TABLE I. Lung Weights of Control Animals and Animals Receiving Anterior Hypothalamic Injections of Aconitine.

Group	Ether, control	Ether, aconitine	Fluothane,* aconitine	Pentobarbital light (35 mg/kg), aconitine	Pentobarbital* (deep)	Ether, posterior hypothalamic lesions, aconitine
No. of animals	10	10	10	8	10	20
Mean wt/100 g rat	.71 ±.047	2.14 ±.911	1.72 ±.821	1.53 ±.466	.91 ±.397	.97 ±.425
P values		<.0005	<.0005	<.0005	<.07	<.055

* Pentobarbital sufficient to give marked depression of respiration and reflexes.

pulmonary edema. Our results showing that the edema produced by injections of aconitine into the preoptic areas is reduced by lesions of the posterior hypothalamus is compatible with their theory. Our observation that deep barbiturate anesthesia reduces the severity of the edema would be explainable on the basis that adequate depression of the "released" areas diminishes their capacity to produce the vascular changes responsible for the edema. It is difficult to account for the fact that drug-induced hypothalamic edema production appears to be a unique property of aconitine.

Summary. Acute pulmonary edema is induced by bilateral injections of dilute solutions of aconitine into the preoptic area of the hypothalamus of rats. The severity of the edema is greatly reduced but not abolished by lesions of the posterior hypothalamus. Deep barbiturate anesthesia greatly reduces aconitine edema. Systemic injections of aconitine up to fatal dose levels do not induce pulmonary edema.

1. Cameron, G. R., De, S. N., *J. Path. Bact.*, 1949, v61, 375.
2. Farber, S., *J. Exp. Med.*, 1937, v66, 397.
3. Gamble, J. E., Patton, Harry D., *Science*, 1951, v113, 626.
4. ———, *Am. J. Physiol.*, 1953, v172, 625.
5. Marie, F. W., Patton, H. D., *ibid.*, 1956, v184, 345.
6. ———, *ibid.*, 1956, v184, 351.

Received February 20, 1962. P.S.E.B.M., 1962, v111.

Effects of 6-Mercaptopurine on Susceptibility of Guinea Pigs to Experimental Allergic Encephalomyelitis.* (27720)

J. D. THOMSON AND R. W. AUSTIN†
Department of Physiology, State University of Iowa College of Medicine, Iowa City

Hoyer, Condie and Good(1) found that 6-MP, given intravenously and daily to rabbits prior to and following inoculation with an emulsion of homologous spinal cord and Freund's adjuvant, reduced the incidence of paralysis of experimental allergic encephalomyelitis, but did not completely prevent it. Larger doses completely suppressed the appearance of the paralysis, which appeared 7 to 10 days after 6-MP was discontinued. Field(2) reported that 6-MP, given to guinea pigs inoculated with brain antigen, not only afforded no protection against EAE, but 6-MP treated animals became paralyzed more quickly than did inoculated animals not given 6-MP.

The aim of the present investigation is to determine the effects of daily administration of a tolerated dose of 6-MP to guinea pigs inoculated with homologous brain plus Freund's adjuvant, particularly on time of appearance and severity of EAE; and to investigate, at

* This investigation was supported wholly by grant from Nat. Multiple Sclerosis Soc.

† Present address: Dept. of Physiology, Univ. of Pittsburgh, Pa.

frequent intervals, peripheral neutrophil and lymphocyte levels, including Cooke-Arneth counts of neutrophils.

Methods. Thirty-five male guinea pigs (English strain) were inoculated with one subcutaneous deposit, in the nuchal region, of guinea pig brain homogenized in saline and emulsified with Freund's complete adjuvant (Difco), with phenol added for bacteriostasis. Each inoculation (1.0 ml) contained 250 mg of brain. Eighteen of the inoculated animals were given 6-MP (0.6 mg/100 g/day, subcutaneously), beginning at the same time as the inoculation of antigen, and 17 of them received no 6-MP. Seventeen other animals were given 6-MP only. Total leukocyte counts and differential leukocyte counts of Wright-stained peripheral blood smears were made each 3-4 days, including Cooke-Arneth counts of neutrophils. A "left shift" in a count (indicative of entry of young neutrophils into the circulation) was considered to have occurred when the sum of categories I + II + ½ of III exceeded 60% of the total number of neutrophils counted ("Arneth Index").

The animals were weighed daily and examined for signs of EAE: paralysis of the hind legs, rapid continuing weight loss, fecal impaction and urinary incontinence. As the animals became paralyzed, terminal total and differential leukocyte counts were made and the animals were then sacrificed. The experiments were terminated on the 28th day. Microscopic examination of sections of brain and spinal cord were not deemed essential for our purpose, since it has been found(3) that, in guinea pigs inoculated with brain plus adjuvant, paralysis rarely occurs in the absence of lesions in the central nervous system.

Results. Fig. 1 and 2 summarize alterations occurring in peripheral neutrophil and lymphocyte levels in guinea pigs receiving 6-MP only, antigen and 6-MP and antigen only. The patterns of leukocyte levels were similar in the 6-MP and 6-MP plus antigen groups. With 6-MP only, a slight initial increase in neutrophils was accompanied by a left shift in the Arneth Index, suggesting an initial stimulation of neutrophil output. Smaller left shifts occurred later, during the maximum depression of neutrophils. In animals given both antigen and 6-MP, left shifts in the Arneth Index appeared during the maximum depression of neutrophils. Administration of antigen only resulted in a bimodal increase in neutrophils; left shifts in the Arneth Index occurred only during the second rise. A late (21 days) increase occurred in the lymphocytes, but it was not significantly higher than the initial level.

