as judged by the formation of characteristic picrate crystals.

The carboxymethylcellulose used in these experiments was prepared from Solka-Floc BW according to the directions of Peterson and Sober(3) and contained approximately 0.75 milliequivalent carboxyl group per gram. The growth hormone was prepared from pig pituitaries as previously described(4), and the corticotropin preparation was purified by adsorption on oxidized cellulose as described by Astwood *et al.*(5).

It is of interest that carboxymethylcellulose adsorbed practically none of the corticotropin preparation from 2.0 M acetic acid but adsorbed 68 mg g of the growth hormone preparation. since it was previously noted that 10.4% carboxyl oxidized cellulose adsorbed. from 0.1 M acetic acid, 340 mg/g of the corticotropin and only 6 mg/g of growth hormone(4).

Summary. A chromatographic system of high resolving power was devised for the purification of growth hormone and was adapted for use in the separation of other peptides.

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Effect of Allergic Shock on Fate of Staphylococci in the Organs of Mice. (22750)

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As recently shown by several investigators (1,2,3) an anaphylactic type of sensitization can be elicited in mice by injecting intraperitoneally certain protein antigens in association with pertussis vaccine. The preliminary results here reported indicate that a mild allergic shock induced in animals so sensitized can modify their resistance to bacterial infections.

Experimental. Specific sensitization of mice to bovine serum. The ability of mice to develop an anaphylactic type of sensitization to bovine serum is illustrated in the following experiments with two different breeds of mice. Female mice of so-called Rockefeller Swiss strain were used when 7 weeks old; their average weight was 31 g. They were fed bread and milk throughout the experiment and sensitized as indicated in Table I. The bovine serum was a sterile filtrate distributed by Difco laboratories. The pertussis vaccine was a preparation distributed for human use by Lederle Laboratories and obtained through the kindness of Dr. Stanton M. Hardy. It contained approximately 60 billion bacterial cells ml. The animals were challenged in groups of 5 by intravenous injection of various amounts of serum. All deaths occurred within 30-60 minutes (Table I).

Irrespective of the amount of serum used for the challenge injection, there were no deaths among the mice pretreated with either physiological saline, or with pertussis vaccine alone (Table I). In contrast, many of the animals that had been pretreated with serum died shortly after the challenge injection, even when the challenge dose was smaller than 0.01 ml. There was evidence furthermore that the degree of sensitization had been increased by injecting pertussis vaccine in addition to bovine serum at the time of sensitization.

The following experiment carried out with another strain of mice provides evidence that

TABLE I. Effect of Pertussis Vaccine on Sensitization of Mice* to Bovine Serum.

Sensitizing treatment (i.p.)		Challenge† (i.v.)	Deaths‡
Serum	Pertussis vaccine	ml serum	(out of 5)
ml			
.5	.05	.1	5
		.01	5
		.003	2
		.001	3
.5	0	.1	3
		.01	2
		.003	1
0	.05	.1	0
		.01	0
Saline controls		.1	0
		.01	0

* Female Rockefeller Swiss mice, 7 wk old.
† Three wk after sensitization.
‡ All deaths occurred within 30-60 min.

the allergic state elicited by serum-pertussis mixtures is specific. Female mice of the CF_1 breed were obtained from Carworth Farms and maintained on a diet of bread and milk. Individual weights ranged from 20-28 g when they received the sensitizing pretreatment. The bovine serum and pertussis vaccine were the same preparations that had been used in the preceding experiment. Fourteen days or 19 days after pretreatment, the animals were challenged with various amounts of bovine serum, rabbit serum, or egg albumin, in a final volume of 0.2 ml (Table II).

The results presented in Table II show that following treatment with bovine serum and pertussis vaccine, CF_1 mice developed a degree of sensitization comparable to that developed by mice of the Rockefeller Swiss breed. With both breeds of mice, intravenous injection of an amount of serum as small as 0.001 ml administered approximately 3 weeks after sensitizing pretreatment was sufficient to cause acute anaphylactic-like death (within 30-60 minutes) of a certain percentage of animals. The specificity of the reaction is indicated by the fact that there were no deaths among animals pretreated with pertussis vaccine alone and challenged with bovine serum, or among animals that had been sensitized with the mixture of bovine serum and pertussis vaccine, but were challenged with rabbit serum or egg albumin.

Effect of allergic shock on fate of staphylococci in the organs of mice. It has been observed in preliminary tests, not reported here, that the susceptibility to tuberculosis of mice sensitized to bovine or rabbit serum can be increased by injecting the tubercle bacilli in association with small amounts of the type of serum used for sensitization. As pertussis vaccine increases the ability of mice to develop the allergic state, an experiment was carried out to determine whether the concentration of this adjuvant used at the time of sensitization with bovine serum would influence the response of animals infected with bacteria resuspended in serum.

