caused by bacterial endotoxins, and dibenamine prevents the development of irreversibility, the blood of dibenaminized rabbits in hemorrhagic shock was tested for the presence of endotoxin and found to be toxin free. But because the blood loss in the dibenaminized animal in shock is less than in the non-dibenaminized animal, the absence of toxin might be due to the lesser hypovolemia rather than to the dibenamine. Accordingly, an additional experiment was performed to see if dibenamine given to the rabbit in reversible shock will permit it to survive a transfusion of toxic blood from an irreversibly shocked donor—a procedure which kills the non-dibenaminized rabbit in reversible shock. This experiment demonstrates that dibenamine blocks the action of the toxin, and thereby not only preserves the responsiveness of the circulation, but also prevents the hemorrhagic lesion in the bowel wall, which is characteristic of irreversible shock.

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Circulatory Reactions of Dogs and Rats to Polyvinylpyrrolidone or Dextran After 48/80. (23814)

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Dogs and rats injected with the potent histamine liberator 48/80 become temporarily refractory to further administration of the same dose(1-2). Temporary tachyphylaxis also occurs in dogs after repeated injections of polyvinylpyrrolidone (PVP) and in rats after dextran(3). These colloids act as histamine releasers in each species respectively(4-5). We have sought evidence of cross-refractoriness using two criteria of sensitivity. One is the appearance of bluing in animals which have been injected with Evans blue (T-1824), and indicates an increase in skin capillary permeability to the albumin-dye complex. The other is a decrease in mean arterial blood pressure, indicative of a systemic reaction.

Methods and results. Details of the experimental procedure and summary of the results appear in Table I. No experiments are included in which the blood pressure at time of challenge was not 90 mm Hg or higher. Evans blue was given usually early in the experiment and in dosages (0.6-2 mg/kg) which produce no cutaneous bluing in untreated animals. In the first experiment 6 dogs received intravenously a series of graded doses of 48/80* as used by Slomka and Goth(6). After 44-112 μg/kg, small foci of blue became visible, surrounded by erythema. Gradually the bluing extended further and the flush faded. Ten minutes after a total of about 600-700 μg/kg, the mean blood pressures measured by catheter in a femoral artery were 20-50 mm

* Available through the kindness of Dr. E. J. de Beer, Wellcome Research Laboratories.

TABLE I. Circulatory Reaction of Dogs and Rats to 48/80 and PVP or Dextran.

No.	Pretreatment (per kg body wt)	No. reacting to last inj. Vasodepression*	Bluing	Challenging treatment (per kg body wt)	No. reacting to challenge Vasodepression*	Bluing
	Dogs conscious or anesthetized with sodium thiopental					
6	48/80, 800-900 μg in 14-17 doses	0	0†	PVP, 35 mg	6	6
6	PVP, 35-70 mg in 2 doses	0	0†	48/80, 100 μg	6	1
6	None			*Idem*	4	2
	Rats anesthetized with sodium pentobarbital					
10	48/80, 600-1300 μg in 4-9 doses	0	1‡	Dextran, 600 mg	0	2
10	Dextran, 1200 mg in 2 doses	0	1‡	48/80, 200-300 μg	2	0
16	None			48/80, 200 μg	13	2

* Decrease in mean arterial pressure of 25 mm Hg or more. † Bluing had appeared in all animals before last inj. ‡ Bluing had appeared in a few animals before last inj.

Hg below their preinjection values. At this time a rapid injection of 200 μg/kg 48/80 produced no further change in pressure or color, showing the animals to be refractory to this dosage. About an hour later (in one animal only 10 minutes later), when the mean pressures were normal, PVP† was injected and the usual vasodepression followed (Fig. 1A). Bluing became more generalized and edema more intense.

Six dogs were shown to be non-reactive to a 2nd PVP injection 1 hour after the first. They were challenged with 100 μg/kg 48/80 and the blood pressures fell 25-60 mm Hg (Fig. 1B). In the first 3 dogs no additional bluing appeared. In the last 3, dye was given just before 48/80 so that an adequate concentration in the circulation was assured, which may not have been the case in the first 3 dogs injected with dye before PVP. A trace of bluing appeared in one. The results in this group resemble in general those seen in 6 additional dogs given 100 μg/kg without pretreatment.

