barely within the sensitivity of those methods which have been used. If a more precise measurement were devised for plasma or serum chromogen levels less than 1.0 mg/100 ml the difference between the reported non-creatinine chromogen proportions might be reconciled.

The presence of non-creatinine chromogen would have a negligible influence upon the creatinine/inulin ratios obtained at high plasma creatinine concentrations. Work which has not been reported previously (because we could not offer a reasonable explanation), but which is based upon 92 animals, resulted in a creatinine/inulin clearance ratio of 1.26 ± .09 within the serum creatinine concentration range of 9.5 to 15.0 mg/100 ml.

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Received October 19, 1953. P.S.E.B.M., 1953, v84.

Effects of Cortisone, Ascorbic Acid and Piromen on Phagocytosis in Mice.*† (20712)

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In considering the role of host mechanisms in resistance to experimentally induced infection, attention was drawn to certain compounds which might augment phagocytic activity. In the experiments described, cortisone, ascorbic acid and Piromen administered parenterally in critical amounts have been shown to alter significantly functional activity of mouse phagocytes *in vivo*.

Methods. Animals. Adult albino (*Mus musculus*) mice of mixed sexes were used in all experiments. The weight range was 24 ± 4 g. *Thorium dioxide analysis.* The ThO_2 uptake by mouse spleens after intravenous injection of Thorotrast‡ was determined by the technic described by Gordon and Katsh(1). Details of technic and precision of the analytical procedure with mouse tissues have been described previously(2). *Peritoneal phagocytosis technic.* The intraperitoneal phagocytosis of *Micrococcus pyogenes* var. *aureus* (FDA 209 P) by phagocytic leukocytes was studied. The organism was grown in (Difco) brain heart infusion broth at 37°C for 18 hours. After 4 hours at refrigerator temperature the culture was diluted with broth to contain approximately 4 x 10^9 organisms/ml which gave a turbidity reading of 650 nephelos in a photonephelometer (Coleman, Model 7). Mice (6 for each determination) were injected intraperitoneally with 0.25 ml of this suspension and sacrificed after 2 hours. Several drops of the peritoneal exudate from each mouse were smeared on a slide and stained with Wright's stain. Exactly 200 phagocytic cells were examined on each slide making a total of 1200 cells for each determination. Activity of these cells was estimated by noting the number of cells containing bacteria (active) or no bacteria (nonactive)(3). In each category the values obtained for the 6 animals were added to give one series of numbers for each determination. Substances tested for effect on

* The work was carried out under research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Department of Health, Education and Welfare.

† Presented before Soc. Am. Bact., Aug. 10, 1953, San Francisco, Calif.

‡ Thorotrast is a stabilized colloidal solution of ThO_2 (24-26% by vol.), generously supplied by the Heyden Chemical Corp., New York.

activity of peritoneal phagocytes were injected subcutaneously 14 and 2 hours before injection of the bacteria. *Maintenance of Klebsiella pneumoniae type A.* The medium and procedure described by Hoogerheide(4,5) was employed. Colonies from freshly incubated plates were seeded into broth and incubated for 7 hours at 37°C. Plate counts of viable organisms were made in (Difco) tryptose phosphate agar. The mouse LD_{50} of *K. pneumoniae* was about 75 viable organisms. Frequent determinations did not reveal any significant change in virulence over an 11-month period. *Drugs.* Cortisone acetate§ was prepared for injection by trituration with a few drops of Tween 80, after which it was suspended in saline. The Piromen‖ preparation employed was a nonantigenic polysaccharide prepared from *Pseudomonas aerugenosa.* The ascorbic acid used was the chemically pure compound purchased from the Nutritional Biochemicals Co. *Analysis of data.* In experiments with ThO_2 the significance of difference between treated and control groups was estimated by calculating "t"(6). The significance of differences obtained in peritoneal phagocytosis experiments was determined by calculating "X^2" (6). This method was also employed in analysing the data obtained in the protection experiments.

Results. Results of the experiment to determine the effect of administration of different amounts of cortisone, ascorbic acid and Piromen on the splenic uptake of ThO_2 by mice are shown in Table I. Cortisone, in the highest dose used (0.1 mg twice daily), caused an increase in ThO_2 uptake which was barely below the .05 level of significance. The uptake in cortisone treated groups was in every case greater than in the control group, and the uptake increased with administration of increasing amounts of cortisone.

