the concept that only a neurogenic component is modified(5). Whether the depressor action of Raudixin would be of greater benefit if the drug were continued for longer periods, or whether it will modify the natural history of the disease, cannot be stated from the present data. The appearance of retinopathy in one patient while under treatment is noteworthy.

The early and temporary urinary sodium retention, observed in 2 hospital patients with nephrosclerosis, was greater than could be explained by chance fluctuation in subjects on a constant fluid and electrolyte regimen; the transitory decrease in the 2 others may be of no significance. The relationship of *Rauwolfia serpentina* to sodium metabolism or the possibility that it may exert temporary effects on renal function will require further elucidation.

Raudixin seems to be a relatively safe drug and may be of value in the treatment of ambulatory hypertensive patients, particularly those who are tense, hyperkinetic and without complications. Its influence on the course of hypertensive vascular disease remains to be established.

Summary. 1. *Rauwolfia serpentina* (Raudixin Squibb) was administered for periods of 2 weeks or more to 10 ambulatory patients with uncomplicated hypertensive vascular disease, 10 bed patients with the accelerated phase of this disorder, and to 4 hospital patients in varying stages of the disease, the last group being studied in detail while on a constant fluid and dietary regimen. 2. Significant depressor effects and bradycardia were observed in the majority of the ambulatory patients; some degree of relaxation was noted in those who were emotionally tense; but little or no effects were evident in those with the accelerated phase or those under hospital supervision. 3. Temporary sodium retention was apparent in 2 hospital patients with nephrosclerosis, but otherwise Raudixin appeared to have no direct hemodynamic, circulatory or metabolic action.

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Phagocytosis Influenced by Growth Media, Filter Paper and *para*-Hydroxybenzoic Acid. (21131)

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Earlier studies(1) demonstrated greater phagocytosis of *Escherichia coli* grown in beef infusion medium than when grown in tryptose medium. Phagocytosis of killed *E. coli* was increased also by previous treatment with filter paper or *para*-hydroxybenzoic acid(2). The latter compound, an active principle in filter paper, was reported by Davis(3,4) as a new bacterial vitamin and antagonist for *para*-aminobenzoic acid. These observations have been extended to *Brucella abortus*.

Materials and methods. Culture media. *Brucella abortus* strain 19 was kept routinely on Bacto Tryptose agar enriched as noted below, and inoculations were made from 24-hour cultures into the media to be tested. In the initial studies on phagocytosis of *Brucella* grown in beef and tryptose broth, the stock inoculum was carried in tryptose broth and transferred every 24 hours. When daily transfers of the stock culture were made in this broth medium, phagocytosis of both beef and tryptose grown bacteria by neutrophiles from normal guinea pigs under standardized

conditions gradually increased from the levels of about 55% usually seen to 94% levels in 2 weeks. Exposure of these animals to *Brucella* was excluded as a cause when blood samples from another group of guinea pigs raised and maintained in a laboratory free from possible contact with *Brucella* showed similarly elevated phagocytic activity. Phagocytosis of a standardized killed suspension of *Brucella*(5) was at normally low levels in the blood of the same animals; thus it appeared that a change in the microorganism was responsible for the above result. Plating the broth cultures of *Brucella* revealed 20% of rough mutant colonies, which were apparently more susceptible to phagocytosis. This change in the microorganism was prevented in subsequent experiments by inoculating the media to be tested from 24-hour stock cultures grown on tryptose agar. A comparison of phagocytosis of *B. abortus* was made between microorganisms grown in Bacto Tryptose broth or agar enriched with 10 g dextrose, 10 mg $FeSO_4$, and 0.1 mg thiamine hydrochloride per l, and those grown in Bacto Beef infusion broth or agar containing 20 g peptone and 5 g NaCl per l. To augment growth, the broth media were used in shallow layers of 25 ml in 250 ml Erlenmeyer flasks. Similar growth augmentation could be obtained in wide tubes agitated during incubation. The bacteria were grown for 20-24 hours at 37°C, harvested by centrifugation or by taking up in saline, washed in 0.9% saline, and resuspended in saline to a concentration of 1 x 10^{10} per ml. The density of the suspensions was measured turbidimetrically with a Coleman Model 11 spectrophotometer, and checked by the dilution plate count method, using the "drop plate" technic of Reed and Reed(6), and Pomales-Lebron and Fernandez(7). For the experiments with filter paper and *para*-hydroxybenzoic acid, the bacteria were grown for 24 hours at 37°C on tryptose agar enriched as noted above, and concentrated to 1 x 10^{10} per ml. The bacteria were killed by treatment with 1% CH_2O for 24 hours at room temperature, washed 3 times in saline, brought to the same concentration, and sterility was checked by plating.

