in the mouse and the hamster is not accounted for by absence of antigen in the mouse tumors because most contained antigen as determined by reactivity with positive hamster sera.

1. Huebner, R. J., Rowe, W. P., Turner, H. C., Lane, W. T., Proc. Nat. Acad. Sci., 1963, v50, 379.
2. Girardi, A. J., Hilleman, M. R., Zwickey, R. E., Proc. Soc. Exp. Biol. Med., 1964, v115, 1141.
3. Huebner, R. J., Casey, M. J., Chanock, R. M., Schell, K., Proc. Nat. Acad. Sci., 1965, v54, 381.
4. Yabe, Y., Samper, L., Bryan, E., Taylor, G., Trentin, J. J., Science, 1964, v143, 46.
5. Lennette, E. H., Diagnostic Procedures for Viral and Rickettsial Diseases, Am. Public Health Assn. Inc., New York, 1964, 51.
6. Habel, K., Virology, 1965, v25, 55.
7. Sarma, P. S., Huebner, R. J., Lane, W. T., Science, 1965, v149, 1108.
8. Trentin, J. J., Bryan, E., Proc. Soc. Exp. Biol. Med., 1966, v121, 1216.
9. Van Hoosier, G. L., Jr., Trentin, J. J., Shields, Jacqueline, Stephens, Kristina, Stenback, W. A., Parker, J. C., Lab. Animal Care, 1966, v16, 119.

Received October 17, 1966. P.S.E.B.M., 1967, v124.

Effect of Pituitary and Gonadal Hormones on Friend Virus Disease in Mice.* (31922)

E. A. MIRAND, N. BACK, J. T. GRACE, JR., AND RITA BUFFET
Roswell Park Memorial Institute, New York State Department of Health, and State University of New York at Buffalo

Friend Virus Disease (FVD) as described by Mirand *et al*(1) is associated with malignant reticulum cell proliferation, a pronounced hypervolemic polycythemia, and progressive hepatosplenomegaly. Although the precise role of an endogenous erythropoietic stimulating factor (ESF) in this disease has not been determined fully, exogenous ESF greatly accelerates the erythropoietic response induced by this viral infection(2). The effect of chemotherapeutic agents on the disease has been studied(3,4,5), but little attention has been given to the effect of hormones. Erythropoiesis was found to be stimulated by androgens(6,7) and adrenal corticosteroids (8), depressed by estrogens(6,9). A mechanism for the depression of erythropoiesis by estrogens in normal and plethoric rodents has been suggested recently(10). The present study reports the effect of several hormones on the erythropoietic response in Friend Virus Disease.

Materials and methods. Ha/ICR Swiss female weanling mice, weighing 24-26 g, were inoculated intraperitoneally with a standard 0.2 ml inoculum of a 10% cell-free spleen homogenate filtrate obtained from Friend virus-infected mice. Methods for preparing the splenic homogenates have been described previously(1).

Mice were divided into groups of 6 mice per group. Drug-treated groups received the hormones subcutaneously once or three times a week for 2 weeks prior to infection with FVD. Drugs then were injected once or three times a week for four consecutive weeks. At weekly intervals, the per cent 24-hour Fe^{59} uptake in blood, liver, spleen, and femur was measured following intravenous administration of the isotope(1). The following measurements also were made weekly: hematocrit, body weight, spleen weight, and liver weight. Mice receiving no drug or virus (Table I), the drug alone or only the virus inoculum comprised the control groups. Mice were maintained in a 24°C temperature-controlled environment on a diet of Derwood-Morris mouse chow and water *ad libitum*.

The following hormones were administered: (a) adrenocorticotropic hormone (ACTH) I.M., 38 mg/kg in saline 3 times/week. The potency of ACTH is expressed in milligrams. An arbitrary standard has been selected (Lot. No. LA-1-A) and all preparations are assayed in terms of milligrams of this standard. The

*This study was supported in part by grants from USPHS (AI-04506, CA-08847 and CA-07745) and John A. Hartford Foundation, Inc.

