Afr. J. Med. Sci., 1956, v21, 9.

8. Magath, Thomas B., Meleney, Henry E., *Am. J. Trop. Med.,* 1940, v20, 211.

9. Miller, Max J., *Nature,* 1953, v172, 1192.

10. Moan, J. C., *Am. J. Trop. Med. and Hyg.,* 1957, v6, 499.

11. Okushima, N., Ohwan, T., Urushibara, T., *Kitasato Jikken Igaku,* 1959, v32, 195.

12. Phillips, Bruce P., *Science,* 1950, v111, 8.

13. Rees, C. W., Bozicevich, J., Reardon, L. V., Jones, F., *Am. J. Trop. Med.,* 1942, v22, 581.

14. Stoll, Norman R., *J. Protozoology,* 1957, v4 (Suppl.), 6.

15. Wells, Ronald, *Med. J. Malaya,* 1956, v11, 93.

Received December 13, 1960. P.S.E.B.M., 1961, v106.

Suppression of Virus-Induced Corneal Toxicity in Rabbits by Pretreatment with Nitrogen Mustard.* (26356)

JANG O. OH AND YONG T. YANG† (Introduced by C. A. Evans)
Department of Microbiology, University of Washington, Seattle

When a large amount of Newcastle disease virus (NDV) is inoculated into the anterior chamber of the rabbit eye, there results a toxic reaction characterized by corneal opacity and characteristic microscopic changes of corneal endothelium(1,2). No evidence of increase in infectious virus is associated with the above reactions. Since an extensive infiltration of leucocytes, particularly polymorphonuclear leucocytes (PMN), into ocular tissues as well as aqueous humor, is a constant feature, it was considered possible that the infiltrating cells might play an essential role in the pathogenesis of the corneal reaction. Because of the known suppressive effect of nitrogen mustard on PMN *in vivo,* this agent was employed in an effort to study the above possibility.

Materials and methods. Virus: The L-Kan 1948 strain of NDV, a heat resistant strain(3) obtained from Dr. H. Rubin, was used in all experiments. It was cultivated in 10-day-old embryonated eggs by inoculating 10^4 plaque forming units (PFU) of stock virus into the allantoic cavity. The allantoic fluid harvested at 48 hours was pooled and centrifuged at 3000 rpm for 5 minutes to eliminate cell debris. It was dispensed into glass tubes in a volume of 1 ml per tube and tightly sealed and stored at –35°C until used. The titer of the virus in this allantoic fluid was 2×10^9 PFU with a hemagglutinin titer of 1280 units per ml. NDV infectivity was determined by using the plaque count technic on cells derived from chick embryo (4).

Rabbit. Normal healthy male albino rabbits weighing between 4 and 4.5 lb were used.

Nitrogen mustard (HN_2). Mustargen hydrochloride (Methyl-bis [beta-chloroethyl] -amine HCl) (Merck, Sharp and Dohme, Philadelphia, Pa.) was used. It was dissolved in sterile double-distilled water just before injection into rabbits.

Intraocular injection of virus. Rabbits were anesthetized with intravenous sodium Nembutal. Two minutes prior to intraocular injection of virus, a drop of Pontocaine HCl (Winthrop Labs, N. Y.) was topically applied to the cornea. The technic for injection of the inoculum into the anterior chamber of the eye has been described(2).

Preparation of corneal endothelium for microscopic examination. Corneal endothelium was stained with a solution of silver nitrate and an entire sheet of stained endothelium was separated from the cornea and mounted on a slide according to methods described previously(2).

Methods of scoring corneal lesions. 1) *Endothelial rosettes.* Microscopic lesions on the silver stained endothelium of the cornea, referred to as endothelial rosettes, were counted by examining the entire endothelium

* This investigation was supported by a grant from Nat. Inst. of Health, U.S.P.H.S.

† On leave from Department of Microbiology, Chonnam Univ., School of Medicine, Kwangju, Korea.

of the cornea mounted on a slide, with a high dry objective at a final magnification of 430. Lesions were recorded as total number of rosettes counted per cornea.

