TABLE I. Renal Excretion of Digitoxin in Dog After Its Injection (0.2 μg/g).

Dog	Wt, kg	Amt digitoxin given, μg	Urine (0-24 hr)		Urine (24-48 hr)		% excreted
			vol., cc	Digitoxin, μg	Vol., cc	Digitoxin, μg	
R-1	9	1800	900	18	450	N.D.*	1.0
R-2	10	2000	1400	35	400	5.	2.0
R-3	15	1500	2220	12	1980	N.D.	0.8

* Non-detectable.

possible, even probable, that a breakdown product of a glycoside might be expected.

The 3 dogs, however, were found to excrete a small amount of digitoxin during the first 24 hours. One of the 3 continued to excrete the drug the second day after its injection. As Table I indicates, the 2 dogs receiving 0.2 μg of digitoxin per g of body weight excreted 18 μg and 35 μg respectively the first 24 hours. The third dog, which received only 0.1 μg of the drug per g of body weight excreted 12 μg. One of the dogs (R-2) excreted 5 μg of the drug during the second 24 hour period of collection. Thus, the total renal excretion of digitoxin in the dog is about 1-2% of a given dose.

Discussion. The above results together with earlier studies(1,2) suggest that differences exist between various species in respect to renal excretion of digitoxin. Thus, whereas the rabbit appears to excrete no digitoxin in its urine, the dog excretes approximately 1-2%, the rat about 3%, and man as much as 40%.

Despite the relative paucity of digitoxin in the urine of the rat(1), rabbit, and dog, considerable amounts of the drug when given intravenously accumulate in the kidney of these animals(3). This is particularly so in the dog whose kidney, for several days after the injection of digitoxin, may contain far more digitoxin than is found in the 24 or 48 hours of urine collection. Moreover, this renal digitoxin is concentrated primarily in the cortical segment of the kidney. These observations suggest the possibility that the kidney of some mammals may destroy more digitoxin than it excretes. This does not appear to be so, however, in man.

Summary. The kidney of the rabbit was not found to excrete significant amounts of administered digitoxin. The dog appeared to excrete approximately 1-2% of an administered dose of digitoxin. The probability of the renal destruction of digitoxin in these species was discussed.

The authors wish to express their thanks to Pamela Angwin and Tadashi Ishida for their valuable aid.

3. Friedman, M., St. George, S., Bine, R., Jr., Byers, S. O., and Bland, C., to be published.

Received September 24, 1951. P.S.E.B.M., 1951, v78.

Effect of Adrenal Hormones on Infection of Mice with Pneumoia Virus of Mice (PVM).* (19121)

J. MACLEAN SMITH,† JAMES S. MURPHY,‡ AND GEORGE S. MIRICK.
(With the technical assistance of Miss Marjorie G. Huck.)
From the Department of Medicine, Johns Hopkins University School of Medicine.

The enhancing effect of urethane on the severity of the infection of mice with pneu-

* This work was carried out in part under contract with the Office of Naval Research, and in part was supported by a grant from the National Cancer Institute, National Institutes of Health, Public Health Service, Federal Security Agency.

† Postdoctorate Research Fellow of the National Cancer Institute.

‡ Fellow, American Cancer Society.

monia virus of mice (PVM) was reported elsewhere(1). Because of certain striking similarities between the pharmacological effects of urethane and those attributed to adrenal cortical stimulation it seemed of interest to investigate possible adrenal effects on PVM infection. Murphy and Sturm(2) noted that the adrenal glands of rats given urethane were 40% heavier than controls. The effects of adrenal stimulation with adrenocorticotropic hormone (ACTH) or adrenal cortical substitution with cortisone have been summarized by Thorn *et al.*(3). Some similar effects of urethane are: Lympholysis(4), eosinopenia (5), suppression of inflammatory reaction(6), and fibroblast growth(7), chloride retention (8), and loss of potassium(9) and calcium (10), alkalosis(4), hyperglycemia(11,12), and increased gastric secretion(13).

In the present paper the results of treating mice with ACTH or cortisone and of adrenalectomy on PVM infection are described.

