the basement membrane have been weakened to the point that plasma and red cells leak into the surrounding tissues. The conspicuous absence of endothelial swelling and leucocyte sticking would seem to argue strongly against the endothelial cell itself being affected sufficiently to account for the changes observed.

The predilection of the capillaries and venules to the disruptive action of Na_4EDTA in adrenally-insufficient rats can be reversed by soluble cortisol in as short a time as 30 minutes. It is difficult to conceive how a direct action on the endothelial cell proper could be reflected on the properties of the basement membrane in such a relatively short period. Inasmuch as there is no evidence that Na_4EDTA acts on the endothelial cell, the blunting of the effects of Na_4EDTA by the cortisol represents further proof for this explanation. In line with the action of Na_4EDTA on the intercellular junction and/or the basement membrane cortisol would appear to have a direct restorative action on these extracellular structures.

Since the topical application of Na_4EDTA results in a localized effect on the mesenteric vessels, a tissue which is relatively devoid of lysosome-rich cells such as leucocytes, macrophages, or reticuloendothelial elements, the present data on the restorative action of cortisol cannot be used as evidence for a stabilizing effect on the lysosomal membrane.

The experiments on lethal endotoxemia were included because this condition is associated with a profound derangement of smooth muscle reactivity(4), and the ability of the cortisol to counteract the lethal action of bacterial endotoxin within as short a period as 30 minutes demonstrates another apparently direct action of corticoids through an action on the cell membrane of these vascular components.

Conclusions. The above experiments have led us to conclude that corticosteroids have a direct chemical action on the extracellular basement membrane or intercellular binding forces which are most probably responsible for the loss of vascular integrity in the adrenally-insufficient rat. It is suggested that a stabilizing action of cortisol on the extracellular basement membrane and intercellular cement of capillaries and venules constitutes a hitherto unreported property of this hormone in relation to its anti-inflammatory action.

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Amniotic Fluid Measurement in Cortisone Treated and X-Irradiated Mice.* (29917)

BRUCE E. WALKER

Department of Anatomy, The University of Texas Medical Branch, Galveston

Cleft palate resulting from puncturing the amniotic sac with a needle has been attributed to leakage of amniotic fluid and consequent constriction of the embryo by surrounding membranes(1). The question can be raised as to whether some other cleft pal-

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TABLE I. Effect of Cortisone and X-Irradiation on Amount of Amniotic Fluid Per Mouse Embryo.

Treatment	No. of embryos	Embryonic wt (mg) Range (selected)	Embryonic wt (mg) Avg (± S.E.)	Amniotic fluid wt (mg) Avg (± S.E.)	Amniotic fluid wt (mg) Range	P	Placenta + membranes + uterus Wt (mg) avg ± S.E.	P
Irradiated	32	<100	80 ± 2.0	162 ± 4.4	122-206		109 ± 3.3	
Control	14	100-199	159 ± 6.0	152 ± 4.7	126-184		150 ± 5.4	
Cortisone	9		164 ± 7.7	146 ± 5.1	126-172	>.10 *	148 ± 5.8	>.50 *
Irradiated	38		143 ± 5.5	169 ± 3.8	120-224	>.02 *	126 ± 3.3	<.001*
Control	20	200-299	235 ± 5.9	206 ± 6.5	162-265	<.001†	229 ± 17.7	.001†
Cortisone	35		249 ± 5.8	137 ± 5.7	38-238	<.001*	150 ± 5.5	<.001*
Irradiated	16		223 ± 7.1	148 ± 3.6	126-176	<.001*	134 ± 3.2	<.001*
Control	8	300-399	360 ± 10.8	190 ± 4.2	134-258	>.10 †	256 ± 11.0	>.10 †
Cortisone	13		338 ± 9.5	135 ± 10.9	106-247	<.01 *	145 ± 7.6	<.01 *
Control	15	400-499	453 ± 9.3	189 ± 5.1	164-224	>.50 †	206 ± 13.1	>.10 †
Cortisone	12		441 ± 6.5	118 ± 4.2	86-136	<.001*	143 ± 12.0	<.01 *
Control	15	500-599	545 ± 7.1	201 ± 5.9	158-242	>.10 †	204 ± 7.2	>.50 †

* Probability relative to control value of same weight group.
† " " " " " " preceding weight group.

ate inducing treatments also act through a reduction in volume of amniotic fluid. Measurement of amniotic fluid has been accomplished by a variety of techniques(2,3,4). A simple procedure is to weigh the embryo and membranes before and after draining off amniotic fluid. A number of teratogens can be used to investigate the mechanism of cleft palate formation. Treating pregnant mice of the A/J strain with X-irradiation or injection of cortisone can produce cleft palate with a frequency as high as 100% by retarding the movement of the palatine shelves from a vertical to a horizontal position(5,6).