2) *Endothelial fusions.* Other microscopic lesions on the silver stained endothelium, called fusions, were scored as follows after examining the entire endothelium mounted on a slide under the microscope with a final magnification of 430: − = no fusion on entire sheet of endothelium, ± = scattered fusions of 2 cells, + = frequent fusions of 3 to 5 cells, ++ = fusions so extensive that only a few cells with cell boundaries are seen in a field, +++ = cell boundaries lost almost completely.

3) *Corneal opacity.* The gross change of the cornea, opacity, was usually scored 24 hours after virus inoculation into the anterior chamber, and at other appropriate times. Degree of corneal opacity was scored as follows: − = no opacity, ± = doubtful opacity, + = definite but weak opacity, outline of iris can be seen easily through cornea, ++ = intermediate degree of opacity between + and +++, +++ = strong opacity, outline of iris cannot be seen through cornea.

Counting leucocytes in aqueous humor. A drop of aqueous humor was placed on a slide, air dried and stained with Wright's solution. The entire drop of aqueous humor was examined under the microscope with a final magnification of 430, and total number of leucocytes present was obtained. Corneal endothelial cells were also seen in aqueous humor as single cells or as groups of cells. These corneal endothelial cells were not included in total leucocyte count of the aqueous humor.

Experimental results. Suppression of leucocytes in blood by intravenous injections of HN_2. This preliminary experiment demonstrated the effects of HN_2 on circulating leucocytes. Six rabbits were injected intravenously twice at an interval of 2 days with HN_2, 0.75 mg per lb of body weight. Total leucocyte and differential counts were carried out at various intervals, on blood withdrawn from the marginal ear vein. Care was taken to avoid unnecessary irritation to the ears. One ear was used solely for HN_2 injection and the other for sampling blood. To prevent bacterial infection during the prolonged period of leucocyte depletion, each rabbit was injected intramuscularly with 10,000 units of procaine penicillin and 10,000 μg of streptomycin given daily for 7 days starting from day of first HN_2 injection. The results of the experiment are summarized in Fig. 1.

The number of circulating leucocytes reached the lowest level, ranging from 400 to 750 per cmm, 2 days after the second injection of HN_2. This severe leucopenia lasted for 2 to 4 days. Then a sharp increase in number of leucocytes followed and there was a return to an almost normal leucocytic level on the 14th day after the first injection of HN_2. Mononuclear cells were shown to be the first cells affected by the HN_2 injection; a drastic decrease to as low as 6% of total leucocytes was noted 24 hours after first HN_2 injection. The relative proportion

TABLE I. Effect of Intravenous Injection of HN_2 on Virus-Induced Corneal Toxicity.

HN_2 injections			Corneal reaction (opaque corneas/corneas used)	
No. of injections	Days before inj. of virus	Exp. No.	HN_2-treated rabbits	Control rabbits*
2	4 and 2	1	0/4	4/4
		2	1/12	5/8
		3	2/6	6/6
		4	0/2	6/6
		5	0/8	6/8
		6	1/4	2/3
		7	1/6	6/6
		Total	5/42	35/41
	2 and 0	1	0/6	6/6
		2	4/6	5/6
		3	4/6	6/6
		Total	8/18	17/18
1	4	1	6/6	5/6
		2	4/4	6/6
		3	5/6	4/6
		Total	15/16	15/18
	2	1	7/8	7/8
		2	3/6	5/6
		3	4/6	6/6
		Total	14/20	18/20
	0	1	6/6	6/6
		2	4/6	5/6
		3	4/6	6/6
		Total	14/18	17/18

* HN_2-untreated.

