TABLE II. Results of Latex Agglutination Tests between Anti-Anaerobic Diphtheroid Strain G Rabbit Antiserum and a Variety of Microorganisms.

Organisms	Results	Organisms	Results
Anaerobic diphtheroids		Actinomyces	
G	+	*A. bovis*	0
M	+	*israeli*	0
E	+		
R_O	+	Others	
T	+	*Nocardia asteroides*	0
R_B	+	*Lactobacillus acidophilus*	0
MOR	+	*Propioni bacterium freudenreichii*	0
Corynebacteriae		*Staphylococcus aureus*	0
C. acne	+	*Beta hemolytic streptococcus* (type 12)	0
hoagii	0	*H. influenza* (type B)	0
xerose	0		
diphtheriae	0		
pseudodiphtheriticum	0		
pyogenes	0		

bic diphtheroids with which it probably shares a common antigen.

Discussion. Latex (polystyrene) particles which are now readily available have become useful serological tools. Singer and Plotz(3) first employed them in a test for rheumatoid arthritis. More recently, there have been reports of their value in study of histoplasmosis (4) and of trichinosis(5). These particles probably make more apparent a precipitinogen-precipitin reaction. Collodion particles were used for this purpose more than 30 years ago, but were of limited value since conditions for preparing and preserving them were very exacting and time consuming(6).

Singer and Plotz(3) stated that the "latex fixation" technic could also be used with Type VII antipneumococcal rabbit serum and Type VII pneumococcal polysaccharide. In this report, we have extended this observation to another bacterial antigen-antibody system, that of anaerobic diphtheroids and their specific antisera, and have investigated optimum conditions for this reaction. Other bacterial antigen-antibody systems, however, may require different conditions.

Summary. A technic for using latex (polystyrene) particles in a serological test with anaerobic diphtheroids is described. Optimum conditions under which latex particles may be used with this bacterial antigen-antibody system are reported. Evidence is presented to indicate a possible immunological relationship between various anaerobic diphtheroids.

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Effect of Phosphorylated Hesperidin and Hyaluronidase on Rate of Erythrocyte Removal from Rat Peritoneal Cavity.* (25952)

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Phosphorylated hesperidin decreases net absorption rate of 0.9% sodium chloride solution from the peritoneal cavity of rats. Hyaluronidase abolishes this effect and by itself increases absorption rate in intact animals(1). The investigations reported below were con-

* Opinions expressed herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or naval service at large.

† This work has been completed during senior author's tenure as Natl. Science Fn. Research Fellow at Wayne State Univ. Coll. of Med. and Detroit Receiving Hosp.

ducted to determine whether phosphorylated hesperidin and hyaluronidase affect rate of absorption of erythrocytes from peritoneal cavity of rats.

Materials and methods. Adult hooded female rats (175-200 g) raised at the Naval Med. Research Inst. were housed in individual cages in an air-conditioned room and had free access to laboratory rat chow and water. Experiments were performed as follows: 1) Animals were injected intraperitoneally (I.P.) with 15 mg of phosphorylated hesperidin (kindly donated by Dr. G. J. Martin, National Drug Co., Philadelphia) in 0.2 ml of 0.9% sodium chloride solution (isotonic saline) at 4 p. m. At 9 a. m. the next day they received a similar I.P. dose of phosphorylated hesperidin followed immediately by an I.P. injection of 6 ml of a suspension of erythrocytes. 2) Hyaluronidase (of bovine testes origin 1550 USP u/mg generously donated by Wyeth Inst. for Med. Research) was administered I.P. in 0.2 ml of isotonic saline, 6000 U.S.P. units per injection. The animals were injected at 9 a.m., 4 p.m. and the next day at 9 a.m. Immediately following last hyaluronidase injection the animals received I.P. 6 ml of erythrocyte suspension. Red blood cell suspension was administered through an 18 gauge needle under ether anesthesia, light enough so that the animals were awake within 2-3 minutes after completion of injection. No untoward reaction to I.P. injection of erythrocytes was observed. Groups of animals were sacrificed at various time intervals following injection of erythrocytes. The abdominal cavity was exposed and the remaining suspension recovered. The diaphragm, peritoneum, thoracic lymph vessels and lymph nodes were thoroughly inspected during autopsy. *Preparation of erythrocyte suspension.* On the day preceding an experiment, blood obtained by heart puncture from adult rats was collected in a syringe containing about 1 ml of 4% sodium citrate for each 10 ml of blood. The blood was centrifuged in a refrigerated centrifuge (4.5°C) at 2500 rpm for 30 minutes. The plasma and most of the buffy coat were discarded. The packed red cells were resuspended in an equal volume of normal saline solution and recentrifuged under refrigeration. The washing procedure was repeated 2 more times and after the last washing the cells were resuspended in an equal volume of isotonic saline and refrigerated until use. *Recovery of red blood cell suspension from peritoneal cavity.* Animals were sacrificed with an overdose of ether. Abdominal cavity was exposed, the remaining cell suspension removed and its volume measured. The abdominal cavity was rinsed twice with 5 ml of isotonic saline which was combined with the recovered suspension. Red blood cells were packed by centrifugation in a Kolmer graduated 10 ml centrifuge tube (Corning No. 8360) at 2500 rpm for 30 mintues. Volume of packed cells was read with an accuracy of 0.05 ml.

