presented in Table IV was planned with this requirement in mind.

It is seen that, whatever the time of infection, more colonies were recovered from the kidneys, livers, and lungs of animals that had been pretreated with bovine serum and with pertussis vaccine than from the organs of animals that had received the vaccine alone.

These findings suggest that the severity of the infectious process had been increased, at least temporarily, by the mild allergic shock resulting from the presence of 1/2000 bovine serum in the diluent used for injecting the bacterial suspension.

Summary. 1) Female albino mice of two different breeds were treated intraperitoneally with a mixture of bovine serum and pertussis vaccine. In confirmation of findings of other investigators, it was found that a large percentage of the animals so sensitized died within 30-60 minutes following the intravenous injection of very small amounts of serum 2-4 weeks after sensitization. The sensitizing effect appeared specific as no deaths occurred when either rabbit serum or egg albumin was used for the challenge injection. 2) Mice pretreated by intraperitoneal injection of bovine serum and pertussis vaccine, or of pertussis vaccine alone, were infected intravenously several weeks later with staphylococci resuspended in 1/2000 bovine serum. When they were sacrificed 24 hours after infection, it was found that many more staphylococcal colonies could be recovered from the kidneys, liver and lungs of the animals that had been sensitized to bovine serum, than from the organs of animals of the control group.

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Effect of Aspirin and Reserpine on Adrenocortical Response to Piromen in Man. (22751)

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The elevated body temperature resulting from parenterally administered pyrogen in man is associated with a rise in the urinary output of 17-ketosteroids, corticosteroids, and an increase in concentration of free 17-hydroxycorticosteroids (17-OHCS) in plasma (1-3). Administration of aminopyrine has been shown to block the febrile response to typhoid-paratyphoid vaccine without altering the associated hemodynamic changes(4,5). Christy *et al.* have shown that aminopyrine also blocks the adrenocortical response to typhoid vaccine(6). Intravenously administered reserpine in dosages up to 1 mg/kg body weight failed to block the temperature elevation resulting from the injection of colon bacilli in rabbits(7). More recently Windle has noted a blocking effect of reserpine on fever production and eosinopenia following Piromen administration in monkeys (personal communication). Other studies in mice have reported that reserpine causes hypothermia (8).

The present study has been designed to elucidate the nature of the adrenocortical response to administration of a bacterial pyrogen, Piromen, and to observe the effects of aspirin and reserpine when given in conjunc-

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tion with Piromen. Specifically, test situations are aimed at clarifying the following points: 1. Is the increase in plasma free 17-OHCS concentration observed following Piromen due to increased adrenocortical secretion or decreased removal from the miscible pool? 2. Is hydrocortisone the adrenocortical secretion responsible for elevated plasma 17-OHCS levels following Piromen administration? 3. What is the effect of aspirin on the adrenocortical response to Piromen? 4. What is the effect of reserpine on the adrenocortical response to Piromen? 5. Is fever the cause of elevated 17-OHCS concentration resulting from the administration of Piromen?