Filter paper experiments. Strips measuring 1 x 4 or 1 x 5 cm of Whatman No. 1 or No. 42 filter paper were rolled and placed in the bottom of 12 ml tapered centrifuge tubes. Dead bacteria contained in 0.5 to 0.8 ml of suspension were pipetted into these tubes and allowed to remain in contact for one to 2 hours, after which the filter paper was squeezed out with glass rods or pipettes and removed. In previous experiments(2), chamber counts showed no significant loss of bacteria after this treatment. *Para-hydroxybenzoic acid experiments.* Killed bacterial suspensions were centrifuged, the saline removed, and the sedimented dead bacteria were suspended in aqueous solutions of Eastman C.P. *para*-hydroxybenzoic acid containing from 0.01 to 1.0 mg per ml. The volumes of suspending solutions were 1, 2, and 6 ml in different experiments, but the concentration of microorganisms exposed to *para*-hydroxybenzoic acid was maintained at 3 x 10^9 per ml. After 1-2 hours in contact, the bacteria were centrifuged, and resuspended in saline to a concentration of 1 x 10^{10} per ml. Controls for the filter paper and *para*-hydroxybenzoic acid-treated experiments were saline-washed microorganisms.

Phagocytosis. The bacterial suspensions contained 1 x 10^9 live or killed *Brucellae* in 0.1 ml of saline. In the growth medium experiments, heparinized guinea pig blood was used. In the experiments with filter paper and *para*-hydroxybenzoic acid, dog blood cells were used after 6 washes in Krebs-gelatin solution(5) and suspension in an equal volume of Krebs-gelatin. Equal volumes of blood cell and *Brucella* suspensions were mixed and rotated for 30 minutes at 37°C. Smears of these rotated mixtures were prepared, stained with Giemsa-Jenner, and the percentage of 50 neutrophiles containing *Brucella* was tallied.

Results. Effect of growth media. In 19 observations, the mean percentage and standard error of neutrophiles phagocytosing live *B. abortus* was 58.7 $\pm$ 4.8 when the organism was grown in tryptose broth, and 53.1 $\pm$ 4.1 when the organism was grown in beef infusion broth. The mean percentage difference and its standard error, 5.6 $\pm$ 6.30, was not statistically significant. In 26 observations, the mean percentage and standard error of neu-

trophiles phagocytosing live *B. abortus* was 52.0 ± 4.8 when the organism was grown on beef infusion agar, and 67.8 ± 3.6 when the organism was grown on tryptose agar. The mean percentage difference and its standard error, 15.8 ± 5.98, was statistically significant. The susceptibility of the bacteria to phagocytosis was the same when grown in tryptose broth, beef infusion broth, or beef infusion agar, but was increased when they were grown on tryptose agar.

Effect of filter paper contact. In 66 observations, previous contact with filter paper resulted in a mean percentage and standard error of 33.3 ± 1.1 of neutrophiles phagocytosing killed *B. abortus,* compared to 22.6 ± 1.2 for saline-treated controls. The mean percentage difference and its standard error, 10.7 ± 1.57, was statistically significant. Whatman No. 1 and No. 42 papers, compared in 33 experiments, had equivalent stimulatory effects.

Effect of contact with para-hydroxybenzoic acid. Previous contact with *para*-hydroxybenzoic acid in concentrations of 0.01, 0.1, and 1.0 mg per ml, in 99 observations showed a mean percentage and standard error of 34.3 ± 0.9 of neutrophiles phagocytosing killed *B. abortus,* compared to 22.6 ± 1.2 for the saline-treated controls. The mean percentage difference and its standard error, 11.7 ± 1.47, was statistically significant.

The stimulatory effect of *para*-hydroxybenzoic acid was greater with increasing concentration. In 33 observations, the mean percentage and standard error of neutrophiles phagocytosing killed *B. abortus* after contact with 1.0 mg per ml of *para*-hydroxybenzoic acid was 37.9 ± 1.6, with 0.1 mg per ml was 34.2 ± 1.3, with 0.01 mg per ml was 30.9 ± 1.4, and after contact with saline was 22.6 ± 1.2. The per cent of neutrophiles phagocytosing bacteria could be plotted as a linear function of the logarithm of the concentration of *para*-hydroxybenzoic acid employed.

Discussion. *B. abortus* 19 was sensitized for phagocytosis by growth on tryptose agar medium, but not by growth on beef infusion agar, tryptose broth, or beef infusion broth. These results differed from those observed with *E. coli*(1), which were phagocytosed more readily when grown in beef broth or agar than when grown in tryptose broth or agar.