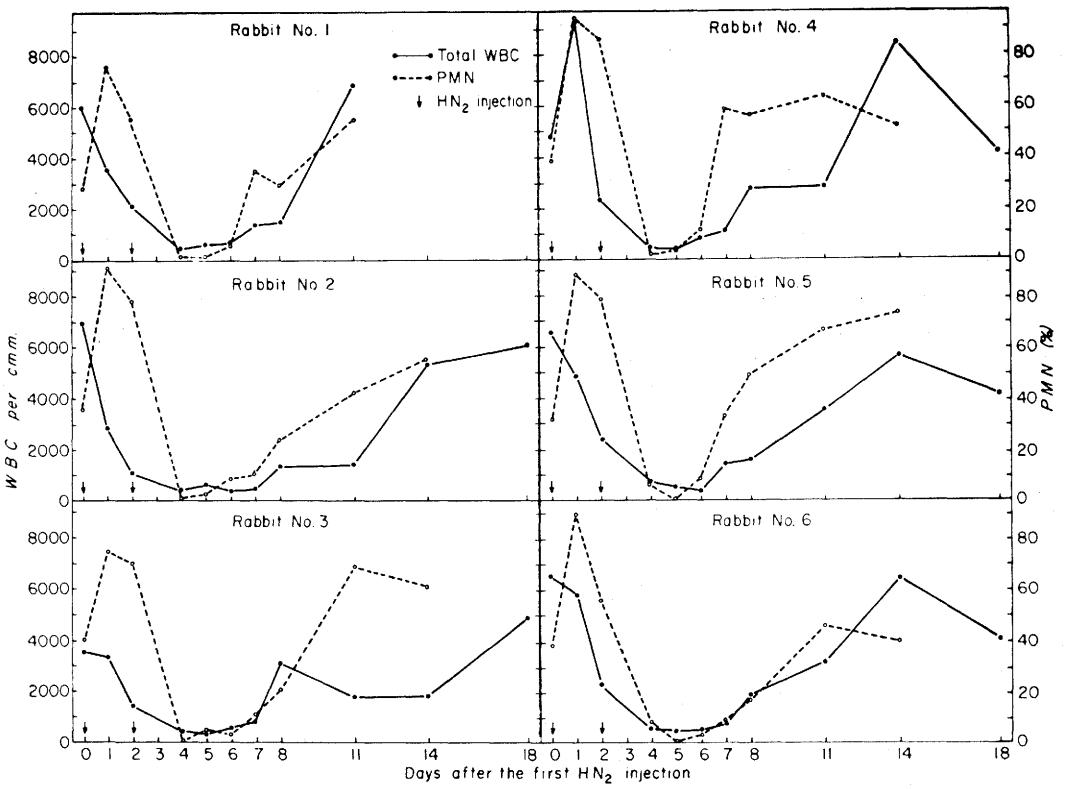

FIG. 1. Suppression of leucocytes in rabbits by 2 intrav. injections of HN_2.

of PMN reached the lowest level, ranging from less than 1% to 8%, 2 days after the second injection of HN_2 and remained at that level for 2 to 3 days. Then the PMN increased rapidly and a normal differential count was restored between the 7th and 11th days after the first HN_2 injection. No corneal opacity was observed in these rabbits throughout the experiment.

Suppression of virus-induced corneal opacity by pretreatment of rabbits with intravenous injections of HN_2. Rabbits were given 2 intravenous injections of HN_2 in the same manner as in the preliminary experiment. Two days after the last injection of HN_2, when the number of leucocytes reached the lowest levels, both eyes were inoculated with 0.2 ml of NDV (4×10^8 PFU). As a control, normal rabbits were simultaneously inoculated with the same amount of virus into their anterior chambers. Frequency of corneal opacity, appearing 24 hours after virus injection, was compared between these two groups. Results are summarized in Table I.

In all experiments, the number of eyes showing corneal opacity was far smaller in HN_2-treated rabbits than in normal controls. Whereas the percentage of corneas showing opacity in HN_2-treated animals in any single experiment ranged from 0 to 33%, it ranged from 63 to 100% in untreated rabbits. In all, only 5 of 42 eyes of the HN_2-treated group showed corneal opacity whereas in the control group, 35 of 41 eyes showed the opaque corneas. It was noted that aqueous humor of these 5 opaque corneas of HN_2-treated rabbits contained few or no leucocytes.

