case of histamine poisoning of mice seems to indicate that different mechanisms are involved. On the other hand, the synergism of histamine and "antihistaminic" substances cannot be explained by a possible histamine-like activity of these "antihistaminic substances." There is no such action in mice. We rather conclude that in the mouse, histamine affects organ systems other than those affected in histamine-sensitive animals, and that in these points of attack, antihistaminic substances are unable to compete with histamine. On the contrary, each poison acts on its own, both toxicities being additive in effect.

It seems quite inconsistent with the synergistic action of histamine and Pyribenzamine that the latter should have a certain protective action in mouse anaphylaxis. The very fact that Pyribenzamine in the mouse does not act as an antihistaminic agent, leads to the belief that this "protection" against mice anaphylaxis is not due to a specific antianaphylactic effect, comparable to that which Pyribenzamine exercises in anaphylaxis of guinea pigs or dogs. It is rather probable that some pharmacologic properties of Pyribenzamine other than its antihistaminic power are responsible for this protection. It may be that it acts here through its local anesthetic power, since it is known that general anesthetics, as well as local anesthetics, have a definite influence upon anaphylaxis (Wolfsohn[16]). The same problem has been discussed in a study concerning the influence of Pyribenzamine upon contact dermatitis in guinea pigs (Mayer[17]). Other experiments by Yonkman, Hays, Chess and Rennick[18] seem to indicate that the general pharmacological activity of an antihistaminic agent such as Pyribenzamine may well include other properties than those associated with antihistaminic power.

Further experiments are necessary to correlate allergy in mice with that of other animals and to identify the "anaphylactic poison" of mouse anaphylaxis, which quite probably is not histamine.

Conclusions. (1) "Antihistaminic substances" act as synergists in the histamine poisoning of the mouse; (2) contrary to the action on histamine poisoning, a certain protective power in mouse anaphylaxis has been observed; (3) the possible conclusions following from these observations are discussed.

16 Wolfsohn, G., *The Palestine and Near East Med. J.*, 1944, **3**, 11.

17 Mayer, R. L., *Ann. Allergy*, in press.

18 Yonkman, F. F., Hays, H. W., Chess, D., and Rennick, B., *J. Pharm. and Exp. Therap.*, in press.

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Concentration and Properties of the Adrenocorticotrophic Substance in Female Human Urine.

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Adrenocorticotrophin has been demonstrated to be present in the blood serum[1] and in the anterior pituitary gland. Its possible presence in the urine has been suggested by the effects of injecting urine from pregnant[2] and nonpregnant[3] women on the adrenal cortex of young guinea pigs. No attempt has been made to identify the substance which may have caused the adrenal hyperplasia. Since the corpus luteum hormone[3] and estrogens[4,5] can induce hyperplasia of the

1 Golla, M. L., and Reiss, M., *J. Endocrinology*, 1942, **3**, 5.

2 de Boissezon, P., *Bull. histol. appliq. physiol.*, 1936, **13**, 129.

3 Blumenthal, H. T., *J. Lab. Clin. Med.*, 1945, **30**, 428.

4 Janes, R. G., and Nelson, W. O., *Am. J. Physiol.*, 1942, **136**, 136.

5 Uotilla, U. Y., *Endocrinology*, 1940, **26**, 123.

TABLE I.
The Effect of Female Urine and Fractions Thereof on the Hypertrophy of Young Male Rat Adrenals.

Exp. No.	No. of rats	Starting wt of rats g	Material tested	Adrenal wt, mg	Mg adrenal per 100 g body wt
I	12	50.9	*Fresh whole female urine*—0.5 cc intraperitoneally, twice daily for 4 days	19.5	36.1
	5	49.6	*Dialysate of above urine*—Administered as above	19.8	35.6
	12	50.8	*Control*—isotonic saline as above	11.2	17.8
II	7	40.7	*Conc. whole urine* (2.5:1)—0.5 cc intraperitoneally, twice daily for 4 days	12.6	24.5
	7	40.7	*Acetone ppt. of dialyzed urine* (Prep. W-1)—6 mg daily, administered as above	14.6	29.3
	7	40.9	*Control*—isotonic saline (pH 7.7)	8.0	15.4
III	7	37.3	*Conc. whole urine*—0.5 cc intraperitoneally, twice daily for 4 days	10.8	27.1
	7	39.0	*Acetone ppt. of dialyzed urine* (Prep. W-2)—6 mg daily, administered as above	14.6	31.1
	6	38.3	*Control*—isotonic saline (pH 7.7)	8.4	17.3

adrenal cortex, the possibility that the cortical hyperplasia might be due to the sex hormones found in the urine has not been eliminated.

