TABLE I. Effect of Fumagillin Administered Orally on *Endamoeba histolytica* Occurring Naturally in Macaques.

Macacus rhesus or *M. philippinensis*	Dose of fumagillin, mg/kg	Duration of treatment, days	Recurrence of *E. histolytica* in stools after treatment
2	50	5	No recurrence after 14 wks
3	50	5	" " 14 "
6	50	5	" " 4 "
			Animal died of TB.
8	100	5	No recurrence after 14 wks
9	100	5	" " 14 "
S	100	5	Recurrence in 12 wks
M	100	5	" 10 "
11	100	5	" 10 "
R	100	5	" 4 "
R_1	125	5	No recurrence after 8 wks
S_1	125	5	" " 8 "
M_1	125	5	" " 8 "
10	125	5	" " 8 "
11_1	125	5	" " 8 "

following therapy; however, during this post-treatment period there was no recurrence of *E. histolytica* in the stools. Monkeys R_1, S_1, M_1, 10 and 11_1 showed no recurrence of *E. histolytica* at the end of 8 weeks.

The effect of fumagillin on non-pathogenic amebas could not be evaluated since only 3 monkeys had such amebas in their stools before the treatment for *E. histolytica* was instituted. However, it was found that 2 of these monkeys were cleared of *Endamoeba coli* during a 14 weeks' follow-up examination period. No toxic manifestations were observed in any of the monkeys treated with the antibiotic. The amount of hemoglobin and the number of erythrocytes and leucocytes remained within normal limits. Bromsulfalein tests indicated a possibility that a dose of 125 mg/kg might cause liver damage. In monkey S there was considerable retention of the dye. This animal has periodic bromsulfalein tests at the present time. Blood urea nitrogen showed an increase in only one monkey; this animal had received 100 mg of fumagillin per kilo of body weight. Electrocardiograms of all the animals were normal following therapy.

Summary. 1. A new antibiotic, fumagillin ("Antibiotic H-3"), was found to be effective *in vitro* against *E. histolytica* at concentrations of 1:10,000,000 to 1:15,000,000. 2. Preliminary *in vivo* studies on monkeys naturally infected with *E. histolytica* indicated that fumagillin possesses considerable amebacidal activity. Further investigations are necessary to evaluate this drug more completely.

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Effect of Sodium Bisulfite and Rutin on Action of Epinephrine. The "Bisulfite Phenomenon" in Man.* (18713)

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Richards(1) reported that sodium bisulfite increased the toxicity of epinephrine if these two compounds were injected subcutaneously or intramuscularly at the same site, but when given intravenously or injected at different sites no increase in toxicity, as measured by lethal doses, was noted. A review of the literature(2) as well as Richards' own studies showed that sodium bisulfite itself is relatively non-toxic. The U.S.P. permits the use of sodium bisulfite in concentrations up to 0.5% as an antioxidant in commercial epinephrine solutions, although not more than 0.2%

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1. Richards, R. K., *J. Pharm. and Exp. Therap.*, 1943, v79, 111.

2. Heffter, A., *Handbuch der experimentellen Pharmakologie*, 3: 1. Berlin, 1927.

is generally used in the 1:1,000 epinephrine hydrochloride solutions. Richards found that not only does sodium bisulfite enhance the subcutaneous and intramuscular toxicities of epinephrine hydrochloride solutions in rats, mice, rabbits and dogs but that non-toxic doses increase the epinephrine effects on the blood sugar curve of dogs and also have an effect on the action of other drugs (neosynephrin(3) and procaine)(4). To show that sodium bisulfite is specific, Richards(1) also tried other drugs and other antioxidants (HCl, ascorbic acid, hypophosphorous acid, sodium hypophosphite, sodium acid phosphate) and these did not influence the resorptive toxicity of the epinephrine solution. As all the previous work has been done on animals we sought to determine if this "bisulfite phenomenon", which presumably acts by increasing the rate of absorption of epinephrine, also occurs in man when ordinary therapeutic doses are used.

Richards and Kueter(5) showed that the increase in epinephrine toxicity in rats caused by sodium bisulfite was abolished by previous administration of rutin, whereas the toxicity of epinephrine solution alone was not affected by rutin. We also tested the effect of rutin on the "bisulfite phenomenon" in man.