For the experiments with dextran adult male and female rats of the Sprague-Dawley or Osborne-Mendel strains were anesthetized with sodium pentobarbital and the usual dose of Evans blue was administered. Pressures were recorded from the common carotid artery. Ten rats received intravenous injections of graded doses of 48/80 until they were refractory to 200 μg/kg. Dextran‡ was then administered, and the vasodepression usual in this species did not occur (Fig. 2A) and additional bluing was seen in only 2 animals.

Ten rats that were non-reactive to a 2nd dextran injection were challenged with 48/80. Only 2 had a significant fall in blood pressure (25 and 35 mm Hg respectively) and none of them had additional bluing (Fig. 2B).

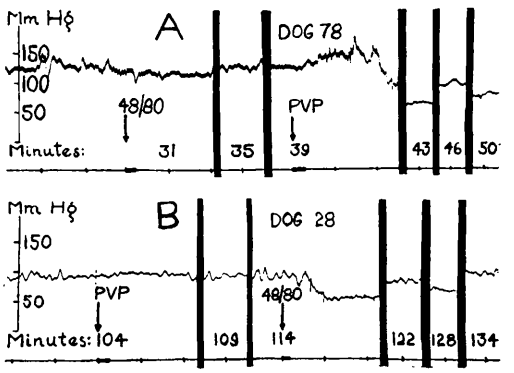

FIG. 1. Mean arterial blood pressure records from 2 unanesthetized dogs. A: dog 78, 12/7/56. Fourth inj. of 48/80 (200 μg/kg) at 30 min. PVP (35 mg/kg) at 39 min. was followed by vasodepression and bluing typical of an initial PVP administration. B: dog 28, 12/6/57. Second inj. of PVP (35 mg/kg) at 104 min. 48/80 (100 μg/kg) at 114 min. was followed by vasodepression and erythema but no bluing.

† PVP-Macrose (3.5% PVP in isotonic salt solution), kindly furnished by Schenley Laboratories.

‡ "Expandex", 6% dextran in isotonic NaCl.

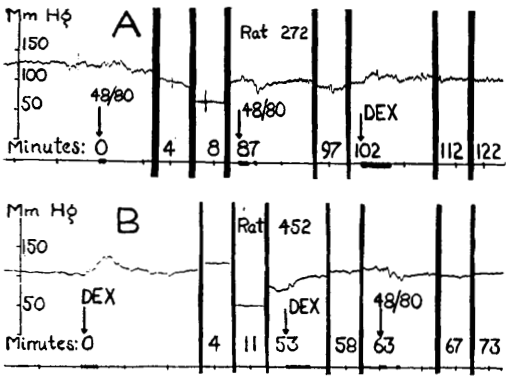

FIG. 2. Mean arterial blood pressure records from 2 anesthetized rats (sodium pentobarbital, about 35 mg/kg i.p.). A: rat 272, 12/19/56. First inj. of 48/80 (200 μg/kg) at 0 min., last inj. at 87 min. (300 μg/kg; total dosage 900 μg/kg). At 98 min. blue seen between toes. At 102 min. dextran (600 mg/kg) was inj., followed by edema and bluing but no fall in blood pressure. B: rat 452, 8/16/57. At 0 min. dextran (600 mg/kg) inj. was followed by the usual prolonged vasodepression and moderate bluing. At 53 min. a similar inj. raised the pressure. At 63 min. 48/80 (200 μg/kg) produced little effect. Compare with A at 0 min.

This is in contrast to vasodepression appearing in 13 out of 16 previously untreated rats.

Discussion. Our finding that dogs refractory to 48/80 still respond to PVP corroborates results with another histamine liberator, Tween 20(6) and substantiates Halpern's statement(7). On the other hand, cross-refractoriness to other histamine liberators has been demonstrated in this species(8-9). Slomka and Goth(6) produced refractoriness in dogs to 48/80 as shown by absence of vasodepression and without increased circulating histamine, but do not mention evidence of local permeability changes. The consistent appearance of bluing in our dogs given large doses of 48/80 is evidence that histamine or some other permeability affective substance is present.

