhibited by antihistaminic drugs.

It has been shown in this study that the isolated uterine strip is sensitive to 0.05γ/ml of serotonin, while a concentration of histamine of 50γ/ml(1) is necessary to produce a reaction. Thus the isolated tissue of the mouse is 1,000 times more sensitive to serotonin than it is to histamine. This sensitivity is completely abolished with the serotonin antagonists LSD and reserpine; and the anaphylactic contraction is completely inhibited in the presence of these drugs. Serotonin is the only substance known to be inhibited by these two drugs. It has also been shown(6) that the tissues of the mouse contain a quantity of serotonin easily sufficient to produce the anaphylactic response. These findings support the hypothesis that the anaphylactic reaction in the mouse is dependent on the release of serotonin.

Summary. (1) The egg white sensitized or normal mouse uterus is 1,000 times more sensitive to serotonin than it is to histamine. (2) This sensitivity is completely abolished in the presence of two known serotonin antagonists, LSD and reserpine. (3) LSD and reserpine, which as far as is known inhibit only serotonin, always and completely prevented the anaphylactic contraction on strips of demonstrably sensitized uterus in the ten trials with each drug.

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Teratogenic Effects of Herpes Simplex, Vaccinia, Influenza-A (NWS), and Distemper Virus Infections on Early Chick Embryos.*† (22580)

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The discovery of a causal relationship between maternal rubella and fetal pathology in human beings(1) has led to an experimental investigation of the role played by infectious agents in the production of congenital malformations. Recent studies concerning viral teratogenesis have resulted in the finding that characteristic developmental abnormalities and death follow infection of early chick embryos with the PR8 strain of influenza-A virus (2,3). Moreover, the induction of these teratogenic effects has been prevented by neutralization of the virus with specific antiserum, thus providing evidence that the virus was the cause of the syndrome(3). In addition, Newcastle disease virus has also been found to produce specific malformations in this embryonic host(4-6). In view of these results, experiments were conducted in this laboratory to determine whether the induction of teratogenic and lethal effects may be a property of other viruses as well. It is the purpose of this paper to describe the effects produced by infection of early chick embryos with herpes simplex, vaccinia, the NWS strain of influ-

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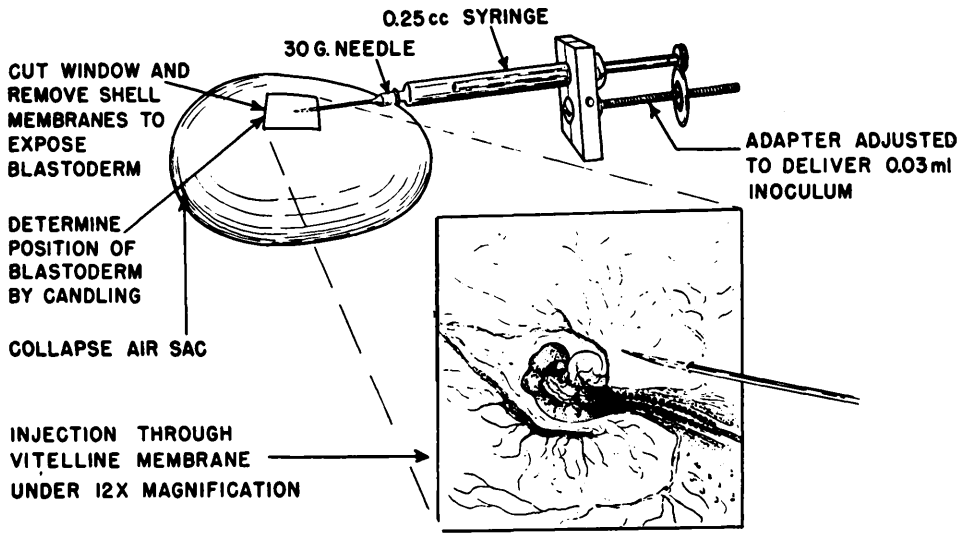

FIG. 1. Schematic presentation of method employed for inoculation of 48-hr chick embryos.

enza-A and distemper viruses.