Results. Effect of phosphorylated hesperidin. In untreated animals volume of fluid and red blood cells recoverable from the peritoneal cavity decreased progressively with time. Rate of removal followed essentially a straight line for the first 4 hours, diminished between 4 and 8 hours after injection (Fig. 1). About 50% of the red blood cells were removed within the first 4 hours, and after 8 hours only 20% of originally administered volume could be recovered from the peritoneal cavity. In the hesperidin treated animals rate of removal of red blood cells and fluid was slow. Over 88% of injected erythrocytes could be recovered 8 hours after injection. Four hours after injection of the red blood cell suspension in untreated animals, diaphragm, thoracic lymphatics and lymph nodes were dark red (Fig. 2a). Microscopic examination of the

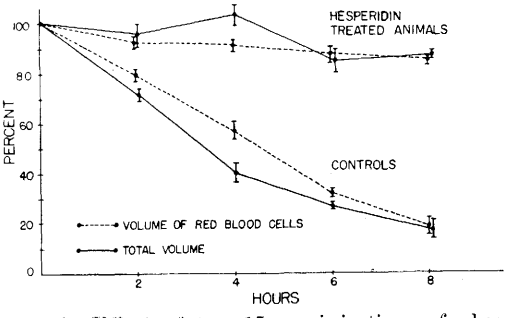

FIG. 1. Effect of two 15 mg injections of phosphorylated hesperidin on absorption of a 50% suspension of red blood cells in physiological saline from the peritoneal cavity of rats. Each point on the curves represents a mean from 12 animals.

TABLE I. Effect of Hyaluronidase on Absorption of Red Blood Cell Suspension (RBC) from Peritoneal Cavity of Rats.

	% of total vol and RBC vol recovered after inj. of 6 ml RBC suspension									
	2 hr		4 hr		6 hr		10 hr		17 hr	
Treatment	Vol*	RBC	Vol	RBC	Vol	RBC	Vol	RBC	Vol	RBC
Hyaluronidase and saline	56.8 ± 3.9† (5)	82.2 ± 3.3	31.9 ± 1.4 (11)	52.4 ± 1.3	20.2 ± 1.8 (12)	33.2 ± 2.2	no free fluid (12)	15.0 ± 1.6	no free fluid (8)	9.0 ± 2.5
	P <.01	.1 > P >.5	P <.01	P <.01	P <.01	P <.05		P <.001		P <.01
Saline	73.2 ± 1.6 (5)	90.4 ± 2.6	40.4 ± 1.3 (12)	61.0 ± 2.1	28.0 ± 1.2 (12)	41.8 ± 3.0	*Idem* (10)	31.0 ± 2.9	*Idem* (8)	20.0 ± 2.2

* Fluid and RBC. † Mean ± stand. error. Numbers in parentheses indicate No. of animals.

FIG. 2. Diaphragms of rats sacrificed 4 hr after intraper. inj. of red blood cell suspension. A. Normal control. B. Hesperidin treated.

material responsible for this discoloring revealed it to be red blood cells. In animals treated with phosphorylated hesperidin and sacrificed 4 hours after injection of a suspension of erythrocytes, the diaphragms were translucent, only a few small areas being filled with blood (Fig. 2b). Microscopic examination of the diaphragms (paraffin embedded and stained with Masson's Trichrom) did not reveal morphologic changes in the peritoneal structures.

Effects of hyaluronidase. Treatment with hyaluronidase produced a small but statistically significant ($P<0.01$) increase of absorption rate of the fluid portion of the red blood cell suspension at all time intervals after injection. The effect on absorption of erythrocytes was also slight. At each examined time interval the hyaluronidase treated animals had fewer erythrocytes recoverable from the peritoneal cavity than controls (Table I). Inspection of diaphragms revealed no difference between hyaluronidase treated and control animals.

Discussion. Absorption of blood from the peritoneal cavity of man and experimental animals was described in the 19th century. The early literature on this subject has been reviewed by Hunter(2). More recent studies revealed that the primary and probably only significant route of erythrocyte absorption is *via* the diaphragmatic lymphatics(2-5). From there the erythrocytes pass into the host's general circulation(6-8) where they survive for a normal life span(9,10). The mechanism of the absorption process, however, is poorly

understood. Simer(11) concluded that small particles (India Ink) can pass from the peritoneal cavity of rats into the subperitoneal lymphatics of the diaphragm between the contiguous borders of the mesothelial cells of the peritoneum and endothelial cells of the lymph vessels. According to Allen(12) the erythrocytes pass through a triple layered membrane (peritoneal mesothelium, a fenestrated basement membrane and endothelium of the lymphatics) separating peritoneal cavity from diaphragmatic lymphatic spaces. The mesothelium and endothelium stretch during expiratory movements of the diaphragm, and openings ("stomata") are formed (up to 25.5 μ in diameter) through which the suspension of particular matter is "sucked" into the lymphatic channels.