The reaction to 48/80 of rats anesthetized with a barbiturate resembles that after dextran. The absence of bluing may be caused by low hydrostatic pressure precluding the passage of dye into tissue spaces or by failure of flow through closed capillaries. Ether has been shown(10) to protect rats from dextran vasodepression as does 48/80, but there is the difference that with ether the incidence of bluing is high. In the experiments of Feldberg and Talesnik(11) rats after extensive 48/80 administration had an attenuated or negative response to egg white. Halpern(7) states that rats depleted of histamine by dextran react to 48/80, which is the opposite of our conclusions using the criteria described.

Separate consideration of the two events, vasodepression and bluing, permits more precise conclusions regarding cross-refractoriness, and hence, mechanisms. The two phenomena may be due to histamine released either at different sites or in different amounts at the same site, or to the presence of some additional substance, such as 5-hydroxytryptamine(12).

Summary. The appearance of cutaneous bluing and vasodepression has been followed after T-1824 and 48/80 injection in dogs and rats subsequently challenged with polyvinylpyrrolidone (PVP) and dextran respectively. The reverse sequence of injection was also tested. Graded doses of 48/80 in dogs resulted in refractoriness to the vasodepressive effects of similar doses, but cutaneous bluing was always seen. This increased skin capillary permeability may be interpreted as evidence of local release of histamine or other substance. In dogs it was confirmed that there was no cross protection between PVP and 48/80. In rats anesthetized with a barbiturate and pretreated with 48/80 the usual vasodepression and bluing after dextran did not occur. Rats refractory to dextran were also refractory to 48/80.

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Selenium and Development of Exudative Diathesis in Chicks.* (23815)

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A number of workers have reported the development of exudative diathesis as a symptom of Vit. E deficiency using the diet of Scott *et al.*(1). It was recently reported that organic complex of selenium was the factor 3 of dried brewers yeast necessary for prevention of liver necrosis in rats fed a similar Vit. E-free Torula yeast diet(2); and selenium was also found to be active in this regard. Schwarz *et al.*(2) have shown that selenium or factor 3 concentrates would prevent the gross symptoms of exudative diathesis in chicks fed Torula yeast. Patterson *et al.*(3) have identified selenium as the active component of casein and defatted pork kidney in preventing development of exudative diathesis in chicks. Previous work has shown that exudative diathesis of the chick or poult resulted in marked diminution of hemoglobin level, total serum protein (albumin level), and total red cell counts. These conditions could be prevented by adding Vit. E or substituting 10% dried brewers yeast in the Torula yeast diet(4,5).

In view of these results it seemed desirable to determine the effect of selenium on occurrence of the blood changes associated with exudative diathesis.

Methods. In the first study, 5 groups of 20 White Rock chicks were placed on the Torula yeast diets supplemented as shown in Table I. The basal diet used in this study is the same as that of Creech *et al.*(5). Selenium was supplied as sodium selenate at levels of 1 and 10 ppm. At the end of 3 weeks, when gross symptoms of exudative diathesis had appeared in birds fed the basal diet, a representative sample (5 birds per group) was selected for the determination of total serum protein, albumin:globulin ratios, red cell counts, hemoglobin, and packed cell volume as previously described(5). The second study in this series employed 20 New Hampshire chicks per group with the basal diet supplemented as shown in Table III. Selenium was supplied as sodium selenate (1, 0.5, 0.1 ppm), as the selenite (1 ppm), and as free selenium (0.5 ppm). At 3 weeks of age a representative sample of birds from each group was removed for the blood analyses previously listed. The birds in both experiments were housed in electrically heated batteries with raised wire floors; feed and water were supplied *ad libitum*. Body weights were determined at weekly intervals throughout the study, and daily observations were made for the appearance of symptoms of exudative diathesis.

Results. The addition of selenium as the selenate either at the level of 1 ppm or 10 ppm completely prevented development of gross symptoms of exudative diathesis up to

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