In this paper, we present evidence that the adrenocorticotrophic activity of human urine does not depend on the ovarian hormones and that it is probably due to a protein which may be the excretory form of pituitary adrenocorticotrophin.

Experimental. Freshly voided human female urine, dialyzed urine and precipitated urinary proteins were tested for adrenocorticotrophic activity in white rats by the methods of Moon[6] and Sayers, *et al.*[7,8] for pituitary adrenocorticotrophins.

Young male rats (23-30 days old) were injected intraperitoneally twice daily for 4 days, with 0.5 cc portions of urine which had been voided and filtered within the previous hour. The urine was obtained by pooling the output of 5 nonpregnant nonmenstruating young women. The control rats received isotonic saline solution at the same time. After the experimental period, the rats were sacrificed and the adrenals removed and weighed. The results of this experiment (I) are shown in Table I. The greater adrenal weight of the experimental rats over that of the controls indicates the presence of adrenocorticotrophic activity in the urine, confirming previous observations.[3]

A sample of urine was dialyzed for 24 hours at 0° to remove the greatest portion of the estrogens present. When the dialyzed urine was tested as in the above experiment, similar results were obtained (Table I, Exp. I). This experiment indicates that the urinary estrogens are not the causative agents for the adrenal hypertrophy.

In order to have a standard supply of urinary adrenocorticotrophin on hand, a concentrate of female urine was made in the following manner. Freshly voided urine was filtered and dialyzed with agitation against 20 volumes of distilled water at 0° for 24 hours. The dialysis was repeated with another 20 volumes of fresh distilled water. The dialyzed urine was then concentrated to approximately ⅕ volume by pervaporation under toluene. To the concentrated urine 5-6 volumes of acetone were added and the mixture kept in the cold room for 48 hours. The precipitate was centrifuged, washed twice with acetone and dried over P_2O_5. A pooled sample of 2.5 l. of urine yielded 505 mg of a light gray powder which gave a strong biuret test and contained 7.34% nitrogen (microkjeldahl, Prep. W-1). When another 1 liter sample of urine was put through this

[6] Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 649.

[7] Sayers, G. M., Liang, T. Y., and Long, C. N. H., *Endocrinology*, 1946, **38**, 1.

[8] Sayer, M., and Sayers, G., *Proc. Fed. Am. Soc. Exp. Biol.*, 1946, **5**, 200.

TABLE II.
Effect of Female Urine and Fractions Thereof on Adrenal Ascorbic Acid of Young Male Rats.

No. of rats	Rat wt, g	Material tested	Ascorbic acid per adrenal μg	Mg ascorbic acid per 100 g adrenal
5	40.2	Fresh whole urine—2.0 cc	16.9	277
5	44.6	Acetone ppt. of dialyzed urine (Prep. W-1); 6 mg in 2.0 cc isotonic saline-phosphate buffer (pH 8.0)	15.5	267
5	36.4	Prep. W-1 heated at 100° for 30 min; 6 mg in 2.0 cc isotonic saline-phosphate buffer (pH 8.0)	45.1	490
4	35.0	Crystalline egg albumin—6 mg in 2.0 cc isotonic-saline-phosphate buffer (pH 8.0)	32.6	473
14	39.5	Control—4 mg gelatin in 2.0 cc isotonic saline-phosphate buffer (pH 8.0)	32.6	501

procedure, a yield of only 52 mg of powder was obtained (Prep. W-2).

A solution of each of the above preparations in isotonic saline-0.01 M phosphate buffer (pH = 7.7; 4 mg per cc) was tested in the manner previously described. The results (Table I, Exp. II and III) show that both preparations have adrenocorticotrophic activity. These experiments are a further demonstration that the urinary steroid hormones do not cause the observed adrenal hypertrophy. Dialysis and the acetone treatment can be expected to remove all but traces of urinary steroids.

The method of Sayers, *et al.*[7,8] depends on the fact that pituitary adrenocorticotrophin will cause a rapid and profound decrease in the ascorbic acid level of the adrenals. The method was modified in the following manner to test for the presence of adrenocorticotrophin in urine. Young male rats were anesthetized with nembutal and 2.0 cc of the solution to be tested were injected intraperitoneally. After 1.5 hours, the animals were sacrificed, the adrenals removed and the ascorbic acid content determined. The ascorbic acid was measured by the method of Carruthers.[9] In our hands, this method was found satisfactory in that known amounts of ascorbic acid could be estimated with an error of less than 7%.