TABLE I. Normal Hematocrit, Liver and Spleen Size and 24-Hour Fe^{59} Uptakes in the Blood, Femur, Liver and Spleen of Control Ha/ICR Swiss Mice.*

	Weeks	Body wt (g)	Hematocrit (%)	Liver wt (g)	Spleen wt (g)
(A)	1	26	45	1.53 ± .36	.18 ± .02
	2	29	43	1.65 ± .14	.14 ± .02
	3	32	45	1.92 ± .21	.15 ± .02
	4	32	46	2.01 ± .24	.16 ± .03

		24-hour Fe^{59} uptake			
	Weeks	Blood	Femur	Liver	Spleen
(B)	1	18.1 ± 2.1	.28 ± .03	12.9 ± 3.2	1.98 ± .04
	2	19.2 ± 1.0	.29 ± .02	11.4 ± 1.0	2.03 ± .24
	3	22.0 ± 2.4	.42 ± .03	9.8 ± 1.2	1.89 ± .14
	4	21.8 ± 1.2	.60 ± .02	10.4 ± 1.3	1.96 ± .08

* Values for each week represent average values for 12 female mice, ± S.D.

TABLE II. Effect of ACTH, Progesterone, Estradiol, and Depo-Testosterone on Hematocrit, Liver and Spleen Size in Friend Virus-Infected and Control Mice.

Treatment		Weeks of treatment	Body wt (g)	Hematocrit (%)	Liver wt (g)	Spleen wt (g) ± S.D.
ACTH	ACTH control	1	26	46	1.44	.18 ± .02
		2	30	47	1.84	.15 ± .02
		3	31	50	1.89	.15 ± .03
		4	32	51	1.98	.13 ± .03
	ACTH + F. V.	1	26	44	1.62	.84 ± .70
		2	27	60	2.06	2.07 ± .70
		3	29	74	2.37	2.70 ± .40
		4	29	84	2.11	2.96 ±1.10
Progesterone	Progesterone control	1	23	45	1.45	.15 ± .01
		2	26	50	1.63	.14 ± .01
		3	32	48	2.06	.16 ± .01
	Progesterone + F. V.	1	26	45	1.73	.79 ± .60
		2	29	61	2.42	1.47 ± .40
		3	32	75	2.65	3.47 ± .04
Estradiol	Estradiol control	1	24	49	1.50	.18 ± .02
		2	22	45	1.37	.14 ± .01
		3	27	49	1.93	.18 ± .01
		4	30	43	2.08	.19 ± .03
	Estradiol + F. V.	1	22	49	1.67	.91 ± .70
		2	20	43	1.83	.58 ± .58
		3	27	40	1.84	.36 ± .09
		4	27	58	3.28	2.02 ± .80
Depo-testosterone	Depo-testosterone control	1	25	44	1.41	.16 ± .01
		2	31	46	1.91	.15 ± .02
		3	32	48	2.00	.17 ± .02
	Depo-testosterone + F. V.	1	25	46	1.73	.98 ± .60
		2	30	51	2.03	1.17 ± .20
		3	31	77	3.26	2.25 ± .30
Control	F. V. control	1	27	43	1.52	1.05 ± .10
		2	26	50	2.24	2.20 ± .50
		3	32	68	3.96	2.71 ±1.40
		4	28	72	5.33	3.27 ± .10

standard is of such activity that when 2 to 4 μg are injected intravenously into hypophysectomized rats, a significant decrease in ascorbic acid content of the adrenals occurs. All preparations are assayed on hypophysectomized rats by the ascorbic acid depletion of the adrenals method; (b) depo-estradiol cyclopentylpropionate in cottonseed oil subcutaneously, 50 mg/kg/week, unless otherwise stated; (c) progesterone benzyl benzoate in cottonseed oil subcutaneously, 33 mg/kg in saline 3 times/week; and (d) depo-testos-

terone cyclopentylpropionate in cottonseed oil subcutaneously, 167 mg/kg/week. An equivalent volume of vehicle was given to the control animals.

To determine the transmissibility of Friend Virus Disease through the blood of Friend virus-infected mice given various doses of depo-estradiol, the following procedure was carried out. Five six-week-old Ha/ICR Swiss female mice were inoculated I.P. with 0.2 ml of Friend virus and 16.6, 33, and 50 mg/kg of depo-estradiol was inoculated subcutaneously into the same mice 2 weeks before virus injection and weekly thereafter for a maximum of 4 weeks. These animals were sacrificed 24 hours after the last treatment with depo-estradiol and 0.2 ml of their blood was injected into Ha/ICR Swiss recipients. The per cent takes, latent periods, and mortality rates of the recipients were followed.