Materials and methods. Inoculation of embryos. Embryonated hens' eggs, which had developed to stages 11-14 as described by Hamburger and Hamilton(7) after incubation at 37.5°C for 48 hours, served as the experimental hosts. The method of preparation and injection is shown schematically in Fig. 1. Following injection, eggs were reincubated and observed daily by temporarily removing the scotch tape "window" covering the site of inoculation. A more complete description of the inoculation procedure as well as the method of observation has been presented in a previous publication(3). *Viruses.* Three of the viruses investigated were obtained from 12-day-old embryonated eggs which had been infected 2 days earlier. Of these, the HF strain of herpes simplex virus and an egg-adapted strain of vaccinia virus were employed in the form of suspensions of triturated chorioallantoic (C-A) membranes, while infective allantoic fluid served as the source of the NWS strain of influenza-A virus. The herpes and NWS viruses had been maintained previously by intracerebral passage in mice. The fourth agent studied, an egg-adapted strain of distemper virus (Lederle), was prepared by trituration of C-A membranes obtained from 14-day-old embryonated eggs which had been infected 7 days previously. All viruses were maintained in frozen storage at —40°C. When required for use, the frozen material was permitted to thaw at room temperature and diluted with Difco Brain Heart Infusion (B.H.I.) medium containing 500 units of penicillin and 100 μg of streptomycin per ml. *Controls.* B.H.I. medium, a 10^{-4} concentration of uninfected allantoic fluid and a 20% suspension of uninfected triturated C-A membranes were employed as control inocula. All dilutions were prepared with B.H.I. medium to which antibiotics had been added. As an additional control, concentrations of virus known to uniformly produce anomalies and/or lesions (10^{-2} herpes, NWS, and vaccinia; 20% distemper) were subjected to heat exposure prior to inoculation of embryos. Heat inactivation was accomplished by placing tubes of virus in a water bath at 60°C for 1 hour. *Virus recovery.* To provide evidence that anomalies and/or lesions were associated with viral infection of the embryo, titration studies of embryonic pools were conducted. Accordingly, 4 groups of 48-hour embryos were inoculated with each of the viruses under study employing as inocula 10^{-4} concentrations of herpes, NWS, and vaccinia viruses and a 20% suspension of distemper virus. C-A membranes of 5 embryos infected with distemper virus were pooled 7 days after inoculation, while pools were made of 5 anomalous dead embryos within each of the remaining 3 groups 2-3

TABLE I. Incidence of Teratogenic and Lethal Effects upon Early Chick Embryos Produced by Infection with Herpes Simplex, Vaccinia, and Influenza-A (NWS) Viruses.

Virus and conc.	No. of embryos	Effects observed*	Days following inoculation 1	2	3	4	5	6-14
10^{-1} Herpes simplex	45	(A)	93	100				
		(D)	4	100				
10^{-2}	32	(A)	100					
		(D)	0	97	100			
10^{-3}	24	(A)	75	96	100			
		(D)	0	79	96	100		
10^{-4}	30	(A)	27	93	100			
		(D)	0	57	93	100		
10^{-5}	22	(A)	9	27	36	55	55	55
		(D)	0	9	23	64	86	86
10^{-6}	20	(A)	0	10	20	20	25	25
		(D)	0	0	15	25	40	50
10^{-7}	19	(A)	0	0	0	5	5	5
		(D)	0	0	0	5	5	5
10^{-1} Vaccinia	10	(A)	100					
		(D)	100					
10^{-2}	17	(A)	94	100				
		(D)	0	100				
10^{-3}	22	(A)	55	100				
		(D)	0	100				
10^{-4}	43	(A)	12	88	98	98	98	98
		(D)	0	58	98	100		
10^{-5}	25	(A)	20	52	56	56	56	56
		(D)	0	16	80	88	100	
10^{-6}	28	(A)	4	11	14	14	14	14
		(D)	0	4	7	11	18	18
10^{-7}	16	(A)	0	0	6	6	6	6
		(D)	0	0	19	19	19	19
10^{-8}	19	(A)	0	0	11	11	11	11
		(D)	0	0	5	11	11	16
10^{-2} Influenza NWS	14	(A)	50	100				
		(D)	0	86	100			
10^{-3}	29	(A)	14	90	100			
		(D)	0	48	93	100		
10^{-4}	27	(A)	26	93	100			
		(D)	0	41	100			
10^{-5}	19	(A)	5	21	47	47	47	47
		(D)	0	5	69	84	89	89
10^{-6}	15	(A)	0	13	20	33	33	33
		(D)	0	0	13	40	47	53
10^{-7}	20	(A)	0	0	0	0	0	0
		(D)	0	0	5	10	20	25
10^{-8}	14	(A)	0	0	0	0	0	0
		(D)	0	0	0	0	0	8

* (A) designates % of anomalous (micrencephaly and/or axial flexion) embryos in each dilution while (D) indicates % dead within that group.

days after inoculation. All embryonic tissue pools were ground and then serially diluted with B.H.I. medium containing antibiotics in preparation for titration. With the exception of the distemper membrane pool, in which case 7-day embryonated eggs were employed, titrations of virus in each embryonic pool were conducted in 11-day eggs. The recovery of herpes, vaccinia, and distemper viruses was established by the detection of the characteristic lesions produced by each on the C-A membrane. The detection of NWS virus in the allantoic fluid was accomplished by the hemagglutinating method.