Method and results. 1. *Action of epinephrine solution using sodium hypophosphite as a preservative.* (a) In 19 male subjects blood pressure and pulse rate determinations were made every 10 minutes for 20 minutes before, and one hour after injection of the drug. One cc of a 1 to 1,000 solution of epinephrine with 0.5% sodium hypophosphite was injected subcutaneously. The systolic blood pressure rose slowly having risen an average of 9.0 mm of Hg at the end of one hour. The diastolic pressure fell an average of 17.2 mm of Hg and the pulse rate increased 8.6 beats per minute (Refer to Fig. 1). (b) In order to follow the blood pressure until it returned to normal and to determine the time of the peak of action, the experiments

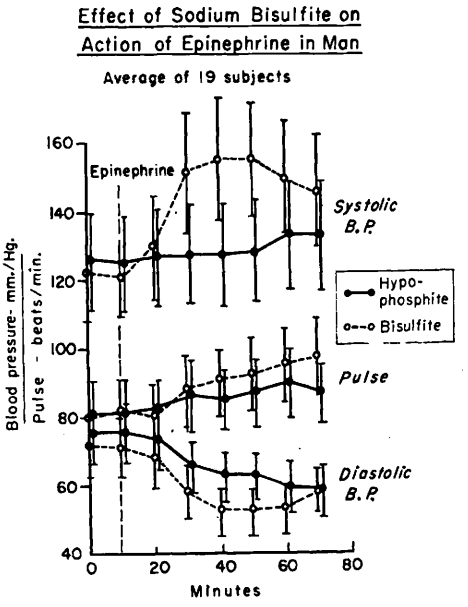

FIG. 1.

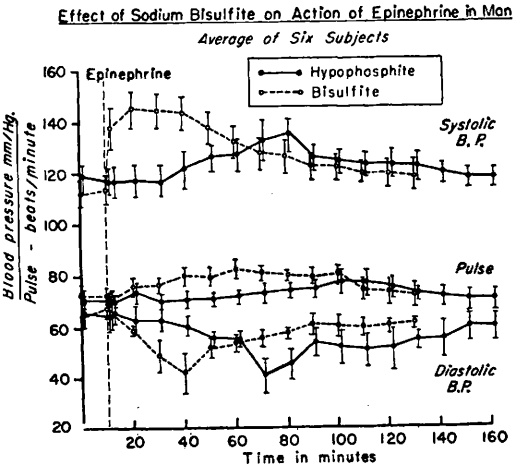

FIG. 2.

The vertical bars represent the standard error of the means.

were repeated on six subjects and the blood pressure and pulse rates were followed until they returned to control levels. These results showed that the systolic pressure peak occurred on the average of 56.7 minutes after injection (Refer to Fig. 2). (c) The same procedure was followed on two subjects using epinephrine powder freshly put into solution with no preservative added and the curve of response was not significantly different from

3. Richards, R. K., *Fed. Proc.*, 1943, v2, 89.

4. Richards, R. K., and Kueter, K. E., *Anesth. and Analg.*, 1943, v22, 283.

5. Richards, R. K., and Kueter, K. E., *J. Pharm. and Exp. Therap.*, 1948, v94, 372.

TABLE I. Effect of Sodium Bisulfite and Rutin on the Action of Epinephrine Average of 6 Subjects.

	Without rutin — Absolute change from control level		Difference		With rutin Absolute change from control level	
	Hypophosphite	Bisulfite	Avg	t	Hypophosphite	Bisulfite
Systolic B/P—mm Hg	20.7	41.7	21.0	5.68	24.4	42.6
Diastolic B/P "	−28.0	−28.0	0	—	−28.0	−28.6
Systolic B/P peak—min.	56.7	14.3	42.4	3.34	26.7	21.3
Diastolic B.P. " "	68.3	30.0	38.3	3.9	68.3	31.8
Pulse—beats/min.	11.0	14.0	3.0	0.26	12.4	12.0

that obtained when sodium hypophosphite was used.

2. *Action of epinephrine solution using sodium bisulfite as a preservative.* (a) The same procedure was followed as in 1 (a) above on the same 19 subjects with the exception that 0.1% sodium bisulfite was used as the antioxidant for the epinephrine. (Refer to Fig. 1.) The systolic blood pressure rose an average of 34.7 mm of Hg, the diastolic pressure fell 19 mm of Hg and the pulse rate increased 15 beats per minute. (b) The same procedure was followed on the same 6 subjects as in 1 (b) above except that sodium bisulfite was used in place of the sodium hypophosphite. The systolic pressure peak occurred in 14.3 minutes (refer to Fig. 2). These results show that the absolute increase in systolic pressure in response to epinephrine solution with sodium bisulfite was 21.0 mm of Hg higher than the sodium hypophosphite ($t = 5.68$); and the time of peak of action was 42.4 minutes sooner ($t = 3.34$). The diastolic pressures were decreased with both drugs to the same degree (−28 mm Hg). The difference in the pulse rate was not statistically significant ($t = 0.26$) (Refer to Table I).