Stained smears and turbidity measurements of live suspensions indicated that *Brucellae* grown on tryptose agar were slightly smaller than those grown on beef infusion agar. On the basis of Fenn's observations that large particles were phagocytosed more readily than small ones(8), the greater degree of phagocytosis of *Brucella* grown on tryptose agar could not be attributed to size. This discrepancy between size and phagocytosis was also noted in the case of *E. coli*(1), where the smaller bacteria grown in beef media were more readily phagocytosed than the larger cells grown in tryptose media.

Thus, the *in vitro* phagocytosis of *Brucella abortus* as well as *E. coli* could be influenced by the medium in which it was cultivated, and the different effects of media with different microorganisms emphasize the importance of control of growth media in the study of these phenomena.

The stimulation of phagocytosis of killed *E. coli* suspensions by filter paper and by *para*-hydroxybenzoic acid reported previously (2) was observed also with *B. abortus* strain 19. This was a direct effect upon the bacteria, since these materials could not have affected the leukocytes under the conditions of the experiments.

Summary. 1. The medium in which *B. abortus* strain 19 was grown influenced the phagocytosis of this microorganism. *Brucellae* grown on tryptose agar were phagocytosed more readily than those grown on beef agar, or in tryptose or beef broth. The effects of the media were different with *Brucella* and *E. coli.* 2. Phagocytosis of formalin-killed *B. abortus* 19 was promoted by previous contact with filter paper or *para*-hydroxybenzoic acid. The latter was effective in concentrations of 1.0 to 0.01 mg per ml, and the stimulatory effect was related in linear fashion to the logarithm of the concentration of *para*-hydroxybenzoic acid. These effects of filter paper and *para*-hydroxybenzoic acid with killed microorganisms were similar to those observed with *E. coli.*

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Effect of Sodium Thiosulfate Upon Mustard-Virus Interaction.* (21132)

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It was previously reported that exposure of influenza virus to appropriate concentrations of mustard resulted in selective inactivation of certain viral attributes such as infectivity and toxicity(1). In analyzing the early stages of destruction of viral toxins by mustard, it was necessary to circumvent the inherent toxicity of mustard itself. The use of sodium thiosulfate in this connection resulted in a rapid detoxification of mustard by thiosulfate(2). This observation along with previous reports of mustard-thiosulfate interaction(3) suggested a possible influence of thiosulfate upon inactivation of virus by mustard. This paper reports the results of such studies.

Materials and methods. Virus. Influenza A (PR8) virus was prepared as described(2). Harvested virus was stored in ampoules in a dry-ice cabinet. When needed, an ampoule of virus was placed in a 37°C water bath for 30 minutes. Following this, the viral suspension was centrifuged 15 minutes at 3500 rpm; the supernatant fluid was removed, allowed to attain room temperature and then used as the source of virus. *Mustard.* The chloroethylamine derivative used in these investigations was cyclohexyl [bis(β-chloroethyl)] amine hydrochloride, kindly supplied by Dr. H. B. Woodruff of Merck and Co., Inc. The chemical solution was prepared in triple-distilled water to yield for the 0.01 M solution of mustard a final pH of 2.6. *Thiosulfate.* Freshly prepared solutions of sodium thiosulfate in distilled water were used.

Exposure of virus to mustard. This was carried out as previously described(2) except that the reaction temperature in these experiments was 25°C. In testing for the effect of thiosulfate upon these mustard-virus systems, sufficient 3 M sodium thiosulfate was added to the samples (before addition of mustard) to yield a final thiosulfate concentration of 0.075 M. *Test of viral toxicity.* Serial 2-fold dilutions of mustard-treated virus were made in normal allantoic fluid; these were tested for residual viral toxicity by injecting 1.0 ml of dilution intravenously into a mouse, 6 mice being used for each dilution. The animals employed in the tests were Swiss mice (Webster strain) 12 to 15 g weight and of random sex. All animals were observed daily for a period of 7 days, and deaths occurring 24 hours or later after intravenous injection were considered to be of viral origin. *Infectivity test.* This was carried out as described previously(2).

Results. Effect of thiosulfate upon destruction of viral toxicity and infectivity by mustard. The effect of sodium thiosulfate upon mustard-virus interaction is shown in Table I. The results presented in this table are representative of a series of similar experiments.

In terms of mustard inactivation of viral toxicity, it is apparent that there was less destruction of viral toxin in the presence of sodium thiosulfate. The greater residual toxicity of virus exposed to mustard in the pres-

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