Limited experiments were carried out to explore the influence of time of injection on the suppressive effect of HN_2 on the toxic reaction to NDV. A suppressive effect was observed irregularly in the rabbits pre-treated with 2 injections of HN_2 at a 48 hour interval and given an intraocular injection of NDV

immediately after the second injection of HN_2. Suppression of corneal opacity was not apparent in the animals which were given intraocular injections of virus immediately, 2 days or 4 days after a single injection of HN_2. It appears that treatment of animals with 2 injections of HN_2 before virus inoculation was an important factor in suppression of lesions.

Effects of pretreatment of rabbits with HN_2 on development of microscopic lesions of corneal endothelium and on virus titer in aqueous humor following intraocular injection of NDV. Ten rabbits were given 2 intravenous injections of HN_2 in the same manner as in the preliminary experiment. On the second day after the last injection of HN_2, 0.2 ml of NDV in allantoic fluid (4×10^8 PFU) was inoculated into both eyes. The same number of normal rabbits served as controls and virus was similarly inoculated. At each interval, 2 rabbits were taken from each group; degree of corneal opacity was checked and animals were immediately sacrificed by an intravenous injection of sodium Nembutal. Aqueous humor, for leucocyte count and virus assay, was aspirated from each eye with a separate tuberculin syringe. Extent of fusion was scored and total number of endothelial rosettes appearing on the entire endothelium was determined. Results are shown in Table II.

Development of corneal opacity was completely suppressed in all HN_2-treated animals of the 12 and 24 hour-groups. Fusions were less extensive than in corresponding control animals. On the other hand, a large number of rosettes still appeared on the corneas of the HN_2-treated rabbits. The number of leucocytes in aqueous humor was much smaller in HN_2-treated animals than in untreated animals. Titer of residual virus in aqueous humor of HN_2-treated animals was not different from that of controls, even though there was a marked suppression on development of corneal opacity in treated animals. These results indicated that suppression of corneal opacity in HN_2-treated animals was probably not due to rapid inactivation of virus in aqueous humor.

From the above results it is reasonable to assume that suppression of corneal opacity and fusion appears to be correlated with, but not necessarily due to, suppression of leucocytes, particularly the PMN, in aqueous humor. However, it is important to note that one rabbit in the 24 hour-control group showed no corneal opacity even though a large number of leucocytes was present in aqueous humor.

Relationship between persistent suppression on production of corneal lesions by NDV and number of leucocytes in aqueous humor following recovery from HN_2-induced leucopenia. If presence of leucocytes, particularly PMN, in aqueous humor is directly related to occurrence of endothelial fusion and corneal opacity, it is reasonable to postulate that when the number of leucocytes in the blood of HN_2-treated animals returned to normal level, intraocular inoculation of virus should cause a large number of PMN to appear in aqueous humor, and corneal opacity and fusion might be expected to develop as extensively as in control rabbits. To examine this possibility experiments were performed as follows:

Five rabbits were injected twice with HN_2 in the standard manner. Two days after the last injection of HN_2 blood leucocyte and differential counts of these animals were determined. At the same time 4 normal rabbits were allocated as controls, and blood leucocyte and differential counts were similarly carried out on these animals. Two-tenths ml of NDV in allantoic fluid (4×10^8 PFU) was inoculated into the anterior chamber of the right eye of each animal in both groups. The uninoculated left eyes served as controls. Twenty-four hours after virus injection, corneal opacity was recorded and 0.1 ml of aqueous humor was aspirated from the injected eye for leucocyte count. On the 13th or 20th day after virus inoculation, blood leucocyte and differential counts were again carried out on these groups of animals and NDV (4×10^8 PFU) was then inoculated into the anterior chambers of both eyes. Twenty-four hours after the second virus injection, corneas of these animals were checked for de-

gree of opacity, and at the same time 0.1 ml of aqueous humor was aspirated to determine total number of leucocytes in a drop. The results are summarized in Table III.

Typical corneal opacity developed after the second virus injection in all 4 control rabbits. However, rabbits treated with HN_2 did not develop corneal opacity even after the blood leucocyte count reached or exceeded a normal value and after leucocytes were present in large number in aqueous humor of these eyes. It was interesting to find that the suppressive effect of HN_2 on development of corneal opacity in response to NDV lasted for at least 13 and 20 days after the first NDV injection. It was considered

TABLE II. Effects of Pretreatment of Rabbits with HN_2 on Production of Microscopic Lesions of Corneal Endothelium and on Viral Titer in Aqueous Humor Following Intraocular Injection of NDV.