Results with Piromen indicate a critical dose-response relationship for uptake of ThO_2; of the 4 doses of this agent used, ranging from 0.01 μg to 10 μg per injection, only the 0.1 μg dose yielded a significant effect on uptake. By contrast, it is seen in Table I that ascorbic acid in doses of 1 mg and 0.1 mg every 12 hours did not significantly affect the splenic uptake of ThO_2.

§ Generously supplied by Merck and Co.

‖ Generously supplied by Travenol Laboratories, Morton Grove, Ill.

TABLE I. Effect of Cortisone, Ascorbic Acid, and Piromen on Splenic Uptake of ThO_2 in Mice.

Treatment and dose/12 hr		mg ThO_2/ spleen	Comparisons to control P
Saline		3.63±.157*	—
Cortisone	.1 mg	4.04±.078	.05+
	.01	3.78±.076	>.1
	.001	3.66±.212	>.1
Ascorbic acid	1	3.29±.225	>.1
	.1	3.36±.190	>.1
Piromen	10 μg	3.76±.664	>.1
	1	3.79±.386	>.1
	.1	4.19±.108	*.05*
	.01	3.30±.366	>.1

* Mean and stand. error.

TABLE II. Effect of Cortisone, Ascorbic Acid and Piromen on Phagocytic Activity of Leukocytes in Peritoneal Cavities of Mice.

Treatment and dose/12 hr		No. of phagocytes Active	Non-active	Comparisons to control P
Saline		256	944	—
Cortisone	.1 mg	261	939	>.1
	1	440	760	<*.001*
Ascorbic acid	1	299	901	<*.05*
Piromen	.1 μg	320	880	<*.01*
	1	239	961	>.1

Table II shows the effect of cortisone, ascorbic acid and Piromen on the phagocytosis of *M. aureus* in the peritoneal cavities of mice. The probability (P) values are based upon the relative numbers of phagocytes in the active or non-active categories in the test groups as compared to the control group. Cortisone in a dose of 1 mg every 12 hours caused a highly significant increase in the number of active phagocytes. Piromen (0.1 μg) also produced a significant increase in phagocytosis, while the higher doses of this agent (1 and 10 μg) were without significant effect. The effective dose of Piromen in this experiment was the same as that which augmented splenic macrophage activity further suggesting a critical dose-response relationship for the action of this agent upon phagocytes. Ascorbic acid (1 mg) caused a slight, but significant increase in phagocytic activity.

Table III summarizes results in a number

TABLE III. Effect of Cortisone and Piromen on Experimental *Klebsiella pneumoniae* Infections in Mice.

Challenge dose and antibiotic treatment	Treatment and dose/12 hr	Mortality (5 days)	Comparisons to control P
5 LD_{50}	Saline	24/40*	—
	Piromen .1 μg	23/40	>.5
	Cortisone .1 mg	34/40	>.5
50 LD_{50}; 1 mg chloramphenicol	Saline	29/40	—
	Piromen .1 μg	28/40	>.5
	Cortisone .1 mg	31/40	>.5
	Piromen .1 μg + Cortisone .1 mg	34/40	>.1
10000 LD_{50}; 100 μg aureomycin	Saline	12/40	—
	Piromen .1 μg	9/39	>.5
	Cortisone .1 mg	8/40	>.1

* Dead/total animals.

of experiments carried out to determine the value in experimental infection of the critical amounts of these agents which apparently potentiated phagocytic activity *in vivo*. In the first experiment groups of mice received subcutaneous injections of these agents 6 hours before intraperitoneal challenge with 5 LD_{50} of *K. pneumoniae* and thereafter at 12-hour intervals throughout the 126 hour observation period. Neither Piromen nor cortisone decreased the mortality of the infected animals.

In the succeeding experiment a modified design was employed; the phagocytosis potentiating agents were tested singly and in combination in mice challenged with 50 LD_{50} of *K. pneumoniae* and treated intraperitoneally one hour later with 1 mg of chloramphenicol. Neither cortisone, Piromen nor the combination of the two drugs produced a significant change in mortality. A third experiment involved the use of chlortetracycline (Aureomycin) in a similar experimental design. Aureomycin could be used in a smaller subcurative concentration than chloramphenicol because of its greater effect on the mouse infection. It is seen that no increased protection was obtained.