Results. Infection with herpes simplex, vaccinia and the NWS strain of influenza-A

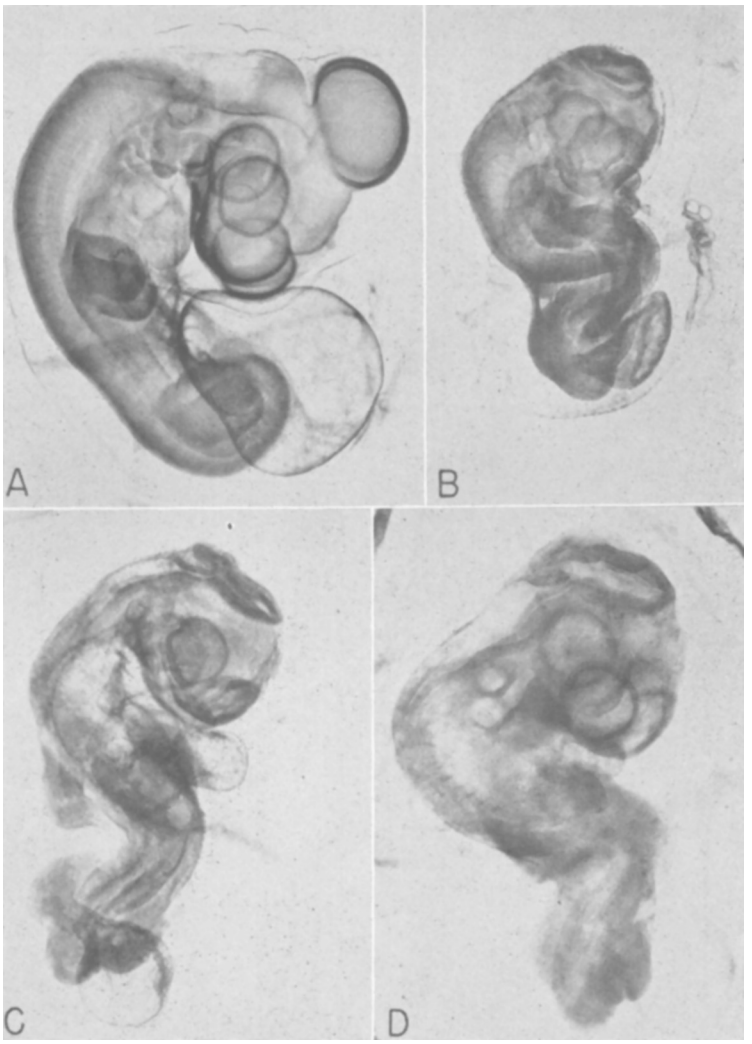

FIG. 2. Embryos after 4 days incubation. A. Uninfected control embryo showing well-developed brain and normal curvature of spinal axis. B. Embryo infected 48 hr previously with herpes simplex virus, showing flattened brain vesicles, axial flexion, and antero-posterior compression of eyes and auditory vesicle. C. Embryo infected 48 hr previously with vaccinia virus, showing micrencephaly, axial flexion, and optic and auditory damage. D. Embryo infected 48 hr previously with influenza-A (NWS) virus, showing swollen auditory vesicle in addition to micrencephaly and axial flexion.

viruses produced teratogenic effects which were quite similar to those previously described for the PR8 strain of influenza-A virus(2,3). In most cases, the first indication of infection was the appearance of a dorsal flexion of the body axis at the level of the wing buds or middle trunk region. The brain subsequently sustained extensive damage leading to more or less pronounced micrencephaly through the collapse of the major brain vesicles, *i.e.* the telencephalon and mesencephalon. The growth of the amnion was retarded in a majority of cases, although it appeared to have closed normally in others. In a few cases, the amniotic sac became turgid through internal fluid pressure. Thus, at the time of death infected embryos typically exhibited extreme axial flexion and advanced micrencephaly shown in Fig. 2. Table I shows that the incidence of anomalous devel-