Hr after virus inoc.	Group	Rabbit No.	Eye	Corneal opacity	Microscopic changes: Fusion	Microscopic changes: Rosette	WBC/drop of aq.h.*	NDV in aq.h.* (PFU/ml)
1½	HN_2†	1	R	—	±	97	0	5.1×10^6
			L	—	±	96	0	5.9×10^5
		2	R	—	±	15	1	1.5×10^6
			L	—	±	16	0	1.2×10^6
	Control	1	R	—	ND	ND	7	4.8×10^6
			L	—	±	79	19	7.5×10^5
		2	R	—	±	110	5	2.9×10^6
			L	—	±	172	1	7.0×10^6
3	HN_2†	1	R	—	±	188	6	5.4×10^5
			L	—	±	110	0	6.4×10^6
		2	R	—	±	132	0	2.2×10^5
			L	—	±	151	0	8.3×10^5
	Control	1	R	—	±	297	974	5.4×10^5
			L	—	±	337	470	ND
		2	R	—	±	191	290	3.0×10^4
			L	—	±	120	540	7.0×10^5
6	HN_2†	1	R	—	+	‡	1	5.4×10^5
			L	—	+	‡	4	2.3×10^5
		2	R	—	± to +	‡	3	2.9×10^4
			L	—	± to +	‡	8	7.3×10^5
	Control	1	R	±	++	‡	610	1.9×10^5
			L	±	++	‡	850	3.0×10^5
		2	R	±	+	‡	534	1.7×10^5
			L	±	++	‡	2216	1.0×10^5
12	HN_2†	1	R	±	++	‡	71	4.0×10^4
			L	±	+	‡	131	4.0×10^4
		2	R	—	+	‡	6	5.8×10^4
			L	—	+	‡	8	5.2×10^3
	Control	1	R	++	+++	‡	241	2.6×10^4
			L	+	++	‡	970	2.1×10^4
		2	R	++	+++	‡	641	9.0×10^4
			L	++	+++	‡	968	2.0×10^4
24	HN_2†	1	R	—	± to +	‡	31	1.1×10^3
			L	—	± to +	‡	4	4.0×10^3
		2	R	—	+	‡	21	1.3×10^2
			L	—	+	‡	4	5.0×10^2
	Control	1	R	—	+	‡	1621	3.8×10^2
			L	—	+	‡	1221	9.5×10^2
		2	R	+++	+++	‡	1451	1.8×10^3
			L	+++	+++	‡	3360	6.2×10^2

* Aqueous humor (aq.h.).
† Two intrav. injections of HN_2 were given 2 and 4 days before intraocular inj. of NDV.
‡ Not suitable to count rosettes due to extensive fusions.
ND = Not done.

TABLE III. Persistent Suppression of Corneal Toxic Reaction to NDV after Termination of Leucopenia Induced by HN_2.

		1st intraocular inj. of NDV						2nd intraocular inj. of NDV					
Group	Rabbit No.	Blood WBC WBC/mm³	Blood WBC PMN (%)	Eye	Inoc. with NDV	Opacity after 24 hr	WBC/drop of aq.h.*	Days after 1st NDV inoc.	Blood WBC WBC/mm³	Blood WBC PMN (%)	Inoc. with NDV	Opacity after 24 hr	WBC/drop of aq.h.*
HN_2 treated‡	1	1825	1	R	Yes	—	31	20	7650	46	Yes	—	930
				L	No		ND				"	—	1160
	2	950	0	R	Yes	±	6	20	10250	47	"	+	730
				L	No		ND				"	—	1500
	3	300	5	R	Yes	—	10	13	4950	40	"	±	1560
				L	No		ND				"	—	2160
	4	525	3	R	Yes	—	1	13	6150	42	"	±	900
				L	No		ND				"	—	916
	5	525	1	R	Yes	+	4	13	4250	39	"	±	761
				L	No		ND				"	—	819
Control	1	4250	39	R	Yes	+++	1714	20	ND	ND	No†		ND
				L	No		ND				Yes	++	2601
	2	6000	47	R	Yes	+++	2160	20	"	"	No†		ND
				L	No		ND				Yes	+++	1900
	3	5100	34	R	Yes	+++	960	13	"	"	No†		ND
				L	No		ND				Yes	+++	3161
	4	8800	40	R	Yes	+++	1500	13	"	"	No†		ND
				L	No		ND				Yes	+++	1916