Materials and methods. Mice and virus. The O'Grady strain of Swiss mice and PVM strain 15 were employed throughout(1). Unless otherwise stated the mice weighed approximately 10 g each at the time of virus inoculation. *Titrations.* The usual methods (14) were used for performing virus titrations and calculating the 50% maximum lesion score (M.S. 50). Unless otherwise stated 20 mice were used in each virus titration. The method of Curnen and Horsfall(15) for performing hemagglutination tests was used. Virus was heated at 80°C for 10 minutes to eliminate the masking tissue component. *Drugs.* The ACTH was Actamour (Armour Laboratories). The cortisone was Cortone R (Merck). Dilutions of each drug were made in 0.85% saline so that the dose used was dissolved or suspended in 0.05 cc to 0.1 cc of fluid. The ACTH was stored, after dilution, at —20°C and the cortisone at room temperature. Each drug was given subcutaneously once daily. *Adrenalectomy.* Young mice weighing approximately 10 g were lightly anesthetized with ether. The adrenal glands were exposed and excised through a single mid-line dorsal skin incision and bilateral muscular incisions. The incisions were then closed with silk sutures and protected with collodion. Mice were maintained on subcutaneous injections of 0.5 cc of 1% glucose in 1.25% saline solution, daily from the time of operation until the end of the experimental period.

Experimental. Effect of ACTH on PVM infection. The effect of ACTH, on both the extent of the lesions resulting from PVM infection and the multiplication of the virus, was tested as follows: In 7 experiments simultaneous duplicate titrations of PVM were made in 2 matched groups of 20 mice weighing approximately 10 g each at the time of inoculation. In each experiment one group served as controls and each mouse in the other group was given a daily injection of ACTH. Twelve days after infection all surviving mice were sacrificed and the 50% maximum score (M.S. 50) was calculated. The results are shown in Table I (Exp. 1-7). It will be seen

1. Mirick, G. S., Smith, J. M., Leftwich, C. I., Jr., and Leftwich, W. B., *J. Exp. Med.*, 1951.

2. Murphy, J. B., and Sturm, E., *Cancer Research*, 1947, v7, 417.

3. Thorn, G. W., Forsham, P. H., Frawley, T. F., Hill, S. R., Jr., Roche, M., Staehelin, D., and Wilson, D. L., *New Eng. J. of Med.*, 1950, v242, 783, 824, 865.

4. Hawkins, J. A., and Murphy, J. B., *J. Exp. Med.*, 1925, v42, 609.

5. Stein, K. F., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 225.

6. Peng, D., *Arch. exp. Path. u. Pharmakol.*, 1930, v151, 270.

7. Lusbaugh, C. C., Green, J. W., Storer, J. B., *J. Nat. Cancer Inst.*, 1948, v8, 201.

8. Fee, A. R., *J. Pharmacol. and Exp. Therap.*, 1928, v34, 305.

9. Boyd, E. M., and Perry, W. F., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 334.

10. Clark, A. S., and White, A. C., *J. Physiol.*, 1930, v68, 433.

11. Conybeare, E. T., Densham, M., Maisels, M., Pembrey, M. S., *J. Physiol.*, 1927, v64, 19.

12. Laughton, N. B., Macallum, A. B., *Biochem. J.*, 1935, v29, 1257.

13. Stein, I. F., Jr., and Meyer, K. A., *J. Am Med. Assn.*, 1949, v140, 522.

14. Horsfall, F. L., Jr., and Curnen, E. C., *J. Exp. Med.*, 1946, v83, 25.

15. Curnen, E. C., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1946, v83, 105.

TABLE I. Comparison of PVM M.S. 50 Scores in Mice Receiving ACTH and Controls (Exp. 1-7) and Amount of Virus in Lungs at 6 Days (Exp. 8-11).

Exp. No.	Treated with ACTH — Schedule	Treated with ACTH — M.S. 50 log	Control, M.S. 50 log	Difference from control, M.S. 50 log
1	(1)	4.21*	4.22	— .01
2	(2)	4.33	4.22	+ .11
3	(3)	4.24	4.61	— .37
4	(3)	4.79	4.57	+ .22
5	(3)	4.05	3.63	+ .42
6	(4)	3.70	3.63	+ .07
7	(5)	3.94	3.63	+ .42
	Avg			+ .10
8	(3)	5.60†	4.17	+1.43
9	(3)	6.53	6.18	+ .35
10	(3)	4.78	4.04	+ .74
11	(5)	4.93	4.04	+ .81
	Avg			+ .83

ACTH schedule	Daily injection	Total dose, γ/g
1	5 γ/g, D—1 to D+4; 20γ/g, D+4 to D+11	190
2	5 γ/g, D+4; 20 γ/g, D+4 to D+11	165
3	20 γ/g, D—2 to D+11	280
4	20 γ/g, D—2 to D+4	140
5	40 γ/g, D—2 to D+11	560

* M.S. 50 score at 12 days in mice treated with ACTH.