Methods. Plasma free 17-OHCS were determined by the method of Silber and Porter (9), as modified by Peterson *et al.*(10). Hydrocortisone and corticosterone were determined specifically by isotope dilution(11,12). Eosinophil counts were made in a Speirs-Levy eosinophil counting slide. A. *Determination of Half-Life of Hydrocortisone.* Plasma half-life of hydrocortisone was determined in 4 young, normal subjects following intravenous administration of 0.3-0.4 μg Piromen/kg/body weight on one day and Piromen placebo on the other day. Three subjects received intravenous injections of 200 mg hydrocortisone sodium succinate and the 4th subject received 20-minute infusions of 200 mg free hydrocortisone in ethanol-water solution. Blood samples were withdrawn before administration of Piromen or Piromen placebo, immediately prior to hydrocortisone administration, and at appropriate intervals thereafter. Plasma 17-OHCS concentrations were determined for all specimens. B. *Determination of Miscible Pool and Turnover Rate of Hydrocortisone.* The miscible pool and turnover rate of hydrocortisone were determined on 1 normal, male subject, age 19, by the method of Peterson *et al.*(13). The study was carried out on each of 2 different days; 1 day the subject received 0.2 μg Piromen/kg body weight 2 hours prior to injection of hydrocortisone-4-C^{14}; on another day, he received Piromen placebo 2 hours before injection of hydrocortisone-4-C^{14}. C. *Effect of Aspirin and Reserpine on Plasma 17-OHCS Levels.* A total of 8 normal subjects, 4 males and 4 females, between 18 and 21 years of age, received in randomized order, saline infusion, Piromen, and Piromen plus aspirin. Observations made on days of saline infusions were used as control data. (The saline infusions do not alter normal diurnal fall in plasma 17-OHCS.) Three of the above subjects also received Piromen plus reserpine. Saline infusions were given from 8 to 10 A.M. at 3 ml/min. Piromen was administered intravenously (0.1 μg/kg body weight) at the beginning of each test (8 A.M.). Days on which the effect of Piromen, or Piromen plus reserpine, was determined, the subject was given 2 lactose capsules (identical in appearance to aspirin capsules) at 7 A.M., 9 A.M., 12 M., and 3 P.M. Days on which Piromen plus aspirin was tested, 2 capsules each containing 0.33 g of aspirin were given at same times. Subjects participating in the Piromen plus reserpine study were primed with oral reserpine in the following manner: first day, 0.25 mg reserpine q.i.d.; second day, 0.5 mg reserpine q.i.d.; third day, 0.75 mg reserpine q.i.d.; fourth and fifth days, 1.0 mg reserpine q.i.d. The test was carried out on the fifth day. The first blood sample was drawn immediately before administration of the test solution (8 A.M.). Blood was then withdrawn at hourly intervals until 12 M. and then at 2 P.M. and 4 P.M. Venous blood was obtained at 8 A.M. and 12 M. for eosinophil counts. Pulse and auscultatory blood pressure recordings were made hourly from 8 A.M. through 4 P.M. Oral temperatures were determined at 8 A.M., 10 A.M., 12 M., and 3 P.M. Fever is defined as an oral temperature above 37°C.

Results. Nature of Adrenocortical Secre-

TABLE I. Effect of Piromen on Concentration of Phenylhydrazine-Reacting Substances, Hydrocortisone and Corticosterone in Plasma.

		Phenylhydrazine method	Isotope dilution method
		μg/100 ml plasma	
Hydrocortisone	Control	15	15
	Piromen	24	25
Corticosterone	Control	—	1.8
	Piromen	—	3.0

TABLE II. Effect of Piromen on Hydrocortisone Half-Life.

Subject	Hydrocortisone preparation	Half-life (min.) Placebo	Piromen
DH	Sodium succinate	78	70
WG	*Idem*	113	110
WM	"	95	64
LS	Free	132	114
Avg		104	90

tion Following Piromen Administration. A comparison of the plasma concentration of 17-OHCS as determined by the phenylhydrazine method with the plasma concentration of hydrocortisone determined by the specific isotope dilution method shows that the concentration of 17-OHCS in the plasma during the control and the Piromen test is equal to the hydrocortisone concentration (Table I). Therefore, the Silber-Porter chromogens will be designated as hydrocortisone. By use of the isotope dilution procedure, it has also been shown that Piromen causes an increase in plasma corticosterone (Table I).

Miscible Pool and Turnover Rate of Hydrocortisone After Piromen. To ascertain whether the increase in plasma hydrocortisone is due primarily to decreased removal from the miscible pool or increased adrenocortical secretion, 2 tests were employed. First, the plasma half-life of hydrocortisone was determined approximately 3 hours following injection of Piromen or Piromen placebo. Table II indicates that the plasma half-life of hydrocortisone is not prolonged by prior administration of Piromen but rather is decreased by an average of 13%. Second, to determine more definitively if the increased plasma hydrocortisone concentration following Piromen administration was due to an increased secretion of hydrocortisone, the miscible pool and turnover rate of hydrocortisone were determined using hydrocortisone-4-C^{14}. Table III

TABLE III. Effect of Piromen on Miscible Pool and Turnover Rate of Hydrocortisone.