* Aqueous humor (aq.h.) was removed at the time when corneal opacity was scored.

† Corneal opacity produced by the first inj. of NDV still persisted.

‡ Two intrav. injections of HN_2 were given 2 and 4 days before the first intraocular inj. of NDV.

ND = Not done.

possible that this late suppressive effect was due to formation of antibodies against NDV. However it was found that there was no significant antibody level against NDV in these animals at the time of second injection of virus. It may be possible that leucocytes at 13 and 20 days after HN_2-treatment are functionally inactive in formation of corneal opacity by NDV.

Discussion. In rabbits pretreated with 2 intravenous injections of HN_2, corneal opacity as well as endothelial fusion was suppressed while rosettes appeared as extensively as in controls. It was reported previously(2) that in the *in vitro* system injection of NDV into the anterior chamber caused formation of numerous rosettes but did not induce either opacity of the cornea or an appreciable degree of fusion of endothelial cells. This experimental evidence suggests that corneal opacity in virus-induced toxic reaction is more closely associated with endothelial fusions than with endothelial rosettes.

Mechanisms by which virus produced these lesions are not understood. However, the present study appears to indicate that leucocytes are not essential for production of a toxic reaction by NDV in the rabbit cornea. This is supported by the following observations: 1) Following intraocular inoculation of NDV, rabbits pretreated with HN_2 occasionally developed opaque corneas. These eyes showed few or no leucocytes in their aqueous humor. 2) Eyes of normal rabbits inoculated with a toxic amount of NDV sometimes failed to produce an opaque cornea and fusions even though there was a large number of leucocytes in their aqueous humor. 3) In the HN_2-treated rabbits, suppressive effect of HN_2-treatment on the development of corneal opacity lasted for at least 13 and 20 days after treatment. This long lasting suppression was observed in eyes which contained numerous leucocytes in aqueous humor. It appears that infiltration of leucocytes into ocular tissues and aqueous humor is secondary to changes induced by the virus in the eye. The role of leucocytes in the toxic cornea is unknown at present.

It has been reported that mustard gas inactivates NDV *in vitro*(5). However titer of residual virus in aqueous humor of eyes of HN_2-treated animals was as high as that of controls at any time. Therefore, suppression of virus-induced corneal toxicity by pretreatment with HN_2 is not due to rapid inactivation of virus in aqueous humor.

Intravenous injection of HN_2 simultaneously with intraocular inoculation of virus failed to suppress development of corneal opacity. It appears that pretreatment of rabbits with HN_2 is an essential factor in suppression of virus-induced corneal opacity. These findings suggest that HN_2 modifies the host's interaction with virus. In virus-induced corneal reaction, however, it is not known whether HN_2 exerts its suppressive action on corneal endothelium, on tissues other than corneal endothelium, or on humoral factors which may play an essential role in the pathogenesis of NDV. It was suggested that in the Shwartzman phenomenon HN_2 exerts its suppressive action on the reticuloendothelial system, primarily the vascular endothelium(6). Possibly a similar modification of corneal endothelium is brought about by HN_2 injection to render host tissue less susceptible to virus activity. Further studies are necessary to make a proper evaluation of this possibility. This attitude is also important in studying the suppressive action of HN_2 on the Shwartzman phenomenon, especially when considering the suggestion of Stetson and Good(7) that inhibition of the Shwartzman phenomenon can be correlated with leucopenia induced by HN_2.