Courtice *et al.*(5) demonstrated that in rats erythrocytes are removed from the peritoneal cavity at a relatively rapid rate, up to 90% of injected erythrocytes being removed within 10 hours. In our experiments rate of removal of erythrocytes was similar in intact animals. However, in phosphorylated hesperidin treated rats, erythrocytes were retained in the peritoneal cavity for periods of up to 8 hours after injection. There was also a block in absorption of the solution in which red blood cells were suspended, confirming previous report by Steinberger *et al.*(1).

Diaphragms of untreated controls were packed with red blood cells while diaphragms of hesperidin treated animals were essentially devoid of grossly demonstrable red blood cells; this suggests that the block was effected at the membrane dividing peritoneal cavity from lymphatic channels of the diaphragm. Considering the mechanisms of erythrocyte removal from the peritoneal cavity proposed in the literature(11,12), hesperidin would appear to exert its effect upon the peritoneal membrane either by inhibiting its ability to form openings during the respiratory excursion of the diaphragm, or by modifying the ground substance between the contiguous borders of the cells, making it less permeable. The effect on the peritoneal structures appears to be local since pilot experiments employing subcutaneous administration of hesperidin did not reveal any effect on peritoneal absorption. Histological examination of the peritoneum revealed no structural changes. A study of a direct local effect of hesperidin on connective tissue is in progress.

The effect of hyaluronidase on passage of red blood cells from the peritoneal cavity was small, but statistically significant. This may indicate that permeability of the membrane was at its peak before treatment with hyaluronidase, and further increase was difficult to achieve. Another, more plausible explanation would be that the lymphatics through which the blood cells are removed have a maximum volume capacity which is the rate-limiting factor not influenced by an increase in membrane permeability. This interpretation is supported by the fact that in untreated control animals the entire lymphatic system engaged in removal of the red blood cells is actually packed with them. The lymph from these lymphatic channels has been reported to contain up to 16 million red blood cells per cmm and to form a thick sludge(12).

Summary. Rate of absorption of erythrocytes from peritoneal cavity of rats was found to be similar to that reported in the literature. Absorption was almost completely blocked by phosphorylated hesperidin. Evidence was presented suggesting that the block is achieved at the membranes separating peritonal cavity from lymphatics of the diaphragm. Hyaluronidase increased rate of absorption only slightly; however, this increase was statistically significant.

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Genetic Susceptibility to Goldthioglucose-Induced Obesity in Mice.* (25953)

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A single injection of goldthioglucose at appropriate concentrations produces a persistent obesity in mice(1,2,3,4,5). The weight gain is correlated with an increase in food intake (6). Lesions found in the hypothalamus of goldthioglucose-injected mice are considered to be the basis for the hyperphagia and ensuing weight gain(7,8,9), and localization of tissue destruction in ventromedial nuclei of the hypothalamus has been related to the postulated existence of a "glucoreceptor" center in this particular area of the diencephalon(7). However, lesions have been found in areas of the brain that lie outside the confines of the hypothalamus following an obesity-inducing dose of goldthioglucose(8,10). Liebelt and Perry(8) have shown a genetic difference in the susceptibility of CBA and C_{57} Black mice to goldthioglucose-induced obesity. The purpose of this investigation was to extend these findings to other strains of inbred mice, thus studying the influence of genetic factors in goldthioglucose-induced obesity.

Methods. All strains of mice used in these experiments had been inbred by brother-sister mating for over 20 generations,† and included C_{58}, CBA, DBA/2, BALB/C, RIII as well as the following F_1 hybrids: (RIII x CBA), (CBA x RIII), (CBA x C_{58}), (C_{58} x CBA), (CBA x BALB/C). Only male mice were used and were housed in groups of 4-6 animals per wooden cage (6 x 12 x 6 in.). Purina lab chow was fed *ad libitum*. Crystalline goldthioglucose (Solganol B)‡ was dissolved in saline, and injected intraperitoneally at 90-110 days of age. All injections were made between 9:00 and 11:00 a. m.; the animals were not starved prior to treatment. A minimum of 10 and a maximum of 40 animals were injected at each of the various dose levels tested for each strain and F_1 hybrid combination; mean weight gain of the surviving animals was determined at 60 days post-injection. Susceptibility to the *toxic effects* of goldthioglucose was expressed only as the LD/100 dose.

Results. Table I shows that both gain in body weight and lethal toxic effects in any given strain vary with dose of goldthioglucose injected. C_{58} mice appear to be most "susceptible" to goldthioglucose in terms of weight gain and toxicity as compared to the more "resistant" BALB/C, RIII and DBA/2 strains. Mice of the CBA strain fell within these two extremes. Weight gain response and mortality were characteristically variable, especially as LD/100 dose was approached, in all except the CBA strain. CBA mice appeared to be unique in that a uniform weight gain response could be predicted for each dose level over a range of 0.2 to 0.6 mg/g body weight with less than 10% mortality at the latter dose.

In general, all animals showed a greater weight gain as dose of goldthioglucose was increased. However, as the LD/100 dose was approached in the DBA/2, RIII, and BALB/C strains, animals actually lost weight when

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