When the concentrated fractions of agglutination factor were lyophilized, all activity was lost. This was unexpected as whole plasma containing the active fraction may be kept indefinitely when treated in this manner.

Summary. The active principle responsible for sheep cell agglutination test for arthritis was purified and concentrated by salt fractionation and by dialysis against solutions of low ionic strength. The most active fractions were precipitated as plasma was adjusted from a concentration of 9% to a concentration of 14% sodium sulfate, or from ionic strength 0.06 to ionic strength 0.04. The electrophoretic fraction most consistently present in the concentrate was beta globulin. The agglutination factor is adsorbed on fibrin and is inactivated during lyophilization of concentrates and by the addition of alcohol to arthritic plasma.

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Effects of Cortisone on Blood Cells after Thermal Stress.* (20769)

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In the course of experiments on inflammatory phenomena (to be published) rabbits were subjected to extensive thermal injuries, and data on erythrocytes, leukocytes, and temperature were recorded. It was considered worthwhile to make a parallel series of records on rabbits treated with cortisone. A comparison of these is the basis for this report.

Methods. Male New Zealand whites weighing 1800 g to 2300 g were used. They were given a routine diet of rabbit pellets, greens and water during a period of one to 3 weeks and frequent blood counts were made to establish the norm for each animal. None was used if significant variations in blood count, temperature, or behavior indicated that the animal's health was not normal. Experiments were made on 4 groups of 10 each. The fur over the back, shoulders, sides and hips was sheared closely with clippers. The rabbit was anesthetized deeply with ether and the areas mentioned were dipped for 5 seconds into water at a temperature of 88° to 95°C. Pain was prevented by frequent injections of morphine during the subsequent 24 hours. Blood counts and temperature were taken at intervals ranging from 30 minutes to 7 hours

* We are indebted to Merck and Co. for generously supplying the Cortone® Acetate.

and again at 24 hours, after which the animal was anesthetized and killed. The scalded areas were estimated at about ⅓ of the total skin surface. One group of 10 rabbits had received cortisone by intramuscular injection as follows: on the day before experimentation, 2 injections of 12.5 mg each, and one injection of the same amount just before the experiment began. The same dosage of cortisone was given to another group of 10 rabbits before thermal injury by freezing. Rabbits were prepared and anesthetized for freezing as described above for scalding. A freezing mixture was made by placing lumps of solidified CO_2 (dry ice) into a basin of 95% alcohol. The back, shoulders, sides and hips of the animal were immersed in this for 60 seconds at temperatures ranging from —50°C to —60°C. Two groups of 10 each were subjected to freezing. Anesthesia, tests and after-treatment were identical to those described for injury by heat.

Results. Erythrocytes. A prompt increase in the count of red cells occurred in every instance. This was evident in some rabbits within 15 minutes after scalding and reached its maximum within one to 6 hours. The average degree of hemoconcentration for each group and the speed of its development are shown graphically in Fig. 1. Fig. 2 portrays the same features after freezing.

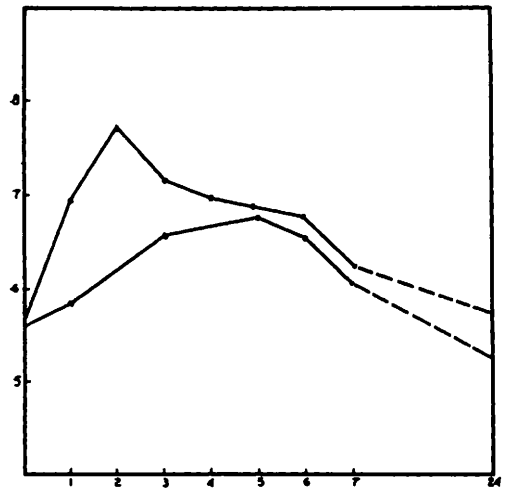

FIG. 1. Hemoconcentration after Stress by Heat. Red cells in millions are shown at the left; elapsed time in hr is shown at the bottom. The upper graph represents untreated animals; the lower graph represents animals treated with cortisone.