	μg % plasma F	Miscible pool, mg	Turnover rate, mg/hr
Control	14	1.5	.75
Piromen	35	7.6	4.70

presents the data in 1 normal subject on a control day and on a day he received Piromen. From this Table, it can be seen that the miscible pool was increased 5-fold and the rate of secretion of hydrocortisone was increased 6-fold.

Influence of Aspirin on Elevated Plasma Hydrocortisone Levels Observed With Piromen. Fig. 1 shows the mean hydrocortisone levels for 8 matched pairs receiving: 1) saline control infusion; 2) Piromen, and 3) Piromen plus aspirin. Intravenous injection of 0.1 μg Piromen/kg causes significant† increases in

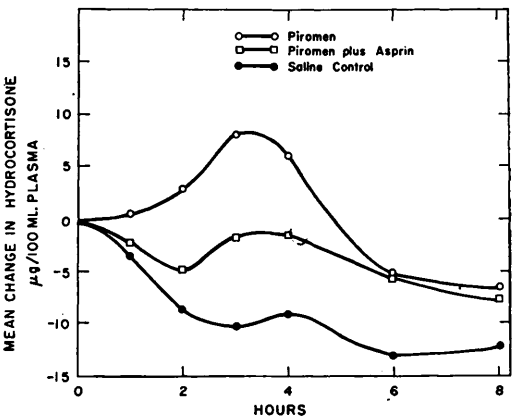

FIG. 1. Effect of Piromen, Piromen plus aspirin, and saline on plasma hydrocortisone levels. Individual points represent mean values for group of 8 normal subjects.

plasma hydrocortisone concentration for 8 hours following its administration. Hydrocortisone levels resulting from concomitant administration of Piromen and aspirin are significantly lower than those observed with Piromen alone at the 2nd and 3rd hours following the injection of Piromen. However, this dosage of aspirin is not sufficient to counteract completely the Piromen effect since there is a significant elevation of plasma hydrocortisone over saline control values at the 3rd through 6th hour of the test and probably a significant elevation at the 2nd and 8th hours ($P < 0.05$).

Influence of Reserpine on Elevated Plasma Hydrocortisone Levels Observed With Piro-

† The term "significant" is used to indicate statistical significance at the 1% level of confidence as determined by the t-test of Fisher.

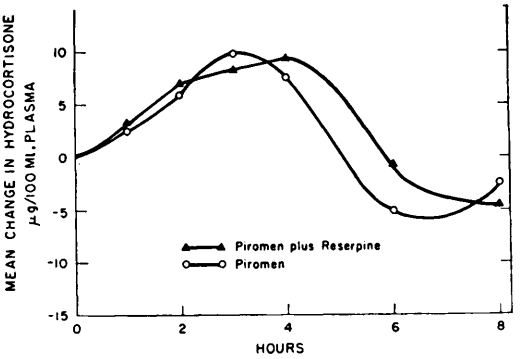

FIG. 2. Effect of Piromen and Piromen plus reserpine on plasma hydrocortisone levels. Individual points represent mean values for group of 3 normal subjects.

men. Fig. 2 shows the mean plasma hydrocortisone levels associated with Piromen and Piromen plus reserpine for 3 subjects. It is apparent that reserpine administered in this manner does not depress adrenocortical response to Piromen.