Results. Table I indicates the normal average values of 12 female mice killed weekly. Table II summarizes the effect of hormone treatment on hematocrit, and liver and spleen weights in control and Friend virus-infected mice. Changes characteristic of Friend Virus Disease seen in the control, infected mice, included a progressive increase in hematocrit levels during the experimental period, hepatomegaly, and splenomegaly. The average liver and spleen weights increased almost 2-25 times respectively. Estradiol was the only agent exhibiting inhibitory effects on the progress of the Friend disease. The Friend virus-induced polycythemia and increased liver and spleen weights were prevented by the estradiol. The effect was evident during the second week of the disease, and continued throughout the full course of the disease. The data in Table III indicate that the proliferation of the virus in infected animals was not affected by various doses of estrogen. The transmissibility of Friend Virus Disease from the blood of Friend-infected donor mice given various doses of depo-estradiol was similar to that observed in the control animals. The slight variations in response of mice receiving blood from Friend virus-inoculated donors, treated with or without estrogen, are not regarded as being significant. The per cent takes were similar for all groups, as were the latent periods and the mortality rates.

TABLE III. Transmissibility of Friend Virus Disease from Blood of Friend Virus-Infected Donor Mice Given Various Doses of Depo-Estradiol.

Friend virus-inoculated donors treated with depo-estradiol (DP)*	No. of recipient mice showing Friend Virus Disease			
	Day of sacrifice of donors†			
	7	14	21	28
Controls	5/5	5/5	5/5	5/5
16.6 mg/kg DP	5/5	5/5	4/5	5/5
33 " DP	3/5	4/5	5/5	5/5
50 " DP	4/5	5/5	5/5	5/5

* 5-6-weeks-old Ha/ICR Swiss female mice inoculated I.P. with 0.2 ml of Friend virus. Depo-estradiol inoculated into mice weekly 2 wk before virus injection and weekly thereafter for a maximum of 4 wk. Animals sacrificed 24 hr after the last treatment with depo-estradiol.

† Several virus-inoculated estrogen-treated mice were sacrificed for inoculation of each recipient. 0.2 ml of donor blood inoculated I.P. into recipient. Period of observation, 180 days.

Progesterone, adrenocorticotropic hormone (ACTH), and testosterone, in the doses used, did not influence significantly the hematologic and organ changes characterizing the disease.

Hormonal effect on Fe^{59} uptake in the blood, femur, liver, and spleen of both control and Friend virus-infected mice is summarized in Table IV. As the disease progresses, there is a trend toward a decrease in the per cent uptake of Fe^{59} in the organs. Correlation coefficients were estimated in an attempt to help determine whether any definitive correlation exists between organ size and per cent radioisotope uptake in that organ. It can be seen (Table IV) that, during the course of the disease, erythroid elements of the spleen are replaced as reflected by the significant negative correlation between spleen size and per cent Fe^{59} uptake (—0.84 and —0.98 at 2 and 3 weeks respectively) in the control virus-infected animals. The ACTH-treated virus-infected mice demonstrated similar negative correlation coefficients at the second and third weeks. No significant correlations were noted in the other drug-treated groups. The estradiol-treated mice exhibited a greater tendency for correlation reversal although these correlations were not significant.

Discussion. Of the hormones studied, only estrogen depressed the Friend virus-induced

TABLE IV. Effect of ACTH, Progesterone, Estradiol, and Depo-Testosterone on Fe^{59} Uptake in Blood, Femur, Liver, and Spleen of Friend Virus-Infected and Control Mice.