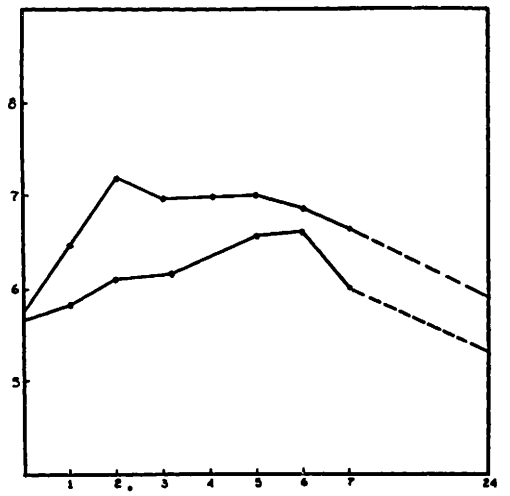

FIG. 2. Hemoconcentration after Stress by Freezing. Details as for Fig. 1.

The curves in Fig. 1 depict the rapid development of hemoconcentration in animals after stress by heat. The average concentration of erythrocytes rose sharply after injury and reached its peak 2 hours later. In contrast, the rise was more gradual in the cortisone-treated animals. The peak was lower and was reached 3 hours later than that of the untreated group. This represents a time lag of 150%. In other words, the development of hemoconcentration was definitely retarded under the influence of cortisone. The variations in hemoconcentration after stress by freezing (Fig. 2) are of the same kind but less in degree than those after injury by heat. Again, the curve in cortisone-treated animals rose more slowly and reached its maximum 3 hours later than that of the untreated group. The fact that these curves are lower than in Fig. 1 may indicate that scalding was a more severe injury than freezing. We have found no reports on the effects either of ACTH or of cortisone upon hemoconcentration during stress of whatever origin.

It is well known that hemoconcentration develops in man after severe burns. Usually this subsides within 2 or 3 days and is succeeded gradually by anemia. Students of capillary physiology have attributed hemoconcentration to endothelial effects. Krogh

(1) and Lewis(2) showed that when epidermal cells are injured by light, heat, trauma, various toxins or chemicals, they release a substance (H-substance—Lewis) which causes dilation and increased permeability of the capillaries locally. This results in hyperemia and edema, due to leakage of plasma into the tissue spaces. Landis(3) stated that any type of injury to endothelium increases the permeability of capillaries to the plasma colloids. Loss of plasma leaves the blood more concentrated. There is evidence(4) that in cases of extensive injury the capillaries in systemic areas are similarly affected, though not everyone supports this belief. Certainly in extensive burns in man, the ensuing hemoconcentration is due largely to local loss of plasma as blister fluid. Burns do not cause blisters nor denuded oozing surfaces on rabbits and other animals, but local edema develops under burned areas. Whether or not superficial burns produce capillary permeability in systemic areas, the present knowledge of capillary physiology supports the explanation that hemoconcentration after burns results from abnormal permeability of endothelium to plasma colloids.