† M.S. 50 score at 12 days in normal mice inoculated with lung extracts from mice treated with ACTH.

that there is no significant difference in lesion scores in the two groups of mice. Since ACTH has been shown to suppress inflammatory reactions of various sorts(16), it seemed possible that the M.S. 50 score might not give the complete picture of ACTH effects on the infection. To test the possible effects of ACTH on virus growth other experiments were carried out. Four other matched groups of 8 mice each were inoculated with PVM 10^{-3}. Half of each group was given ACTH subcutaneously and the other half served as controls. Six days after virus inoculation the mice were sacrificed. The lungs from each group were pooled and extracted separately with trypticase soy broth (Baltimore Biological Laboratory) and the amount of virus in each pool was titered for infectivity in a fresh group of 20 mice. These results are also shown in Table I (Exp. 8-11). It will be seen that the lung extracts from the ACTH treated mice gave an average M.S. 50, 0.83 log higher than those from controls. To test the reproducibility of this type of assay, 5 other groups each comprising 4 normal mice were inoculated at the same time with PVM, dilution 10^{-3}. At 6 days the mice were sacrificed and the virus infectivity titer in pooled lung extracts from each group was measured in the usual fashion. The mean M.S. 50 was 6.61 logs and the standard deviation 0.061 log. For the purpose of this investigation a difference of 0.70 log or greater between control and experimental titers of virus in the lungs at 6 days is considered significant. Although the difference 0.83 log in virus titer in the lungs of ACTH treated and control mice is not great it is thought to be significant. It was decided to repeat the above experiments using cortisone rather than ACTH.

Effect of cortisone on PVM infection. Before testing the effects of cortisone on PVM infection it seemed important to determine, first, the toxicity of this substance. Following a single injection into 10 g mice, the M.L.D. 50% was found to be 400 γ/g. Mice which died showed pulmonary edema, vascular dilatation, and hemorrhage. Uninfected mice which received smaller sublethal amounts (15 γ/g) once daily for 6 days showed, on sacrifice at 12 days, no gross lesions in the lungs. However, the lung weight/body weight

16. Kass, E. H., and Finland, M., *New Eng. J. Med.*, 1951, v244, 464.

TABLE II. Comparison of PVM M.S. 50 Scores in Mice* Receiving Cortisone and Controls (Exp. 1-7) and Amount of Virus in Lungs at 6 Days (Exp. 8 and 9).

Exp. No.	Treated with cortisone: Schedule	M.S. 50 log	Control, M.S. 50 log	Difference from control, M.S. 50 log
1	(1)	6.00†	3.63	+2.37
2	(2)	4.36	3.63	+ .73
3	(3)	5.00	3.73	+1.27
4	(4)	6.50	4.54	+1.96
5	(4)	5.34	4.82	+ .52
6	(5)	3.10	2.15	+ .95
7	(6)	2.59	2.15	+ .44
	Avg			1.18
8	(2)	5.59‡	4.04	+1.55
9	(2)	5.28	4.04	+1.24
	Avg			1.39

Cortisone schedule	Daily injection	Total dose, γ/g
1	50 γ/g, D—2 to D+11	700
2	50 γ/g, D—2 to D+4	350
3	25 γ/g, D 0 to D+11	300
4	12.5 γ/g, D 0 to D+6	87.5
5	9 γ/g, D 0 to D+6	63
6	5.6 γ/g, D 0 to D+6	39.2

* All mice were 3 to 4 wk old and weighed about 10 g each except those in exp. 6 which were 4 mo old and weighed 35-40 g and those in exp. 7 which were 6 mo old and weighed 40-50 g each.
† M.S. 50 score at 12 days in mice treated with cortisone.
‡ M.S. 50 score at 12 days in normal mice inoculated with lung extracts from mice treated with cortisone.

ratio in these mice was 17% greater than in controls. This suggests that even these smaller doses of cortisone resulted in some pulmonary edema or congestion and is reminiscent of the findings in urethanized mice(1). The M.S. 50 scores observed when PVM was titered simultaneously in matched groups of mice, given cortisone and control, are shown in Table II (Exp. 1-7). The cortisone treated mice received 5-50 γ/g of the hormone subsubcutaneously once daily. In the same table (Exp. 8, 9) are recorded the M.S. 50 scores of virus in the lungs of cortisone treated and normal control mice tested at 6 days after inoculation with PVM 10^{-3}. These mice received 50 γ/g of cortisone subcutaneously once daily. It will be seen that the average lesion score in cortisone treated mice was 1.18 logs greater than in controls and that the amount of virus at 6 days was also greater in the cortisone treated mice by 1.39 logs. The amount of virus at 2, 4, 6, and 8 days after inoculating PVM 10^{-3} into normal, ACTH treated and cortisone treated mice, was also tested by the capacity of heated lung extracts to agglutinate mouse erythrocytes. The results are recorded in Fig. 1. It is seen that none of the heated extracts of lungs removed 2 days after virus inoculation caused hemagglutination. At 4, 6, and 8 days the average titer in ACTH treated mice was about equal to that in controls but in cortisone-treated mice was about 1 log greater than in the controls. This, again, is similar to the finding in mice which were treated with urethane(1).