Material and methods. Pregnant mice of the A/J strain were treated, as described previously, with 4 doses of 2.5 mg cortisone acetate at 11⅓ to 14⅓ days postconception(5) or 300 r of X-irradiation at 11⅓ days postconception(6) to induce cleft palate in 100% or 99% of the offspring, respectively. At 14 to 16 days after observing a vaginal plug, the mice were killed, the uterus dissected free and blotted to remove blood. Embryos were separated by cutting through the uterus. The combined embryo, membranes and surrounding piece of uterus were blotted to remove draining blood and then weighed. The uterus and membranes were cut open, escaping fluid was absorbed on filter paper and discarded, the total tissue mass was weighed again and then the embryos were dissected free and weighed separately. This procedure was carried out on 228 embryos from 40 litters ranging in age from 13 days 13 hours to 16 days 14 hours postconception, including cortisone treated, X-irradiated and control mice. Specific gravity was calculated by draining amniotic fluid into a dish and using this pooled sample to fill a 200 lambda ultramicro pipette. The measured volume of fluid was then weighed.

Results. Control embryos from 200 to 599 mg did not differ significantly in amount of amniotic fluid per embryo, despite the differences in embryonic weight (Table I). Cortisone reduced the amount of amniotic fluid from all embryonic weight groups except the lightest (Table I). X-irradiation also reduced amniotic fluid, but the validity of comparison with controls on an embryonic weight basis is uncertain due to the pronounced reduction in embryonic weight caused by this treatment. Among embryos weighing over 200 mg, there was some overlapping in the range of amniotic weights from control and cortisone treated embryos. Only 3 control weights fell below 160 mg (158, 155, 134 mg) and 4 from cortisone treated mice were above 160 mg (187, 221, 238, 247). Among X-irradiated embryos with body weight exceeding 200 mg, 4 had amniotic fluid weights above 160 mg. Embryos with cleft lip were excluded from Table I. Specific gravity of sone treated litters at 14½ days postconcep- amniotic fluid from both normal and corti-

tion was 1.01. The combined weight of membranes, placenta and uterus was roughly proportionate to amniotic fluid weight, being less than the controls after cortisone treatment in the embryonic weight groups over 200 g and less after irradiation in all groups.

Discussion. Control and treated embryos were compared on the basis of embryonic weight, rather than age, since previous work had shown this to give the best correlation with developmental age(6). Furthermore, embryos of the same weight would be expected to need the same amount of amniotic fluid to protect them from compressing effects of surrounding membranes. As long as the embryo is completely bathed in, and surrounded by amniotic fluid, any compressing force exerted by membranes would be equally distributed to all parts of the body surface by hydrostatic pressure. If fluid were deficient to the extent that the membranes could come in contact with the head and rump, then compression of the lower jaw against the chest could occur and lead to cleft palate formation, as shown previously(1). The amount of fluid necessary to effectively surround an embryo is obviously dependent on the size of the embryo, so embryos have been grouped by weight in Table I, even though both cortisone and X-ray treated fetuses are noticeably smaller than normal at term and X-irradiated embryos are lighter for a given morphological rating at the time of normal palate closure(6). There was no significant difference in amount of amniotic fluid between 4 control groups of embryos from 200 to 599 mg, a relationship reported earlier by McCafferty(2). Since the palate is closed in normal embryos of about 250 mg or more (6), the decrease in proportion of amniotic fluid for embryos over 300 mg should not influence palate development. The combined weight of fetal membranes, placenta and uterus was reduced relative to embryonic weight by both teratogenic treatments. This reduction paralleled the reduction in amount of amniotic fluid and perhaps was an expression of generalized growth inhibition by these teratogens.