Treatment		Weeks of treatment	% Fe^{59} uptake (± S.D.) Blood	Femur	Liver	Spleen	Correlation coefficients: Liver Fe^{59} uptake and wt	Spleen Fe^{59} uptake and wt
ACTH	ACTH	1	16.7 ± 4.2	.28 ± .03	14.9 ± 4.3	11.9 ± 2.6	—.66	+.66
	control	2	15.2 ± 4.6	.34 ± .04	10.5 ± 2.0	13.7 ± 3.4	—.09	+.26
		3	21.0 ± 3.3	.78 ± .02	7.9 ± 1.3	14.5 ± 4.4	—.74*	—.88*
		4	18.0 ± 4.0	.52 ± .01	6.8 ± .80	13.8 ± 5.5	—.91*	+.21
	ACTH +	1	14.8 ± 4.9	.30 ± .03	14.7 ± 5.0	12.7 ± 3.5	—.81*	—.13
	F. V.	2	23.8 ± 4.3	.14 ± .04	5.0 ± 1.4	7.1 ± 1.9	+.02	—.87*
		3	26.0 ± 3.0	.21 ± .02	4.0 ± .60	6.5 ± 1.7	—.97*	—.76*
		4	18.4 ± 2.7	.14 ± .02	3.2 ± 1.1	4.9 ± 1.7	—.49	—.63
Proges-	Proges-	1	19.5 ± 4.5	.34 ± .02	11.4 ± 3.4	16.7 ± 3.1	+.34	+.21
terone	terone	2	15.4 ± 8.3	.43 ± .06	12.0 ± 4.3	16.0 ± 4.6	—.92*	—.07
	control	3	23.9 ± 9.2	.53 ± .05	8.6 ± 3.2	17.1 ± 7.9	—.80*	+.51
	Proges-	1	17.9 ±12.9	.33 ± .06	11.2 ± 4.2	13.0 ± 8.0	—.36	—.70
	terone	2	19.7 ± 7.7	.22 ± .02	7.4 ± 4.4	8.9 ± 1.7	—.33	+.24
	+ F. V.	3	24.9 ± 5.9	.14 ± .00	3.4 ± 1.0	3.8 ± 1.3	—.18	—.65
Estradiol	Estradiol	1	14.8 ± 1.1	.53 ± .30	18.7 ± 2.2	13.7 ± 2.1	—.84*	—.55
	control	2	13.3 ± 3.9	.30 ± .07	20.6 ± 4.9	17.3 ± 5.8	—.43	+.12
		3	14.7 ± 6.8	.28 ± .03	18.6 ± 5.2	16.7 ± 4.4	—.36	—.10
		4	19.0 ± 7.6	.21 ± .06	10.5 ± 3.9	26.3 ± 7.0	—.95*	—.41
	Estradiol	1	11.7 ± 2.8	.41 ± .20	22.2 ± 6.1	9.4 ± 1.0	—.69	—.42
	+ F. V.	2	3.4 ± 2.6	.34 ± .01	20.3 ± 3.7	15.6 ± 9.1	—.56	+.24
		3	17.5 ±11.9	.23 ± .06	17.5 ± 8.5	15.7 ± 6.2	+.26	—.27
		4	15.5 ± 5.8	.17 ± .09	5.2 ± 2.1	8.1 ± 3.0	—.54	—.05
Depo-tes-	Depo-tes-	1	16.0 ± 4.5	.28 ± .06	16.0 ± 4.3	12.9 ± 3.3	+.33	—.32
tosterone	tosterone	2	21.7 ± 4.4	.30 ± .09	8.7 ± 5.1	17.8 ± .70	+.06	+.14
	control	3	22.6 ± 3.1	.57 ± .20	7.4 ± 1.2	16.5 ± 1.6	—.79*	—.33
	Depo-tes-	1	11.7 ± 3.0	.26 ± .03	15.2 ± 1.8	11.1 ± 2.7	—.00	—.95*
	tosterone	2	21.9 ± 6.6	.18 ± .08	7.7 ± 1.8	11.2 ± 1.3	+.06	+.04
	+ F. V.	3	28.1 ± 4.9	.15 ± .01	3.6 ± 1.4	5.1 ± .60	—.84*	—.27
Control	F. V.	1	12.7 ± 3.6	.43 ± .02	16.0 ± 3.5	8.7 ± 1.3	—.12	+.62
	control	2	17.5 ± 3.6	.14 ± .10	7.8 ± 2.9	7.3 ± 2.1	—.77*	—.84*
		3	24.9 ± 3.8	.22 ± .10	3.2 ± .8	4.8 ± 3.2	—.93*	—.98*
		4	10.8 ± 3.5	.18 ± .04	3.3 ± .0	6.1 ± .90	—	—

* Indicates correlation coefficient is significantly different from zero at 5% level.

reticulum cell proliferation and polycythemia. Noted was the decreased hematocrit, the decrease in Fe^{59} uptake by the erythroid organs, and the reduction in hepatosplenomegaly. The erythroid proliferative character of the disease thus appeared to be checked. Progesterone, ACTH, and testosterone did not alter the progress of the disease.

In light of recent studies on the mechanism of estrogen action on erythropoietin and erythropoiesis(10,11), it is unlikely that the inhibitory effect of estrogen is mediated by action on the invading virus. The data in Table III substantiate this since various doses of depo-estradiol had no effect on the transmissibility of Friend Virus Disease. Rather, the erythropoietic depression is due probably to estrogen inhibition of response of target cells for the virus and/or cells involved in ESF production.