Effects of adrenalectomy on PVM injection. Since both ACTH and cortisone treatment seemed to result in increased amounts of PVM in the lungs of mice at 6 days after virus inoculation, and the latter also accentuated the lesions observed at 12 days, it seemed of interest to test the effects of adrenalectomy on PVM infection. In three experiments the adrenal glands were removed from each of 24 10 g mice. Seven to 10 days after operation PVM was titered in the usual fashion in 20 of the mice. Simultaneous duplicate titrations were done in intact control mice of the same age. Eight additional mice, 4 adrenalectomized and 4 intact, were inoculated in each experiment with virus dilution 10^{-3}, sacrificed at 6 days and extracts of their pooled lungs compared for infectivity. The results are shown in Table III. It will be seen that the average

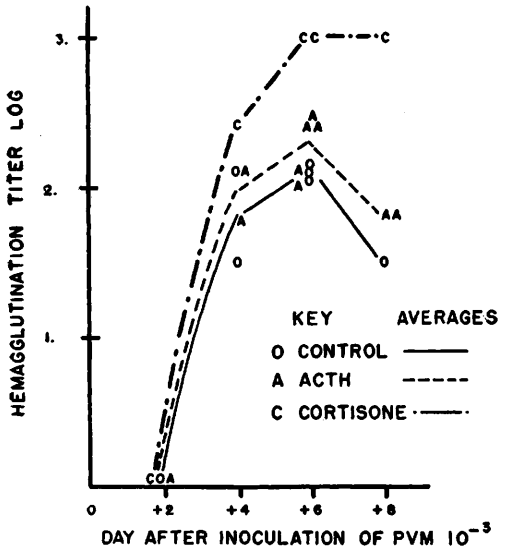

FIG. 1. Hemagglutination titers of heated lung extracts from mice treated with ACTH or cortisone and controls. Most mice died on the 7th or 8th day after virus inoculation.

M.S. 50 score in the adrenalectomized mice was slightly (0.33 log) less than in controls. The average titer of virus at 6 days was 0.89 log less in the adrenalectomized mice. These differences are not great but are consistently in the opposite direction from the findings in mice which received ACTH or cortisone. Taken together the findings strongly suggest that adrenal stimulation and especially cortisone administration enhances PVM infection and adrenal extirpation has the opposite effect.

Effects of ACTH and cortisone on pulmonary tumors. The similar effect of ACTH, cortisone and urethane on PVM infection suggested that prolonged treatment of mice with these drugs should be tested for possible oncogenic action. Twenty-five mice one month old were injected thrice weekly with 400 γ of ACTH for 3 to 5 months. Forty-three other mice were similarly treated thrice weekly with 250 γ of cortisone. These mice and 110 untreated controls were sacrificed at 6 months. The tumor incidence in the ACTH and cortisone treated groups was 36% and 25%, respectively, as compared with 31% in the controls. It is concluded that ACTH and cortisone in the doses tested had no oncogenic effect.

Discussion. The effects of ACTH and cortisone have been tested in a variety of bacterial infections(16); in general, treatment with these drugs has resulted in some suppression of the inflammatory reaction but enhancement and dissemination of the infection. The influence of ACTH on certain virus infections of man has been reported. Finland *et al.*(17) described the symptomatic improvement of a patient with primary atypical pneumonia treated with ACTH. However, there was no apparent effect on the course of the disease. Treatment with ACTH may have alleviated the severity of acute viral hepatitis (18), but this drug did not influence the course of poliomyelitis in human beings(19) when given after the onset of symptoms

A number of reports have appeared recently concerning ACTH and cortisone effects on experimental virus infections in laboratory animals. ACTH has been found to have little or no influence on experimental infections in mice with influenza virus(20,21), the Lansing

TABLE III. Comparison of PVM M.S. 50 Score in Adrenalectomized and Control Mice (Exp. 1-3) and Amount of Virus in the Lungs at 6 Days (Exp. 4-6).