Amniotic weights from embryos in the weight range of 200 to 599 mg were significantly different between control and cortisone treated mice. Therefore, cortisone treatment caused a reduction in amount of amniotic fluid. However, since 3 control animals not developing cleft palate had as little amniotic fluid as cortisone treated embryos that were developing cleft palate, and since 4 cortisone treated embryos had more amniotic fluid than the average normal embryo, the lack or abundance of amniotic fluid could not be directly responsible for development of cleft palate. Instead, the decreased fluid in the average cortisone treated embryo must be an additional concurrent effect of the excess cortisone.

The quantity of amniotic fluid associated with X-irradiated embryos was intermediate between control and cortisone treated embryos and overlapped with the controls. Therefore, this treatment also reduced amniotic fluid volume without the latter being responsible for development of the cleft palates that occur in these embryos.

Gulienetti *et al*(3), using a riboflavin-deficient regimen known to produce a high frequency of cleft palate, found an increased volume of amniotic fluid bathing the treated embryos. Similarly, meclizine treatment of rats, which led to palatal clefts in almost all offspring, also produced an increased volume of amniotic fluid at the time of palate closure(4). Thus, cleft palate can be produced in the presence of decreased or increased quantities of amniotic fluid. Cleft palate can be produced by mechanically reducing the volume of amniotic fluid(1), but such a reduction does not constitute a common mechanism for all cleft palate producing teratogens. Considering that loss of amniotic fluid was not the mechanism of action for the 4 cleft palate-producing teratogens investigated so far, it is probably an infrequent, perhaps rare, cause of cleft palate.

Summary. The amount of amniotic fluid associated with mouse embryos at the normal time for palate closure was determined in cortisone treated, X-irradiated, and control embryos. Both of these cleft palate-inducing teratogens caused a significant reduction in amount of amniotic fluid. However, a few embryos destined to have cleft palate had as much amniotic fluid as most normal embryos, and a few normal embryos had as

little amniotic fluid as most treated embryos. Therefore, the reduction in amniotic fluid caused by these teratogens is not the effect which is primarily responsible for development of the cleft palates.

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In vitro Production of an Interferon-Like Inhibitor of Viral Multiplication by a Poikilothermic Animal Cell, The Tortoise (*Testudo greca*).* (29918)

E. FALCOFF AND B. FAUCONNIER (Introduced by Hilton B. Levy)
Centre de Recherches sur les Virus, Groupe de Recherches de l'Institut National de la Santé et de la Recherche Médicale, Hôpital Saint Vincent de Paul, Paris and Service des Virus, Institut Pasteur, Paris

The capacity of animal cells to react to viral infection by synthesizing substances which inhibit viral multiplication, appears to be a general phenomenon in cells derived from warm-blooded animals. Protein factors (interferons) which inhibit intracellular viral synthesis have been previously demonstrated in infected homeothermic animal cells, *i.e.*, chick embryonic cells(1), many different types of normal and malignant human cells (2,3,4), monkey kidney cells(5), rabbit kidney cells, and calf kidney cells(6).

Although all interferon-like substances (ILS) act during the intracellular phase of viral synthesis, important differences concerning physico-chemical properties such as ether sensitivity and heat stability have been reported. Since the synthesis of these substances appears to depend on the cellular and not on the viral genome, such physico-chemical differences may be attributed to the cell type from which they originate. This may also explain the relative species specificity which has been previously observed(7,8).

Since interferon-like substances are liberated by almost all types of virus-infected cells, while antibody is produced only by immunologically competent cells, one may speculate that such factors may be produced by infected cells at different levels of the zoological scale, perhaps even by unicellular organisms. To test the interferon-producing capacity of cells derived from cold-blooded animals, tortoise cells have been chosen because of previous experience with these cells in culture(9).

Material and methods. Cell culture: The technics of propagation of tortoise kidney cells in culture and their viral susceptibility have been described elsewhere(10). Kidney cells were dispersed by 0.25% trypsin in buffered saline solution (BSS) and monolayers were obtained in Roux bottles and Kahn tubes by growing the cells at 30°C in a casein hydrolysate culture medium supplemented by 5% heat inactivated calf serum.

Monolayers of chick, mouse embryo, human amnion, guinea pig and rat kidney cells were grown according to standard methods using the same casein hydrolysate medium with 10% calf serum.

Continuous cell lines of human KB, monkey MA 104 (received from Microbiological Associates, Bethesda, Md.), and hamster BHK 21(11), were maintained in casein or Eagle's medium supplemented with 10% calf serum.

Virus strains: Parainfluenza type 1 (Sendaï) virus was used as inducing agent in production of interferon. The virus was an egg-

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