It has been suggested that estrogens possibly inhibit the production of a precursor substance from the liver required for renal activation or production of ESF. This indirect mechanism has been proposed because estrogen exerts its erythropoietic inhibitory action on nephrectomized as well as intact mice. Estrogens have been reported to depress protein metabolism by the liver(12).

Androgens and adenohypophyseal hormones did not affect erythropoiesis in Friend virus-infected mice. In normal and plethoric

rodents, androgens had an augmenting action on erythropoiesis(10). This action required the presence of the kidneys, suggesting a renal mechanism, although direct action of testosterone on erythrogenic tissue grown in tissue culture has been reported(13). It is possible that a lack of an enhancing effect by androgens on erythropoiesis in Friend virus-infected mice may be due to a dose response.

Summary. The effect of pituitary and gonadal hormones on Friend Virus Disease in mice has been studied. Estradiol inhibited the virus-induced hypervolemic polycythemia, hepatomegaly, and splenomegaly. The effect was most evident during the second week following injection. Progesterone, adrenocorticotropic hormone, and testosterone did not influence significantly the hematologic and organ changes characterizing the disease. A possible mechanism of action of estrogen-induced suppressions of erythropoiesis in this disease is discussed.

We extend thanks to Dr. P. Trudel, and to Edmund Dywinksi, Andres Bulba, Joyce Jividen, Ruth Deckert, Basilius Sywenkyj, A. G. Mirand, and W. Trudel.

1. Mirand, E. A., Grace, J. T., Jr., Acta Union Internationale Contre Le Cancer, 1963, v19, 351.
2. ———, Accademia Nazionale Dei Lincei, 1964, v65, 9.
3. Mirand, E. A., Back, N., Prentice, T. C., Ambrus, J. L., Grace, J. T., Jr., Proc. Soc. Exp. Biol. Med., 1961, v108, 360.
4. Mirand, E. A., Back, N., Grace, J. T., Jr., Exp. Med. & Surg., 1965, v23, 239.
5. Sugiura, K., Stock, C. C., Acta Union Internationale Contre Le Cancer, 1960, v16, 780.
6. Mirand, E. A., Prentice T. C., Slaunwhite, W. R., Ann. N. Y. Acad. Sci., 1959, v77, 677.
7. Mirand, E. A., Gordon, A. S., Wenig, J., Nature, 1965, v206, 270.
8. Gordon, A. S., Piliero, S. J., Landau, D., Endocrinology, 1951, v49, 497.
9. Dukes, P. P., Goldwasser, E., ibid., 1961, v69, 21.
10. Mirand, E. A., Gordon A. S., ibid., 1966, v78, 325.
11. Mirand, E. A., Conference on Murine Leukemia, Philadelphia, NCI Monograph No. 22, 1966, 483.
12. Puchol, J. R., Carballido, A., Med. Exp., 1959, v1, 384.
13. Erslev, A. J., Proc. IX Cong. Internat. Soc. Hematol., 1962, v3, 143.

Received October 25, 1966. P.S.E.B.M., 1967, v124.

An Immunofluorescent and Histopathological Study of Respiratory Syncytial (RS) Virus Encephalitis in Suckling Mice.*† (31923)

JOSEPH J. CAVALLARO,‡ HUNEIN F. MAASSAB, AND GERALD D. ABRAMS
(Introduced by Thomas Francis, Jr.)
Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, and Department of Pathology, Medical School, Ann Arbor

In our initial investigation of the adaptation of the Long strain of respiratory syncytial virus(1) to newborn mice, we reported that the mouse-passed RS virus could not be passed intracerebrally in mice 3 to 4 weeks of age(2). Subsequently, the susceptibility of suckling mice of different ages was examined periodically during the serial intracerebral passages. The present investigation indicates that the neuropathic strain of RS virus may be propagated in 7- and 9-day-old mice, and describes the application of histologic, virus assay and direct immunofluorescent staining techniques to the study of the pathogenesis of the infection produced in suckling mice by the neuropathic strain of RS virus.

*This paper is part of a dissertation submitted by Joseph J. Cavallaro in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan. Supported in part by Graduate Training Grants from Nat. Inst. of Allergy I Infect. Dis., NIH, 2 T1 AI-60 and 5 T1 AI-60.

† Conducted under sponsorship of Commission on Influenza, Armed Forces Epidemiological Board, and supported by U.S. Army Medical Research and Development Command, Dept. of the Army, under research contract DA-49-193-MD-2066.

‡Present address: Medical Research Laboratories, Chas. Pfizer Co. Inc., Groton, Conn.