TABLE I.

Diagnosis	Avg rise in blood pressure in cold pressor tests —MM Hg			
	Control		After benzodioxane	
	Systolic	Diastolic	Systolic	Diastolic
Normal	6	11	3	14
Chronic glomerulonephritis	14	25	23	33
Essential hypertension	19	29	30	25
" "	30	25	18	14
" "	21	15	22	10
" "	6	13	16	16

ever, Goldenberg *et al.*(5) have demonstrated that benzodioxane abolished the rise in systolic blood pressure that followed the intravenous infusion of epinephrine. The dose of epinephrine which they used was adjusted to cause a 25% increase in systolic pressure. This increase is comparable to the increase in systolic blood pressure noted on cold stimulus in our experiments. The fact that benzodioxane did not block the cold pressor response suggests that this response is not due to the release of epinephrine. The fact that cold stimulation causes a rise in both systolic and diastolic blood pressure also suggests that this response is on a neurogenic rather than an epinephrine-induced basis.

Conclusions. Benzodioxane, administered intravenously in dosage of 10 mg per sq m of body surface, does not inhibit the cold pressor response.

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Adsorption by Erythrocytes of Antigens of *Pfeifferella mallei* and *Pfeifferella whitmori.** (17658)

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During the course of experiments on bacterial haemagglutination it has been observed that erythrocytes of various species, under certain conditions, adsorb serologically specific substances from a solution of mallein (a Seitz E-K filtrate of a steam-killed 6 weeks culture of *Pf. mallei* in synthetic broth, concentrated to 2/5 of the original volume). Thus it was found that human, horse, guinea pig, sheep and fowl red cells exposed in contact with mallein were rendered agglutinable by the sera of horses, rabbits and other animals immunized against mallein. Ox red cells were shown to adsorb antigen from mallein solutions, and then, when treated with anti-mallein sera, to adsorb antibodies responsible for the agglutination of sensitized horse cells. However, neither ox nor pig cells exposed to mallein could be agglutinated by such antisera.

Sheep and fowl cells required about 8 times, and guinea pig cells 2 times more mallein to sensitize them than did horse and human cells. Different batches of mallein varied considerably in their sensitizing potency, some being twenty times more active than others in this respect. There was no quantitative relationship between the amount of sensitizing antigen and the amount of antigen responsible for complement fixation present in the various preparations.

Sensitization of horse red cells with a minimum sensitizing dose of antigen took 3 hours to reach completion at room temperature

* This work has been carried out at the Institute of Animal Pathology, Cambridge, England, on a grant from the Animal Health Trust, London.

TABLE I.
Agglutination of Horse Erythrocytes Sensitized with Mallein by Normal and Immune Sera.

Serum dilutions	Horse serum		Rabbit serum		Human serum	
	Normal (1:10)	Anti-mallein (1:5120)	Anti-*Salmonella pullorum*	Anti-mallein	Normal	From patient infected with *Pf. whitmori* (640)
80					—	++++
160					—	++++
320	++++	++++	—	++++	—	++++
640	++++	++++	—	++++	—	++++
1,280	—	++++	—	++++	—	++++
2,560	—	++++	—	++++	—	++++
5,120	—	++++	—	++++	—	++++
10,240	—	++++	—	++++	—	++++
20,480	—	++++	—	+	—	—
40,960	—	+++	—	—	—	—
81,920	—	++	—	—	—	—
123,840	—	—	—	—	—	—
327,680	—	—	—	—	—	—

Note: The figures in parentheses refer to the titers of the sera by the Conglutinating Complement Adsorption Test [Hole and Coombs(2)].

(about 21°C) whereas, at 37°C the reaction was complete in 15 minutes or less.

Horse red cells were generally used in serum titration experiments for a number of reasons, *e.g.*, they were readily available in quantity, they required less antigen than other species to sensitize them, and positive sera agglutinated sensitized horse cells to a higher titre than sensitized sheep or fowl cells.