In each of the challenge-treated experiments described, the cumulative survival time of the mice in each of the test groups was compared to the control group. In no case was any significant difference noted.

Discussion. A number of workers(7,3,8) have directed their attention to the specific problem of finding agents which augment phagocytic function. Since no term has been proposed for such agents, it is suggested that the word *auxophagocytic* (Gr. *auxo* = to increase) be employed to indicate agents which specifically augment phagocytic activity by their direct or indirect action on phagocytes. The antonymic structure would be *meiophagocytic* and would indicate agents which depress phagocytic action.

It appears from the results presented that in certain dose ranges cortisone, ascorbic acid and Piromen may be auxophagocytic in mice. The agents varied in effect not only with dose but also with site of action, *e.g.*, ascorbic acid did not significantly affect ThO_2 uptake by splenic macrophages but augmented peritoneal phagocytosis. The auxophagocytic action of cortisone may help to explain some of the seemingly contradictory reports concerning the efficacy of cortisone in treating microbial infections.

Ascorbic acid was observed to cause a slight increase in the phagocytic activity of leukocytes. Nungester and Ames(9) showed that the leukocytes from scorbutic guinea pigs were much less active than those from normal animals. Merchant(10) noted a direct correlation between the phagocytic capacity of leukocytes suspended in serum and the concentration of ascorbic acid in the serum until the normal ascorbic acid concentration was reached. The effect of ascorbic acid noted in the present investigation was small and would not appear as a significant increase unless a large number of leukocytes were observed.

Piromen has been shown here to increase the phagocytic activity of both fixed macrophages and leukocytes. While no beneficial effect was noted in mice infected with *K. pneumoniae* the ability of this drug to increase phagocytic activity *in vivo* suggests that it should be evaluated under a variety of experimental conditions.

Summary. Cortisone, ascorbic acid and Piromen were tested in mice for their effect upon macrophage activity as determined from the splenic uptake of colloidal ThO_2 and upon the phagocytic activity of leukocytes in the peritoneal cavity against *Micrococcus aureus*. Cortisone in high doses (0.1 to 1 mg every 12 hours) significantly enhanced phagocytic activity of both macrophages and leukocytes. Ascorbic acid in doses as high as 1 mg every 12 hours had no effect on the activity of macrophages but this dose did significantly enhance the phagocytic ability of leukocytes in the peritoneal cavity. Piromen (0.1 μg every 12 hours) significantly increased the phagocytic activity of both types of cells. However, doses above or below this value were without significant effect in either type of experiment. The more potent auxophagocytic agents (cortisone and Piromen) were without salutary effect when tested singly and in combination in protection experiments in mice challenged with predetermined doses of *Klebsiella pneumoniae*.

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Received October 19, 1953. P.S.E.B.M., 1953, v84.

Differentiation of Streptomycin and Dihydrostreptomycin Inhibition, by Means of Reversing Metabolites.* (20713)

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The close chemical relation between streptomycin (SM) and dihydrostreptomycin (DSM) together with the close resemblance of their antimicrobial spectra with respect both to wild type organisms and to strains developed for resistance to each respectively, has led to the widespread current belief that these antibiotics inhibit bacteria by identical mechanisms. The activity of DSM has even been postulated to result from its oxidation to SM by the bacteria(1).

SM and DSM have been extremely difficult to distinguish microbiologically, if indeed it has, with assurance, been possible at all, and frequently the two have been regarded as identical in antimicrobial growth effects(2-6) and in enzymatic effects(7), as well as in dependency experiments(8). Also SM and DSM appear to be absorbed and excreted by the mouse in the same way(6).

On the other hand, there is some evidence suggesting that SM and DSM may not be identical in their antibacterial action. This evidence rests principally on small differences in relative amounts of the two antibiotics required for inhibition of a number of different organisms tested in parallel(6,9). Of interest in this connection is the fact that the neurotoxic threshold dose (especially for the eighth nerve) of DSM in animals is distinctly less than for SM(10).

* This work supported in part by funds from Atomic Energy Commission; Office of Naval Research; American Cancer Society recommended by the Committee on Growth of the National Research Council.