Kendall(5) demonstrated that one important function of adrenal cortical hormones is to govern permeability and to control the movement of water and of electrolytes between the blood and the tissues. This has been substantiated by Selye(6) and others, and it has been shown that Compound E (cortisone) is largely responsible for this effect. Best and Taylor(7) state that the cortical steroids reduce capillary permeability. Ebert(8) and Lurie(9) reported that cortisone has this effect in reactions to injury. Balourdas and Chambers(10) found that cortisone caused a striking reduction of capillary leakage in the exteriorized mesoappendix of rats. We(11) found a definite decrease in the amount of edema formed in experimental inflammation under cortisone treatment. This was attributed to lessened capillary permeability. Whitelaw(12) used ACTH in the treatment of patients suffering extensive burns; he reported that oozing from burned surfaces decreased progressively. Hemoconcentration after burns and early in secondary shock from other causes is accompanied by a decrease in the volume of circulating blood. The evidence cited indicates that this is due to leakage of plasma through abnormally permeable endothelium and that cortisone tends to lessen this effect. Hence cortisone tends to sustain the blood volume and to retard hemoconcentration. We have found no report that cortisone actually increases blood volume except after prolonged use, when signs of Cushing's syndrome develop. Finch *et al.* (13) recorded blood volumes and other items in 20 patients during several courses of cortisone treatment; no significant changes in blood volume were found. We believe that the retarded hemoconcentration in our experiments was due to the effects noted by these authors. We have no evidence whether the lessened permeability caused by cortisone was other than local.

Leukocytes. Cortisone caused a significant reduction in the total count of white cells in the blood. This leukopenia explains the low initial point for the leukocytic curves of cortisone-treated animals in Figs. 3 and 4. In 17 rabbits, differential counts had been made both before and after cortisone was given and prior to thermal injury; in 3, only total white counts had been made. The average white cell count of this group was 6,350.

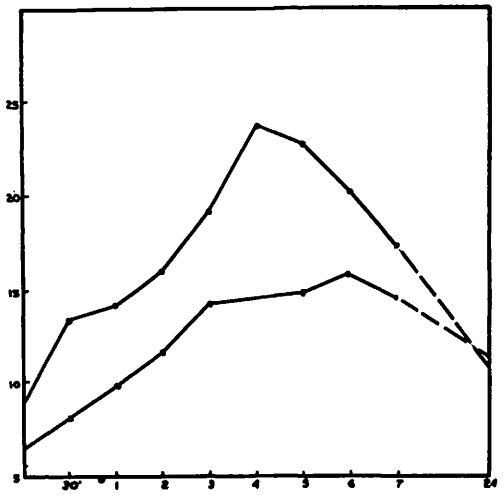

FIG. 3. Leukocytosis after Stress by Heat. Leukocytes in thousands are indicated at the left; elapsed time in hr is shown at the bottom. Untreated and cortisone treated animals are shown on the upper and lower graphs respectively.

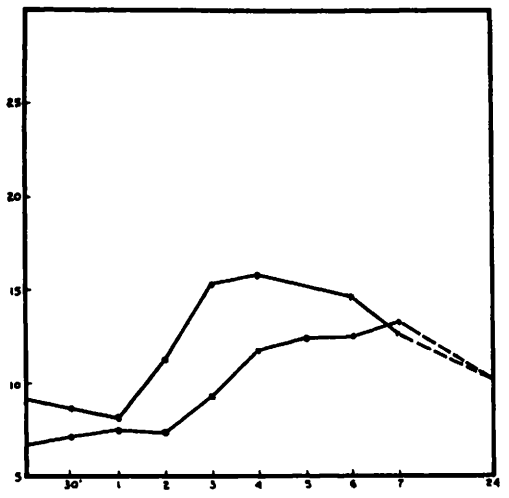

FIG. 4. Leukocytosis after Stress by Freezing. Details as in Fig. 3.

This decline is attributed largely to lymphocytopenia after cortisone treatment. The average percentage of lymphocytes in this group was 58%; after cortisone injections and before injury, the lymphocyte count dropped to 27%—a difference of 31%. Now, if 8,775 is reduced by 30%, it leaves 6,143 which is near to 6,350—the average total white count found after cortisone treatment. The lymphocytopenic effect of cortisone has been observed by other workers (see Dougherty)(14). It is well known that extensive superficial burns in man cause an immediate leukocytosis. We have not found similar reports after exposure to severe cold, though this is certainly a powerful stressing agent. In the experiments here described, white cell counts were made both before and at intervals after thermal stress, both heat and cold, in 10 cortisone-treated and in 10 untreated rabbits. The results are shown graphically in Figs. 3 and 4.