The following technique was used to titrate sera for antibody against the adsorbable antigens. Two minimum sensitizing doses of mallein (0.01 ml of a potent preparation) were added in 1 ml of saline to 1 ml of a 5% suspension of horse red cells (previously washed at least 4 times in saline); this mixture was incubated for one hour at 37°C. The cells were then washed twice and resuspended in 1 ml saline. One drop (0.05 ml) of this suspension was added to each tube (0.8x7 cm) of a row containing twofold dilutions in 0.5 ml quantities of the test serum. The tubes were then shaken and allowed to stand for one hour at room temperature. Agglutination was manifest by the formation by the cells on the bottom of the tubes of a pattern similar to that seen characteristically in haemagglutination by the influenza group of viruses [Salk(1)].

Some samples of sera, especially from the horse, contained substances inhibiting the formation of the typical agglutination pattern. This effect was observed in dilutions of serum up to 1:1280. In such cases, the agglutinated cells settled to the bottom of the tube more quickly and could be seen flocculating together in grossly visible aggregates which were readily dispersible on shaking. If the tube was allowed to stand undisturbed for some time, these floccules tended to slide down the sloping surfaces of the bottom of the tube to the lowest and central point, so that the final picture was similar to that seen in the saline controls and with negative sera. Kaolin, in the proportions of 1 g to 10 ml of serum dilution 1:10 in phosphate buffer (pH 7), was found capable of completely adsorbing the inhibitory factor, while causing only a slight drop in antibody titre.

Some typical results are shown in Table I.

The agglutination of mallein-sensitized cells by the serum of the patient suffering from infectious melioidosis is of interest. This serum has also been shown to agglutinate cells sensitized with a similar antigen preparation from *Pf. whitmori*, although this preparation failed to sensitize the cells to agglutination by anti-mallein serum except when used in much higher concentrations (100 fold). Even then,

1. Salk, J. E., *J. Immunol.*, 1944, v49, 87.

2. Hole, N. H., and Coombs, R. R. A., *J. Hyg. Camb.*, 1947, v45, 480, 490.

the agglutination was weak and uncertain, and only occurred in sera in relatively high concentrations.

Little work has so far been carried out on the chemical nature of the adsorbable antigen and of the receptor on the red cell responsible for the adsorption. The experiments of Keogh and North(3) with *H. influenzae* and other organisms, and of Middlebrook and Dubos(4) using saline extracts of tubercle bacilli after extracting with 90% phenol, suggest that a bacterial polysaccharide, either alone, or in combination with some other substance, may be involved.

In connection with the receptor on the red cell, the following experiment may be worth reporting. An alcohol-ether (3:1) extract of 10 ml of washed packed horse red-cells was prepared: the extract was evaporated and the residue resuspended in 25 ml saline with the aid of a little ether. Two rows of tubes were set up each containing twofold dilutions in 0.5 ml of the red cell extract suspension, and were allowed to stand at 37°C to let the ether evaporate. To the tubes of the first row were added 2 sensitizing doses (for 1 drop 5% red cells) of mallein, in 0.1 ml saline; after one hour at 37°C one drop of 5% horse red cells were added, and the mixture incubated for another hour. In the second row, the red cells were added first, and the sensitizing antigen at the end of the first hour. Then all the tubes were centrifuged and the cells washed twice, before adding to each two agglutinating doses of an anti-mallein serum, contained in 0.5 ml saline. The results were as follows:

Tube No.	1	2	3	4	5	6	7	8	9	10
Row 1	—	—	—	—	—	—	2+	3+	4+	4+
Row 2	—	+	+	+	2+	4+	4+	4+	4+	4+

The alcohol-ether extract, therefore, contained some substance or substances capable of inhibiting the sensitization of horse red cells by mallein to agglutination by anti-mallein sera.

The difference between the rows 1 and 2 is consistent with the interpretation that this inhibition is due to an affinity of the inhibitor for the antigen rather than to a mechanical blocking effect resulting from a coating of the red cell surface. In other words, there appears to be true competition between the extracted substance and the red cells for the antigen.

Summary. Erythrocytes of the horse and some other species treated with mallein were rendered thereby agglutinable by high dilutions of anti-mallein sera and by the serum of a human patient suffering from infectious melidioidosis. Erythrocytes of ox and pig could not be sensitized to agglutination under the same conditions. The sensitization by mallein of horse red cells to agglutination by antimallein sera was inhibited by an alcohol-ether extract of these cells.

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3. Keogh, E. V., North, E. A., and Warburton, M. F., *Nature,* 1948, v161, 687.

4. Middlebrook, G., and Dubos, R. J., *J. Exp. Med.,* 1948, v88, 521.