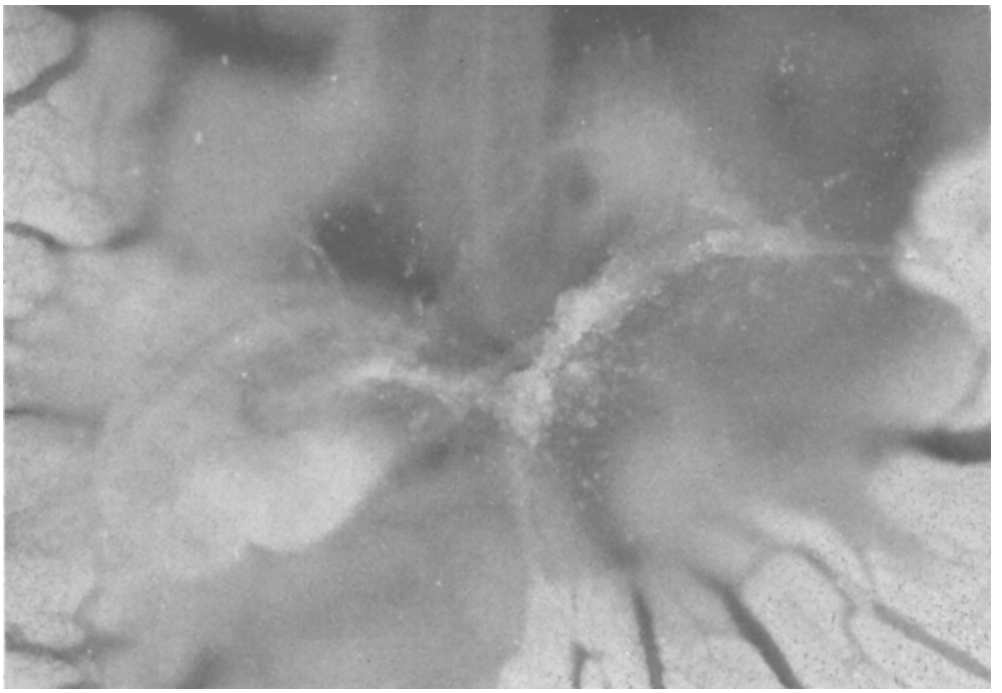

FIG. 3. Lesions on chorionic membrane directly overlying 4-day-old embryo inoculated 2 days previously with influenza-A (NWS) virus.

opment, time until appearance of anomalies, and time until death were functions of the virus dilutions employed. Once anomalies became apparent, subsequent growth of the embryo proceeded at a slow rate until death supervened. Infected embryos which failed to show anomalies by the sixth day appeared normal at death.

There were, however, minor differential effects which were characteristic of each virus. *Herpes simplex virus.* When herpes simplex virus was used as the inoculum, the major damage was similar to that produced by the other viruses, but the eyes tended to become compressed along their antero-posterior axis in a higher percentage of cases than was observed with the influenza-A or vaccinia viruses (Fig. 2B). The auditory vesicles of practically all herpes-infected embryos underwent severe damage and became very small white masses of tissue during the infection. *Vaccinia virus.* When embryos were infected with vaccinia virus, the eyes occasionally became flattened medio-laterally, and the auditory vesicles were in many instances either small and opaque or markedly compressed (Fig. 2C). The amnion sometimes appeared abnormally opaque along its free border with pox-like, raised lesions observable at the time of death. *Influenza-A (NWS strain) virus.* A striking effect, not noted when any other virus was employed, could be observed in embryos inoculated with NWS strain virus. About 2 days after injection, small, discrete, white lesions appeared on the extra-embryonic membranes in most cases, and frequently became contiguous and much enlarged by the time of death (Fig. 3). In addition, patches of whitish tissue could sometimes be seen along the borders or within the limb buds, in the branchial area, as well as near or in the somites and neural tube walls. The developing eyes frequently appeared flattened medio-laterally and were irregular in outline. The auditory vesicles were swollen in many cases, a condition not noted in embryos infected with the other viruses employed (Fig. 2D).