Four groups of female mice, 5 weeks old, of the so-called Rockefeller Institute Swiss breed, were pretreated by intraperitoneal injection of 0.5 ml bovine serum, with or without admixture of different amounts of pertussis vaccine (Sharp and Dohme). The preparation of pertussis vaccine used in this experiment was an experimental batch (not packaged for human use) which had been kindly supplied by Dr. J. Munoz of Merck, Sharp and Dohme. It must be noted that this preparation of vaccine was much more concentrated (approximately 15 times) than

TABLE II. Specific Sensitization of Mice* to Bovine Serum.

Sensitizing treatment (i.p.)		Challenge inj. (i.v.)		
Serum (ml)	Pertussis vaccine (ml)	Time after sensitization (days)	Material inj. (ml)	Deaths† (out of 5)
.5	.05	14	.2 bovine ser.	5
			.1	5
			.05	3
			.02	4
			.005	4
			.001	0
0			.2	0
0			.1	0
.5	.05	19	.01 *Idem*	5
			.001	3
			.001	1
			.0005	0
			.2 rabbit ser.	0
			.01 " "	0
			.2 1% egg alb.	0
			.05 " "	0

* Female CF_1 mice, 7 wk old.
† All deaths occurred within 30-60 min.

TABLE III. Effect of Amount of Pertussis Vaccine Used as Adjuvant on Susceptibility of Mice to Staphylococcal Infection.

Sensitizing treatment,* pertussis vaccine (ml i.p.)	Challenge infection† (i.v.): 0.1 ml staph. culture + 0.1 ml 1/1000 bovine serum										
	Deaths within 4 hr	No.‡ of staph. colonies recovered from individual organs									
		Kidneys					Lungs				
.02	3/5	2	12				4	10			
.006	0/5	1	70	400	1100	3000	10	10	10	300	300
.0006	1/5	5	100	1700	5000		100	240	250	3000	
0	0/5	11	17	100	300	1200	30	30	30	290	500

* All animals received 0.5 ml bovine serum i.p.
† 27 days after sensitization.
‡ Figures to be multiplied by 1,000 for whole lung, and by 10,000 for whole kidney.

the one from Lederle Laboratories used in the 2 preceding experiments. Twenty-seven days later, all animals received a mixture containing 0.1 ml of culture of *Staphylococcus aureus* (Smith strain) and 0.1 ml of 1/1000 bovine serum in saline. The characteristics and behavior of this staphylococcal strain, *in vitro* and *in vivo*, have been reported earlier(4). The animals were sacrificed 24 hours after infection and the numbers of staphylococcal colonies that could be recovered from their organs were determined by technics which have been described elsewhere(5). The results are presented in Table III.

Three of the animals which had received the largest amount of pertussis vaccine along with the sensitizing dose of bovine serum died acutely upon receiving the 1 2000 serum dilution used as diluent for the infective dose 27 days after sensitization. It is probable that this finding can be explained by the fact that the degree of allergic sensitization to bovine serum was the highest in animals that had received the largest amount of vaccine.

There was no evidence that the allergic shock had affected the response to infection, as no striking differences could be detected between the numbers of staphylococcal colonies recovered from the organs of animals sensitized with bovine serum alone and from animals having received in addition .006 or .0006 ml pertussis vaccine. While this experiment was in progress, however, the results of other unrelated studies disclosed that vaccination of mice with pertussis vaccine had a marked and lasting protective effect against infection with staphylococci. Evidence of this protective effect has been presented in a recent publication(6). Suffice it to state here that mice vaccinated with an amount of pertussis vaccine as small as 0.0006 ml (Sharp and Dohme preparation) still exhibit several weeks later a much increased resistance to staphylococcal infection. In view of this fact, it became evident that the protective effect of pertussis vaccine alone would have to be controlled in any study of the influence exerted by allergic shock on susceptibility to infection of animals sensitized with the help of this material as adjuvant. The experiment

TABLE IV. Effect of Allergic Shock on Fate of Staphylococci in the Organs of Mice.

Sensitizing treatment,* bovine serum (ml i.p.)	Time (days) between sensitizing and challenge	Challenge infection (i.v.): 0.1 ml staph. culture + 0.1 ml 1/1000 bovine serum											
		Nos. of staph. colonies† recovered from individual organs											
		Kidney				Liver				Lungs			
.5	10	4	14	85		27	39	103		13	22	31	
0	"	.2	1	12		53	140	148		.2	2	?	
.5	18	8	55	160	1900	24	210	310	1100	3	6	17	210
0	"	.1	1	5	35	5	48	56	230	0	0	.4	2
.5	23	560	3200	3500		78	170	300		0	1	4	
0	"	25	80	750		12	17	47		.1	1	1	

* All animals received .05 ml pertussis vaccine (Lederle) i.p.
† To be multiplied by 10^3 for whole lungs, and by 10^4 for whole liver and kidney.

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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