Exp. No.	Adrenal-ectomized, M.S. 50 log	Control, M.S. 50 log	Difference from control, M.S. 50 log
1	2.69	3.38	—.69
2	2.63	2.67	—.04
3	3.17	3.43	—.26
	Avg		—.33
4	4.14	5.05	—.91
5	3.39	4.16	—.77
6	4.96	5.95	—.99
	Avg		—.89

17. Finland, M., Kass, E. H., and Ingbar, S. H., *Proc. First Clinical ACTH Conference,* The Blakiston Co., p529.

18. Colbert, J. W., Jr., Holland, J. F., Heissler, I., and Knowlton, M., *Proc. Second Clinical ACTH Conference,* Vol. 1, John R. Mote, Editor, The Blakiston Co., 1951.

19. Corriell, L. L., Siegel, A. C., Cook, C. D., Murphy, L., and Stokes, J., Jr., *J. Am. Med. Assn.,* 1950, v142, 1279.

20. Loosli, C. G., Hull, R. B., Berlin, B. S., and Alexander, E. R., *J. Lab. and Clin. Med.,* 1950, v36, 956.

21. Kalter, S. S., Smolin, H. J., McElhaney, J. M., and Tepperman, J., *Bact. Proc.,* 1951, p86.

strain of poliomyelitis virus(22,23), or the virus of western equine encephalomyelitis(23). Ainslie *et al.*(24) reported that large doses of ACTH in monkeys seemed to make them more susceptible to infection with the Minnesota strain of poliomyelitis. Cortisone has been shown to markedly enhance poliomyelitis infection in mice and hamsters(22). It increased the susceptibility of old mice to Coxsackie virus(25), of mice to influenza virus (26), and to West Nile, Ilheus and Bunyamwera viruses(27), and of guinea pigs to vaccinia virus(28). Moreover, cortisone treatment of embryonated eggs has resulted in significantly greater concentrations of both influenza and mumps viruses in the allantoic fluid(29).

Our findings that ACTH, in the doses employed, produced little effect on PVM in mice whereas cortisone treatment resulted in rather striking increase in the severity of the infection are in general agreement with the findings reported in other virus infections. It is quite possible that the ACTH employed may have lost potency through storage and that larger dosage or more frequent treatment might have resulted in effects similar to those of cortisone. The mechanism through which this effect takes place is obscure but the evidence presented in this paper and elsewhere(1) suggests that the enhancing effect of urethane on PVM infection may work through a similar mechanism.

Summary. The treatment of mice with ACTH did not influence their susceptibility to PVM infection but slightly enhanced virus multiplication. Cortisone treatment enhanced both the virus infection and the multiplication of virus to a greater degree. Adrenalectomy rendered mice slightly less susceptible to infection and retarded virus growth.

22. Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 835.

23. Milzer, A., *J. Inf. Dis.*, 1951, v88, 54.

24. Ainslie, J. D., Francis, T., Jr., and Brown, G. C., *Bact. Proc.*, 1951, p94.

25. Kilbourne, E. D., and Horsfall, F. L., Jr, PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 135.

26. Kass, E. H., Ingbar, S. H., Lundgren, M. M., and Finland, M., *J. Lab. and Clin. Med.*, 1951, v37, 780.

27. Southam, C. M., and Babcock, V. I., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 105.

28. Kligman, S. M., Baldridge, G. D., Rabell, G., and Pillsbury, D. M., *J. Lab and Clin. Med.*, 1951, v37, 615.

29. Kilbourne, E. D., and Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 116.

Received September 24, 1951. P.S.E.B.M., 1951, v78

Growth Studies on *Polytomella agilis*. (19122)

P. A. LITTLE, J. J. OLESON, AND J. H. WILLIAMS.

From Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

The nutrition and metabolism of phytomonads and related species have been studied by several investigators. Doyle(1) has reviewed the problem of physiological classification of protozoa on the basis of their known nutrition. That ethanol and acetate are utilized by various species has been known for many years(2,3). A number of green and colorless flagellates such as Euglena, Astasia, Polytoma, Polytomella, Chlorogonium, and Hyalogonium have been found to be unable to utilize sugars and hence are dependent on simple acids and alcohols. Lwoff, Ionesco, and Gutmann(4) showed that *Polytomella caeca* lacks the hexokinase required for utilization of exogenous sugars. Albaum *et al.*(5) investigated this problem in Euglena.

1. Doyle, W. L., *Biol. Rev.*, 1943, v18, 119.

2. Loefer, J. B., and Hall, R. P., *Arch. f. Protistenk.*, 1936, v87, 123.

3. Hall, R. P., *Vitamins and Hormones*, 1943, v1, Academic Press, 249.

4. Lwoff, A., Ionesco, H., and Gutmann, A., *Biochim. et Biophys. Acta*, 1950, v4, 270.