When tissue pools obtained from anomalous dead embryos infected with herpes, vaccinia, and NWS viruses were titrated it was

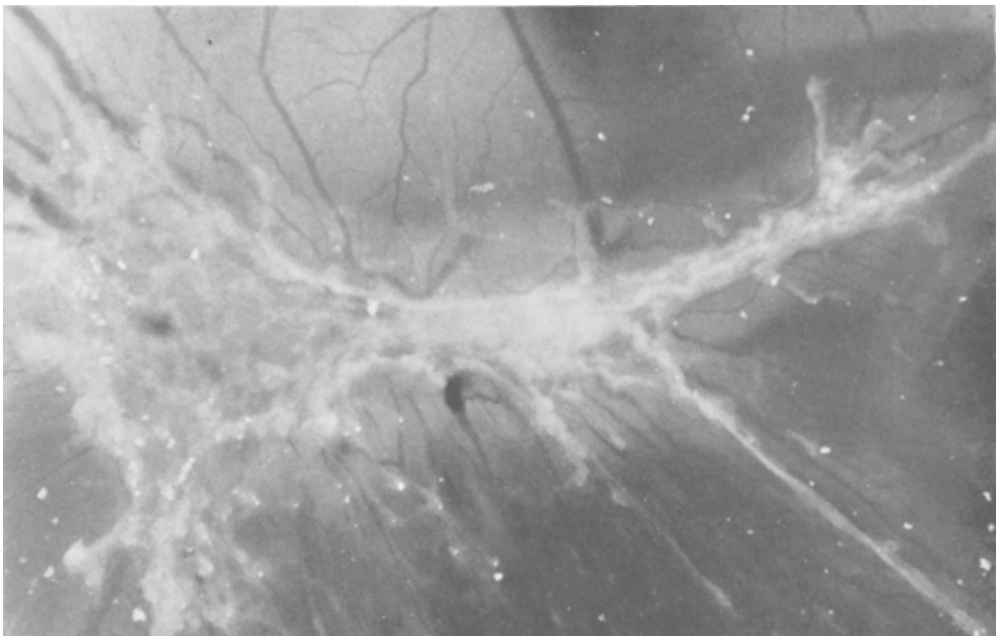

FIG. 4. Lesions on C-A membrane of 8-day-old embryo inoculated 6 days previously with distemper virus.

found that all 3 agents had undergone multiplication in the embryo during the period of "acquired" teratism to the extent that such pools could be diluted at least 10^6 and still yield detectable virus. Significantly, these 3 viruses, following heat inactivation, failed to evoke teratogenesis.

Unlike the other 3 viruses, distemper virus suspensions failed to produce in the embryo itself any gross morphological anomalies observable under the dissecting microscope. However, 4 or 5 days after injection of a 20% suspension of virus, small spot or thread-like lesions began to appear on the C-A membrane. During the next few days, these lesions grew in size and coalesced to form the large, ulcerative areas on the surface of the membrane (Fig. 4) which characterize distemper infection in older embryonated eggs (8). In a few cases, similar though smaller lesions could be detected on the amnion as well. It can be seen from Table II that almost all embryos possessed C-A lesions 7 days after inoculation with the 20% virus suspension, and that many had succumbed to the infection by that time. Most of those remaining had died by the tenth day, and all were dead 2 weeks after injection. Similarly, all embryos which developed C-A lesions following inoculation with 2% and 0.2% suspensions subsequently died of the infection. However, a number of embryos apparently

TABLE II. Effect of Distemper Virus Infection upon Early Chick Embryos.

Virus conc. (%)	No. of embryos	Effects observed*	Days following inoculation 3	4	5	6	7	8	9-13	14-18
20	43	(L)	0	2	37	60	98	100		
		(D)	0	0	0	2	21	74	98	100
2	21	(L)	0	10	19	29	67	81	81	81
		(D)	0	0	0	0	19	43	67	81
.2	8	(L)	0	0	0	13	38	38	38	38
		(D)	0	0	0	0	0	13	38	38

* (L) designates % with C-A lesions in each dilution group while (D) indicates % dead within that group.

TABLE III. Effect of Control Inocula upon Early Chick Embryos.

Inoculum	No. of embryos	% dead on days following inoculation 1	2	3	4	5	6-14
B. H. I. medium	40	3	5	8	8	8	8
Normal allantoic fluid (10^{-1})	43	0	0	5	5	5	5
Normal C-A membranes (20% susp.)	47	2	2	9	9	9	9
Total	130	1	2	7	7	7	7

escaped infection completely, with the percentage of survivors inversely proportional to the virus concentration employed. The C-A lesions which were produced as a result of distemper infection in 48-hour embryos were shown to contain active virus. Inoculation of such membrane pools into 7-day embryos produced the characteristic lesions of distemper (8). Significantly, heat inactivated distemper virus was not capable of producing C-A lesions.