Observations on Temperature, Eosinophil Counts, Pulse, and Blood Pressure. Fig. 3 shows the temperature course during the different test procedures. Compared with the control observations, Piromen causes a significant elevation in temperature at 12 M. Aspirin administration causes a significant decrease in the temperature response to Piromen at 12 M. and 3 P.M. The 8 A.M. temperatures observed on 5 subjects reserpinized

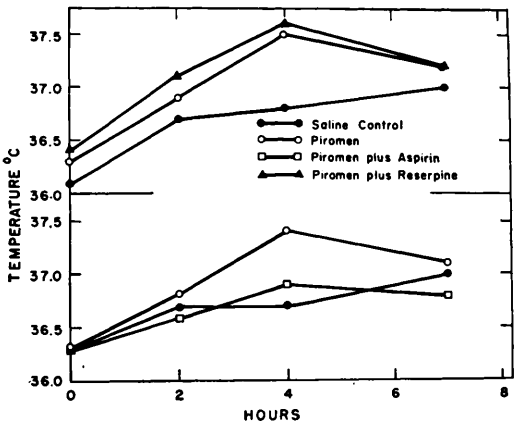

FIG. 3. Effect of saline, Piromen, Piromen plus aspirin, and Piromen plus reserpine on temperature. Upper graph represents mean values of 3 subjects. Lower graph represents mean values of 8 subjects.

in the manner described (the 3 subjects included in the adrenocortical evaluation and 2 additional) were not lower than the 8 A.M. temperatures on these same individuals on control and Piromen test days. Reserpine exerts no blocking effect on the temperature response to Piromen. The average percentage fall in the circulating eosinophils 4 hours after the beginning of the test was: saline, 39%; Piromen plus aspirin, 39%; Piromen, 58%; and Piromen plus reserpine, 57%. The 4-hour fall in the number of circulating eosinophils following Piromen is significantly greater than that following saline or Piromen plus aspirin. This dosage of Piromen causes a significant tachycardia from the 3rd through 7th hour of the test when compared with control values. Aspirin administration with the Piromen significantly lowers the pulse rates from the 4th through 8th hour. Reserpine causes bradycardia but the percentage increase in pulse rate following Piromen administration is not altered. No differences in the base-line blood pressure or blood pressure responses following Piromen or saline were noted between any experimental groups.

Relationship Between Body Temperature and Plasma Hydrocortisone Concentration. Analysis of individual relationship between plasma hydrocortisone concentrations and temperature rise 2 hours after Piromen administration indicates that only 1 of the 8 subjects revealed a temperature rise greater than 37°C. There is no significant difference in the mean 10 A.M. temperature between Piromen and control groups, while the plasma hydrocortisone levels are significantly higher with Piromen administration (mean difference is 12.9 μg%). Two hours after combined Piromen and aspirin administration, 2 of the 8 subjects showed a rise in plasma hydrocortisone (albeit less than they showed with Piromen alone) with a temperature less than 37°C. A 3rd subject manifested a temperature greater than 37°C, but there was no associated rise in the plasma hydrocortisone concentration.

Two hours after Piromen administration, 2 of the 3 subjects receiving reserpine manifested temperatures above 37°C, but all 3

subjects showed a rise in plasma hydrocortisone levels.

Comments. One of the observations of major interest in this study was the appearance of significantly increased adrenocortical secretory activity distinctly before the appearance of fever. Indeed, only one of the subjects receiving Piromen manifested an oral temperature above 37.6°C at the height of the temperature response. It is apparent that the effect of Piromen on adrenocortical activity is not dependent on fever production. It appears that the elevation of temperature and of plasma hydrocortisone levels are separate manifestations of the effect of Piromen. The inference of Christy *et al.*(6) that fever is the cause of increased plasma concentrations of 17-OHCS secondary to pyrogen administration is not supported by our observations.

The likelihood of salicylates in the dosages employed in this study causing a significant alteration in plasma 17-OHCS levels may be discounted on the basis of studies of Done *et al.*(14). These investigators were unable to show a significant rise or fall in plasma 17-OHCS levels associated with the administration of 1 gram of sodium salicylate administered hourly for 8 hours. Furthermore, Peterson and Bunim (unpublished observations) have shown that salicylates in a dose of 8 g/24 hrs exert no effect on the turnover rate of hydrocortisone in human subjects.

The difference in effect of reserpine on pyrogenic and eosinopenic effect of Piromen in monkeys observed by Windle (personal communication) and in our human subjects may be explainable by the dosages used. Windle employed a dose of 20 mg reserpine/kg body weight and a dose of 0.5-1 μg Piromen/kg body weight.