Fresh female urine and Prep. W-1, when tested in the above manner, showed a marked effect in depressing the adrenal ascorbic acid as compared to the controls (Table II). The control animals received 4 mg of gelatin in 2 cc of isotonic saline-phosphate buffer (pH = 8.0). The gelatin was used as a foreign-protein control. When a solution of Prep. W-1 was heated in a water-bath at 100° for 30 minutes, it no longer possessed any depressing effects on the adrenal ascorbic acid level. Since Prep. W-1 is also nondialyzable, this would seem to indicate that the active material is protein in nature. That nonspecific antigenic proteins are probably ineffective in this test was shown when egg-albumin (3X recrystallized) was assayed and found to have no effect on the adrenal ascorbic acid level.

A solution of Prep. W-1 was also tested on hypophysectomized rats. One of the adrenals of the hypophysectomized animals was removed and a solution of 6 mg of Prep. W-1 in isotonic saline-phosphate buffer was injected. After 1.5 hours the second adrenal was removed and the ascorbic acid level in each determined. A depression in adrenal ascorbic acid (145 mg/100 g adrenal tissue) was found. This indicates that the urinary adrenocorticotrophin acts in the same manner as the pituitary adrenocorticotrophins in that both decrease the ascorbic acid level of the adrenals markedly in a relatively short time. Further, the urinary adrenocorticotrophin acts without the mediation of the pituitary.

It can, therefore, be seen that the urinary and pituitary adrenocorticotrophins are qualitatively similar in their effects. This establishes the possibility that they are identical, or, at least, closely related, and that the urinary adrenocorticotrophin is an excretory form of the pituitary adrenocorticotrophin.

[9] Carruthers, A., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 826.

The determination of the presence of adrenocorticotrophin in male urine and the identification of the adrenocorticotrophic protein in female urine are now under investigation.

Summary. Normal female urine contains adrenocorticotrophic activity which has been shown not to be due to its estrogen content. Being nondialyzable and thermolabile, the active material appears to be a protein. It is similar to pituitary adrenocorticotrophin in its ability to produce adrenal weight increases and to depress the adrenal ascorbic acid level in normal and hypophysectomized male rats.

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Effect of Boric Acid on Avian Malaria.

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The recent paper by Hardcastle and Foster[1] reporting encouraging results with the use of borax in the control of cecal coccidiosis in poultry suggested the possibility that boron derivatives might be effective as chemotherapeutic agents in certain types of avian malaria.

Trophozoite-induced *Plasmodium gallinaceum* infections were established in S.C. White Leghorn chicks weighing 50 g by intravenous inoculation of 200,000,000 parasitized erythrocytes per kg; trophozoite-induced *P. cathemerium* infections were induced in Peking ducklings weighing 50 g by the intravenous injection of 500,000,000 parasitized erythrocytes per kg. Sporozoite-induced *P. gallinaceum* infections were used in prophylactic tests and were established in chicks of 50 g weight by injecting intravenously 0.2 cc per chick of a suspension of sporozoites prepared by grinding 100 infected mosquitoes in 20 cc of chicken plasma.

[1] Hardcastle, A. B., and Foster, A. O., *Proc. Helminthological Soc. of Washington*, 1944, **11**, 60.

TABLE I.
Effect of Boric Acid on Trophozoite and Sporozoite Induced Avian Malaria Infections.

No. birds	Drug	Dose	Average % erythrocytes parasitized at peak of infection
	I. Trophozoite-induced *P. cathemerium* infections in ducklings.		
4	Boric acid	2.0% in diet	1.3
4	" "	1.0 " " "	6.6
3	Quinine	80 mg/kg p.o.	5.6
3	"	40 " "	3.1
6	Controls	—	25.6
	II. Trophozoite-induced *P. gallinaceum* infections in chicks.		
5	Boric acid	2.0 % in diet	2.4
5	" "	1.0 " " "	31.9
3	Sulfadiazine	0.05 " " "	<0.1
3	"	0.025 " " "	0.5
6	Controls	—	66.1
	III. Sporozoite-induced *P. gallinaceum* infections in chicks.		
4	Boric acid	2.0% in diet	0.01*
4	" "	1.0 " " "	1.7
4	Sulfadiazine	0.1 " " "	0.00
4	Controls	—	30.0
4	Quinine	0.4 " " "	22.0
4	Atabrine	0.4 " " "	19.7
4	Sulfadiazine	0.2 " " "	0.00
8	Controls	—	17.6

* One chick died, two showed zero counts.