It is seen (Fig. 3) that a sharp rise in the total white count occurred immediately after injury by heat, and reached its peak 4 hours later. In the cortisone-treated animals the rise was more gradual, the peak was not so high and was reached 2 hours later than in the untreated group. Differential counts showed the increase to be in the percentage of polymorphonuclear cells. There was a moderate decline in the percentage of lymphocytes found in the blood of the untreated animals. Before stress by heat, the lymphocytes were 54.7% in this group; 4 hours later it had declined to 42.2%. Probably this has little significance, since the high increase in neutrophiles would result in a lower relative percentage of lymphocytes. Leukocytosis in each group had decreased greatly in 24 hours, but had not subsided completely. No examinations of blood were made during the interval between 7 and 24 hours.

We(15) have shown evidence that substances derived from damaged cells attract leukocytes into areas of local injury, as after burns, freezing or trauma. One of us(16) advanced the explanation that probably the same substances produce a systemic mobilization of leukocytes when released in large amounts by extensive injuries of the same kind. The fact is well attested that extensive burns or trauma are followed promptly by leukocytosis. This occurs too rapidly and subsides too soon to be caused by infection. Our results indicate that cortisone retards the leukocytosis which follows burns.

After freezing, blood counts made at 30 minutes and at 60 minutes showed a *decline* in white cells in the 10 untreated animals (Fig. 4). This was followed by a sharp rise reaching its peak 4 hours after the injury. In the cortisone-treated animals, no significant changes occurred until 2 hours after freezing, and the peak was reached at 7 hours—3 hours later than in the untreated group. Apparently, cells injured by freezing do not so promptly liberate substances which affect the count of leukocytes, as do cells injured by heat.

Summary. 1. Our results indicate that cortisone inhibits and retards the development of hemoconcentration after thermal stress in rabbits. We attribute this to its property of maintaining endothelial impermeability. 2. After injections of cortisone the total white cell count is lowered, due largely to severe lymphocytopenia. This finding agrees with previous reports by others. 3. Thermal stress in rabbits is followed by leukocytosis. This is of less degree and develops more slowly under the influence of cortisone.

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Comparison of Hypotensive Action of Sodium Azide in Normotensive and Hypertensive Patients.* (20770)

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In the course of investigations on the effects of various inhibitors on human cancer patients (1), it was noted in cases with co-existent hypertension that the acute administration of sodium azide caused a temporary lowering of the blood pressure toward normotensive levels. In contrast, comparable doses of azide produced no appreciable change in the blood pressure of normotensive individuals. These incidental observations led to a more extended study of the hypotensive effect of sodium azide in both man and experimental animals. A review of the literature indicated that Graham(2) had made an intensive study of the pharmacological effects of sodium azide. However, he made no mention of its effect on hypertensive patients. Page and Olmsted(3), as an incidental finding in studies on vascular reactivity, mentioned the greater susceptibility of dogs with either renal or neurogenic hypertension to the acute hypotensive effects of sodium azide.

* This investigation was supported by a grant from the Leukemia Research Foundation.

In view of the finding that sodium azide, a known metabolic inhibitor(4), produced a more pronounced lowering of blood pressure in hypertensives than in normotensives, it appeared of direct interest to use sodium azide as a tool for investigating hypertensive disease in man and experimental animals. Furthermore, the possibility was entertained that repeated administration of azide to hypertensives might so alter tissue metabolism as to result in a sustained lowering of the blood pressure. Although the drug is usually considered to be highly toxic, it should be noted that the lethal range (10 mg/kg subcut.)(5) is far in excess of the oral dosage required to produce a fall in blood pressure (.01-.02 mg/kg).

Materials and methods. A comparison was made between the effects of sodium azide in normotensive and hypertensive individuals. Included in the normotensive series are normal healthy controls (students, laboratory personnel), as well as patients suffering from diverse types of cancer. The patients with