As shown in Table III, about 7% of control embryos inoculated with B.H.I. medium or suspensions of uninfected allantoic fluid or C-A membranes subsequently died within 14 days. Indications of retarded embryonic development or operational injury (*i.e.*, damaged blastoderm) were almost always associated with these incidental deaths. Although such deaths were occasionally accompanied by aberrant embryonic development resembling virus disease, these anomalies were readily distinguishable from the latter. Similar incidental mortality occurred in about 7% of those embryos which were inoculated with virus suspensions, and such cases were therefore not included in the data presented in Tables I and II. Deaths occurring after 14 days were randomly distributed in control and virus groups alike. The majority of such deaths are probably due to incubation difficulties associated with collapse of the air sac, such collapse being a requisite of the operational procedure.

Discussion. Infection with herpes simplex, vaccinia, and the NWS and PR8 strains of influenza-A viruses evokes teratogenesis and death in the early chick embryo. The fact that these viruses all produce the same major syndrome, including micrencephaly and axial flexion, indicates that they may share the ability to attack the same morphogenetic centers during critical stages of embryonic development. However, minor differences in pathology were observed which suggest that the teratogenic effects of one virus may, in some instances, be differentiated from those of another. For example, embryos infected with the NWS strain of influenza-A characteristically developed lesions on the extra-embryonic membranes as well as in certain regions of the body itself while those inoculated with the PR8 strain never exhibited this effect(2,3). Similarly, compression of the eye, when it occurred, was generally medio-lateral in the case of vaccinia and the influenza viruses while it was usually antero-posterior in the case of herpes simplex. Moreover, other investigations showing that infection with Newcastle disease virus preferentially affects the lens, auditory vesicles, and other structures to a lesser degree(4-6) provide even more striking evidence that different viruses may attack different sites.

The phenomenon of "acquired" teratism appears to be dependent upon the presence of an active viral agent since herpes, vaccinia, and NWS viruses all failed, following heat inactivation, to produce anomalies in susceptible embryos. That these agents undergo multiplication in the embryo was borne out by titration studies which showed that the virus concentration rose at least 100-fold during the period in which teratogenesis occurred.

The ability of some viruses to produce anomalous development of the early chick embryo should not imply, however, that teratogenesis may result from any virus infection. Distemper virus, for example, evoked no gross teratogenic effects but produced a lethal infection characterized by ulcerative lesions on the C-A membrane. Similar lesions are known to result when the egg-adapted strain of distemper virus is inoculated onto the C-A membrane of older embryos(8), but death rarely occurs. The ability of the infection to kill younger but not older embryos may lie in the greater susceptibility of the metabolic

processes of the early chick embryo to the pathogenic effects of the virus(9).

It is noteworthy that the virus strains which have been shown in this laboratory to produce anomalous development of early embryos following injection through the vitelline membrane were also capable of producing a lethal infection in older (9-10 day) embryos when introduced extra-embryonically. Newcastle disease virus also shares this property(5,6). On the other hand, distemper virus, which did not regularly produce death in older embryos, has not been shown to produce teratogenic effects in early embryos, under the experimental conditions so far employed. The results observed with distemper are quite similar to the data reported for mumps virus(2). The latter agent, although undergoing multiplication in the allantoic sac, did not kill 8-day-old embryos, nor did it prove teratogenic in 48-hour embryos, even though these younger embryos succumbed to the infection within 5 days. It is possible, however, that teratogenesis might also result from infection with these viruses following sufficient modification of such factors as the time selected for inoculation, or the volume and concentration of the inoculum.

Summary. Herpes simplex, vaccinia and influenza-A (NWS) viruses produced teratogenic and lethal effects in the early chick embryo. The primary teratogenic effects of all 3 viruses were micrencephaly and axial flexion, but minor characteristic differences could be detected in embryos infected with each of these agents. Distemper virus failed to produce any gross embryologic changes, although its injection led to the development of ulcerative lesions on the C-A membrane and death of the embryo.

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Free and Apparent Amino Acids in Deproteinized Plasma of Normal and Uremic (Nephrectomized) Dogs.* (22581)

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It would be of physiological and clinical importance to determine whether the disturbances of urea and protein metabolism, known to occur in uremia, are accompanied by abnormal levels of plasma amino acids. Although total amino nitrogen is not elevated consistently in the blood of uremic patients (1,2), it appears possible that individual amino acids may vary markedly. Levenson *et al.*(3) observed that plasma amino nitrogen was near normal in patients with severe battle wounds and renal failure although plasma urea concentrations were as much as 30 times normal. All plasma amino acids were not depressed or elevated at any time but the concentration of some individual

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