Rose and Gellhorn(8) failed to modify influenza virus-induced pulmonary lesions in mice by pretreating the animals with daily intraperitoneal injections of HN_2 beginning 2 days before or immediately after virus inoculation. However, in view of the suppressive action of HN_2-treatment on NDV-induced toxic reaction, it is desirable that HN_2 should be further explored for its possible effectiveness in preventing lesions in other tissues infected by NDV or other viruses known to produce a similar toxic effect in animals.

Summary. Pretreatment of rabbits with 2 intravenous injections of nitrogen mustard (HN_2) partially suppressed production of

NDV-induced toxic corneal reactions. In most animals this treatment suppressed development of opacity and reduced markedly the extent of fusions of endothelial cells. It did not inhibit formation of endothelial rosettes. The suppressive effects in the HN_2-treated animals lasted for at least 20 days, well after leucopenia had disappeared. Decreased response to injected virus is neither a result of suppression of circulating leucocytes nor of rapid inactivation of virus in aqueous humor.

Treatment of rabbits with a single intravenous injection of HN_2 4 days, 2 days, or immediately before virus injection caused no demonstrable suppression of virus-induced toxic reactions of rabbit corneas. Leucocytes appeared to play no essential role in production of corneal reaction.

The authors wish to express their deep gratitude to Dr. C. A. Evans for advice and criticism during this investigation.

1. Wilcox, W. C., Wood, E. M., Oh, J. O., Everett, N. B., Evans, C. A., *Brit. J. Exp. Path.*, 1958, v39, 601.

2. Oh, J. O., Evans, C. A., *Virology*, 1960, v10, 127.

3. Hanson, R. P., Upton, E., Brandly, C. A., Winslow, N. S., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 283.

4. Dulbecco, R., *Proc. Nat. Acad. Sci. U. S.*, 1952, v38, 747.

5. Herriott, R. M., *J. Gen. Physiol.*, 1948, v32, 221.

6. Becker, R. M., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 247.

7. Stetson, C. A., Good, R. A., *J. Exp. Med.*, 1951, v93, 49.

8. Rose, H. M., Gellhorn, A., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 83.

Received December 15, 1960. P.S.E.B.M., 1961 ,v106.

Correlation of Pituitary Ribonucleic Acid Levels and ACTH Production Following Adrenalectomy and Cortisone Administration.* (26357)

MELVIN HESS, JAMES J. CORRIGAN, JR.† AND JOHN A. HODAK‡
(With the technical assistance of James E. Bird)
Department of Anatomy, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

It has been inferred that withdrawal of adrenal cortcial hormones enhances both release and synthesis of pituitary adrenocorticotrophic hormone (ACTH), but with a predominent effect on synthesis. The latter mechanism serves to explain the increased concentration of ACTH in pituitary tissue following adrenalectomy(1,2,3). The inhibitory effect of adrenal cortical hormones on ACTH content of pituitaries and plasma likewise has been attributed to alteration of rates of trophic hormone production and release (3,4).

Since the relationship of ribonucleic acid (RNA) and protein synthesis has been shown to be applicable to pituitary hormone production following adrenalectomy(5), one can correlate fluctuations in tissue content of ACTH following adrenalectomy and cortical hormone administration, as demonstrated by Fortier(1,4), with RNA levels in the hypophysis. This report, based on pituitary RNA content following adrenalectomy or adrenal cortical hormone treatment, is an attempt to correlate protein synthetic activity with conent of trophic hormone in the tissue.

Methods. Male rats of the Holtzman strain were bilaterally adrenalectomized *via* dorsal approach and maintained on Purina Laboratory Chow pellets and 1% NaCl *ad lib*. Unoperated rats of the same age, receiving tap water in place of salt solution, served as controls. At intervals of time from ½ hour to daily and weekly post-operative periods (Table I), adrenalectomized and control rats were sacrificed. Other rats received

* This investigation was supported in part by research grant from Nat. Inst. of Arthritis and Metab. Dis., P.H.S., and in part by Arthritis and Rheumatism Foundation.

† Ciba Pharmaceutical Co. Medical Student Research Fellow.

‡ Medical Student Fellow of National Foundation.