3. *Action of epinephrine solution after the injection of rutin.* (a) Using sodium hypophosphite as a preservative the same procedure was followed on the same 6 subjects as in 1 (b) above with the exception that 125 mg of rutin (solubilized with piperazine) in 20 cc of saline was given slowly intravenously over a period of 4 minutes before the epinephrine with sodium hypophosphite was given. The blood pressure and pulse rates taken directly after the rutin showed no change and the epinephrine was injected immediately therafter. The systolic pressure rose an average of 24.4 mm of Hg with the peak occurring in 26.7 minutes. The diastolic pressure fell 28 mm of Hg and the pulse rate increased 12.4 beats per minute. (b) Using sodium bisulfite as a preservative the same procedure with rutin was followed on the same 6 subjects as in (a) above with the exception that sodium bisulfite was used as the preservative for the epinephrine solution. The systolic blood pressure rose an average of 42.6 mm of Hg with the peak occurring in 21.3 minutes. The diastolic pressure fell 28.6 mm of Hg and the pulse rate increased 12 beats per minute. These results with the rutin are not significantly different from those without the rutin.

Richards(3) found that a dose of about 350 mg per kg of rutin was required to counteract the "bisulfite effect" in rats. Since our dose in human subjects was only about 2 mg per kg, it is not surprising that the rutin failed to alter significantly the "bisulfite effect".

A number of studies including a recent one by Crismon and co-workers(6) have shown that intravenously injected rutin potentiates the action of topically applied epinephrine on the vessels of the rat's mesoappendix. Crismon *et al.*(6) found a dose of 2.5 mg/kg adequate to produce this action. It is interesting to note that in the present study doses of 2 mg/kg given intravenously in human subjects failed to alter the action of epinephrine on blood pressure. In studies on anesthetized dogs we have found that rutin in doses as

6. Crismon, J. M., Berez, R. R., Madden, J. R., and Furhman, F. D., *Am. J. Physiol.*, 1951, v164, 391.

high as 50 mg/kg given intravenously does not alter the pressor response to small doses of epinephrine.

Summary and conclusions. The subcutaneous injection of epinephrine solution containing sodium bisulfite as an antioxidant produced a more rapid and higher rise in systolic blood pressure than did the same amount of epinephrine in a solution containing sodium hypophosphite as a preservative. Small doses of rutin given intravenously immediately before the epinephrine did not significantly alter the "bisulfite effect" nor did the rutin influence the effect of epinephrine.

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Leukocyte Response to Stress in Normal and Adrenalectomized Rats Pretreated with Ascorbic Acid. (18714)

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Ascorbic acid treatment to animals exposed to stress has been observed to exert certain protective actions which are related to adrenal cortical function(1-3). This ability of ascorbic acid has been established by survival studies, and by studies on the functional status of the cortex. It was demonstrated that ascorbic acid pretreatment to intact rats prevented the eosinopenia following epinephrin stress; this was associated with a prevention of the depletion of adrenal steroids(3).

This paper reports results which indicate that there may exist an ascorbic acid-eosinophil alliance independent of the adrenal.

Materials and methods. Adult female Osborne-Mendel rats were used in this study. Twelve sham-adrenalectomized rats (7-8 days post-operative) were divided into 2 groups. Group I animals were pretreated with ascorbic acid (Vitamin C injectable, sodium ascorbate, Roche), 87.5 mg/100/g in two intra-peritoneal injections; Group II rats received pretreatment with saline solution. Two groups of adrenalectomized rats, (7-8 days post-operative) were given respectively, pretreatment with ascorbic acid, (Group III), and pretreatment with saline (Group IV). About 6-8 hours after the last pretreatment injection, all animals were given single subcutaneous injections of epinephrin at the rate of 0.03 mg/100 g. Total and differential leukocyte counts (Randolph's method)(4) of the tail blood were made of all animals in the following instances: Count (a) before pretreatment, Count (b) 3 hours after the injection of epinephrin, and Count (c) 24 hours after epinephrin. The mean differences of Counts (b), and (c) from Count (a) were calculated and are presented along with the standard errors in Table I. Counts made previous to the experiment indicated that the operation of sham-adrenalectomy did not disturb the leukocyte picture, but adrenalectomy led to a significant leukopenia, lymphopenia, and eosinopenia. Adrenalectomy did not however, affect the polymorphonuclear count.

Results. Both groups of sham-adrenalectomized rats (I and II) exhibited a significant decrease of lymphocytes 3 hours after the injection of epinephrin and returned to prestress levels 24 hours after the stress. The injection of epinephrin to adrenalectomized rats (III and IV) failed to alter the number of circulating lymphocytes. The expected rise in polymorphonuclear leukocytes (neu-

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2. Dugal, L. P., and Leblanc, J., *Rev. canad. de biol.*, 1949, v8, 440.

3. Bacchus, H., and Toompas, C. A., *Science*, 1951, v113, 269, 367.

4. Randolph, T. G., *J. Allergy*, 1944, v15, 89.