The studies on the plasma half-life of hydrocortisone and the miscible pool and turnover rate of hydrocortisone-4-C^{14} discount the possibility that factors other than increased adrenocortical secretion are responsible for the increased plasma 17-OHCS levels resulting from Piromen administration. It is apparent that the increased plasma hydrocortisone half-life which follows Piromen administration to anesthetized dogs(15) does not occur in unanesthetized humans.

Conclusions. 1. Intravenous administration of 0.1 μg Piromen/kg body weight to young, normal male and female subjects causes a significant rise in body temperature and plasma hydrocortisone levels. 2. Increased concentration of plasma free 17-OHCS following Piromen administration has been shown to be the result of increased secretion of hydrocortisone. 3. The rise in plasma hydrocortisone distinctly precedes the elevation of body temperature above 37°C. Fever is, therefore, not the cause of the elevated plasma hydrocortisone levels. 4. The increases in plasma hydrocortisone levels, eosinopenia, temperature, and pulse rate caused by Piromen administration are significantly lessened by the concomitant administration of aspirin. 5. Administration of reserpine in a dose of 4 mg/24-hr period caused no hypothermia in normal human subjects. 6. Pyrogenic, eosinopenic, and adrenocortical stimulatory effects of Piromen are not altered by the prior and concomitant administration of reserpine.

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Effect of Synthetic Lysine-Vasopressin on Plasma Hydrocortisone Levels in Man. (22752)

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Previous studies in this laboratory(1) have demonstrated that intravenous administration of highly purified arginine-vasopressin and highly purified lysine-vasopressin, as well as Pitressin, cause increased plasma hydrocortisone concentrations in man. Because of the possibility of ACTH-releasing or adrenotrophic substances contaminating these vasopressin preparations, a comparative study has been made of the effects of synthetic lysine-vasopressin and Pitressin on plasma hydrocortisone levels.

Methods. Five normal subjects, aged 18 to 21 years, were assessed for plasma hydrocortisone responses to intravenously administered Pitressin and synthetic lysine-vasopressin† in the following manner: At 8 a. m. on 2 different test days, each subject received intravenously 2 ml of vasopressin diluent (0.5% chlorobutanol solution adjusted to pH 3.6 with acetic acid). At 8:30 a. m., the subjects received intravenously on 1 test day 2 U. Pitressin and on the other test day, 2 U. of synthetic lysine-vasopressin. The order in which each subject received the 2 vasopressin preparations was randomized. Venous blood samples of 20 ml each were obtained through an indwelling Cournand needle prior to administration of the diluent (8 a. m.), 30 minutes following injection of diluent (*i.e.*, immediately preceding injection of the vasopressin preparation), and 30 minutes following the injection of vasopressin (9 a. m.). Plasma hydrocortisone concentrations were determined in duplicate on all samples by the method of Silber and Porter(2), as modified by Peterson *et al.*(3). Pulse rates and auscultatory blood pressures were determined at 1-minute intervals before, during, and after injection of Pitressin and synthetic lysine-vasopressin.

TABLE I. Effect of Pitressin and Synthetic Lysine-Vasopressin on Plasma Hydrocortisone Concentrations.

Time of blood withdrawal	Plasma hydrocortisone, μg/100 ml plasma	
	2 U. Pitressin	2 U. synthetic lysine-vasopressin
0′ (Pretest value)	16.6	18.0
30′ Following inj. of vasopressin diluent	14.3	16.6
30′ Following inj. of vasopressin preparation	25.7	25.6

Results. Table I shows the mean plasma hydrocortisone‡ concentrations for the 5 subjects on the Pitressin test day and on the synthetic lysine-vasopressin test day. There is no significant difference between the plasma levels elicited by 2 U. of Pitressin and 2 U. of synthetic lysine-vasopressin. The mean blood pressure response to both vasopressin preparations was the same (approx. 20 mm Hg increase) while the pulse rates were un-

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‡ It has been shown in other studies(4,5) that the Silber-Porter chromogens are equivalent in concentration to free hydrocortisone.