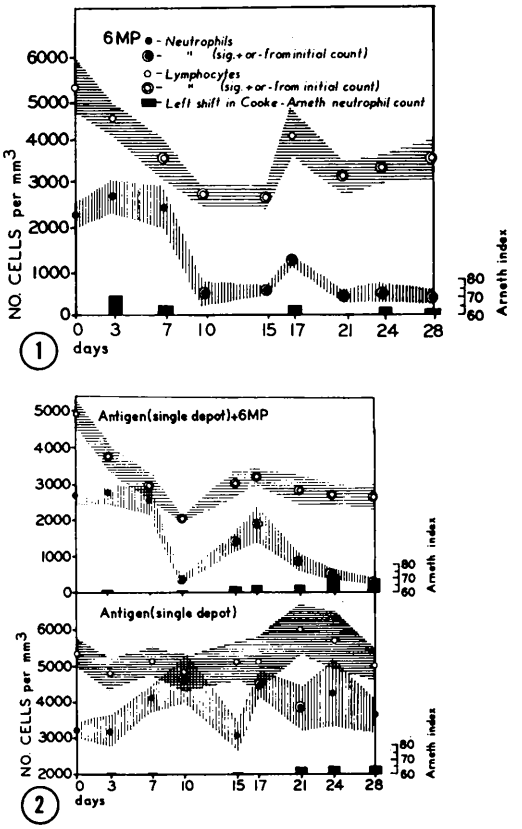

FIG. 1. Leukocyte patterns in guinea pigs receiving 6-MP only. Open circles represent lymphocytes; closed circles, neutrophils. Points with outer circles represent mean values significantly ($p = 0.10$ or less) greater or less than initial values. Hatched areas include ± 1 stand. error from mean. Black bars at bottom represent magnitude of Arneth Index.

FIG. 2. Leukocyte patterns in guinea pigs receiving antigen + 6-MP and antigen alone. Symbols as in Fig. 1.

Table I summarizes the incidence of terminal symptoms in both groups of guinea pigs. Equal numbers of animals in both groups were symptom-free; symptoms of urinary incontinence and rapid weight loss were nearly 3

TABLE I. Mean Incidence of Terminal Symptoms.

Treatment	No. animals	No. of animals affected: No symptoms	Paralysis	Urin. incont.	Fec. impac.	Wt loss
Antigen + 6-MP	18	1/18	6/18	3/18	3/18	3/18
" only	17	1/17	4/17	1/17	3/17	1/17

TABLE II. Mean Day of Onset of Symptoms.

Treatment	No. animals	Paralysis	Urin. incont.	Fec. impac.	Wt loss
Antigen + 6-MP	18	24.5	23.7	28.0	23.3
" only	17	30.5	30.0	27.3	27.5
Antigen + 6-MP *vs* antigen only (*p* values)		.35	.47	.78	.77

times more frequent in animals receiving both 6-MP and antigen than in those receiving antigen alone; frequency of paralysis and fecal impaction was about the same in both groups. Table II presents the mean days of onset of symptoms, together with a statistical analysis (*p* values) of the differences between the means of the 2 groups. Although animals receiving antigen and 6-MP showed slightly earlier onset of paralysis, urinary incontinence and weight loss, these differences were not statistically significant.

Summary. Guinea pigs injected with both antigen (homologous brain plus Freund's complete adjuvant) and 6-MP showed a slightly higher incidence of urinary incontinence and rapid weight loss but no definite difference in incidence of paralysis than did animals given only antigen; no change in mean day of onset of symptoms was evident. While antigen alone increased the number of circulating neutrophils (but not of lymphocytes), antigen and 6-MP together and 6-MP alone produced similar leukocyte patterns: delayed but marked neutropenia, and a marked rapid decline in lymphocytes. In animals given both 6-MP and antigen, a left shift in the Arneth Index of neutrophils occurred, in spite of the reduced level of circulating neutrophils.

1. Hoyer, L. W., Condie, R. M., Good, R. N., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 205.
2. Field, E. J., *Proc. Roy. Soc. Med.*, 1961, v54, 15.
3. Alvord, E. C., Jr., Kies, M. W., *J. Neuropathol. and Exp. Neurol.*, 1959, v18, 447.

Received March 19, 1962. P.S.E.B.M., 1962, v111.

Mechanism of Excretion of Urea by the Kidney in the Rabbit.* (27721)

D. R. DRURY, M. C. ALMEN AND K. W. BALLARD
Department of Physiology, School of Medicine, University of Southern California, Los Angeles

Urea, according to the traditional view, is excreted by the kidney as a result of glomerular filtration and partial passive reabsorption in the tubules. Schmidt-Nielsen(1) in a recent review cites observations which necessitate modification of this theory. One such observation is the steep concentration gradient of urea in the medulla of rats and other rodents, increasing from the cortical border up to the papilla. Gottschalk(2), following the theory of Wirz, Hargitay and Kuhn(3), proposes that sodium chloride is actively transported in the ascending limb of the loop and that urea also may be, although he considers it more likely that the urea gradient re-

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