Discussion. Our glass purification experiments have indicated an origin of macrophages from monocytes(6) and of PHA-cells from lymphocytes. It is our impression that macrophages represent highly differentiated, specialized end cells. The PHA-cell, on the other hand, would seem to be a reversion to a less differentiated cell type. Its formation from lymphocytes is significant in the light of the opinions of such observers as Yoffey and Courtice(10) and Maximow(11) that the lymphocyte is a multipotential cell capable of becoming the stem blood cell. The PHA-cell may be related to hematological blast cells, but there is no evidence for its ability to transform into a more differentiated cell. We are in accord with McCulloch and Parker (12) who suggested caution in applying hematological terminology to altered cells in tissue culture.

We observed that blood cells of patients with chronic lymphocytic leukemia usually failed to transform to PHA-cells. If PHA-cells are derived from lymphocytes, it may be concluded that leukemic lymphocytes had an impaired ability to transform into blastlike PHA-cells.

Further experiments are in progress to study the PHA-cell and it has been found that a similar type of cell could be obtained with tuberculin-purified protein derivative(13).

Summary. Suspensions of normal human blood cells were incubated at 37°C with phytohemagglutinin (PHA) in slide chambers. In 2 to 4 days, the reagent stimulated the formation of large blast-like cells (PHA-cells) which could be readily differentiated from macrophages. PHA-cells developed in purified suspensions of lymphocytes and many intermediate cells were seen between PHA-cells and lymphocytes. PHA-cells did not develop in blood cell suspensions of some patients with chronic lymphocytic leukemia nor in suspensions of rat blood cells. The findings suggest that PHA-cells were derived from normal human lymphocytes and should be differentiated from macrophages.

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Chlorpromazine in Protection from Acute Effects of Extensive High Temperature Skin Injury in Rats.* (28318)

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The mortality from high temperature skin injury can be greatly reduced by proper fluid management. Thermal skin injury may nevertheless, despite extensive fluid therapy and according to the degree and area of the burn, result in a gradual impairment of homeostasis which progresses to a point of insufficiency similar to the fatal peripheral circulatory collapse following mechanical trauma and hemorrhage. In common with other forms of shock,

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extensive thermal injury is associated during the early period with a generalized peripheral vasoconstriction which is believed to be a major factor contributing to the ensuing fatal condition(1). Pretreatment with procedures and agents which favor vasodilation through neurogenic or a direct action on smooth muscle has been reported to protect from the lethal effects of various shock initiating procedures other than burns(2-7). In contrast, administration of the vasodilating agents after shock has developed fails to reduce mortality in experimental animals(8-10).

The shock condition following extensive thermal injury usually develops more slowly than shock initiated by other experimental procedures(11). It was therefore of interest to determine what effects interference with metabolism *after* a lethal burn might have on the mortality in the subsequent shock phase. In this study chlorpromazine, 2-chloro-10-(3-dimethylaminopropyl) phenothiazine, was used on the rationale that it may generally attenuate metabolic activity and increase peripheral circulation by counteracting sympathetic vasoconstriction.

Materials and methods. Four hundred young adult male albino rats of the Wistar strain, weighing 250-300 g were used in these experiments. Full thickness high temperature skin burns were produced under ether anesthesia by a standardized technic for quantitative thermal injury, exposing (3 seconds on the abdomen, 5 seconds on the flanks and on the back) a calculated area closely shaven skin to a thermo controlled (250°C) metal surface through asbestos frames.§ The animals were divided into 5 groups. Both experimental and control animals were weighed and shaved on the day before the burn procedure. Two groups (IV and V), received chlorpromazine (Smith, Kline, and French Laboratories) intraperitoneally in doses of 1.5 mg/kg and 0.5 mg/kg respectively immediately after burn and on the 2 following days after injury. All groups of animals were anesthetized and Groups III, IV and V were given dextran 6% in saline (Abbott) intraperitoneally in a dose of 30 ml/kg on the day of injury immediately after the burn procedure and in the morning on the following 2 days. They were then returned to individual cages with water and food *ad libitum* in a special recovery room kept at 20°C and with 40% relative humidity. Group I was kept under conventional laboratory conditions. Extreme cleanliness was observed in all these experiments to minimize bacterial infection. Coding of animals and statistical treatment of the data were carried out according to current practice in biology(12). Autopsies were performed on all animals.

Results. Environmental Factors. Group I, which received no treatment, was kept under conventional laboratory conditions showed a high mortality on the first day. The ambient temperature in these experiments ranged from 16° - 28°C and humidity was high and varied accordingly. A substantial increase in survival was afforded, also without treatment, in Group II by controlling the environmental temperature and humidity. (Fig. 1).

Fluid Treatment. Survival during the first 12-48 hours was increased further in Group III when fluid therapy was given. The differences in survival after 6 hours were significant at the 95% level (Fig. 1).

Chlorpromazine and Fluid Therapy. When chlorpromazine was administered in a dose of 1.5 mg/kg rat, no deaths occurred immediately after injury and the 24 hour mortality fell to 4.8% or one-fourth of the mortality in the group with fluid treatment alone. By 48 hours, however, the chlorpromazine-treated rats showed a sharply diminished incidence of survival below that of animals receiving fluid therapy alone, and about the same as that of the untreated group kept under conventional conditions.

Chlorpromazine in a dose of 0.5 mg/kg rat also circumvented the high 24 hour mortality. Although the lesser dose of the drug appeared to be better tolerated at 48 and 72 hours, cumulative mortality was somewhat higher than fluid treatment alone (Fig. 2).

The differences between the 24 hour cumulative mortality percentages of Groups III and IV, Groups III and V, and the difference

§ This technique was originated in collaboration with O. J. Malm.

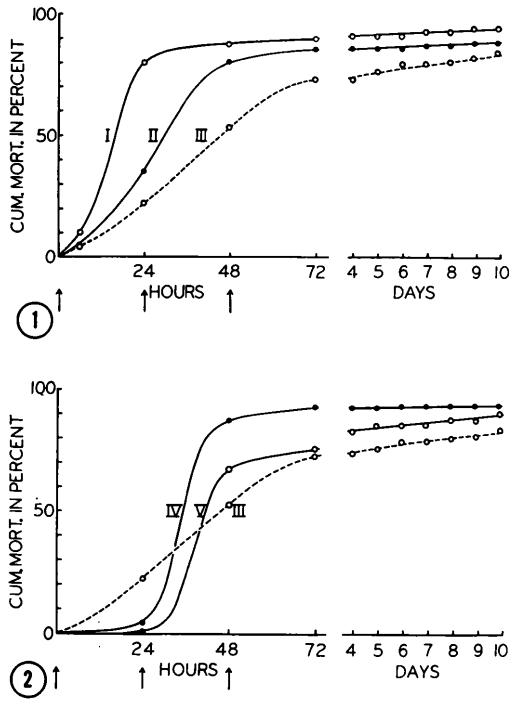

FIG. 1. Group I O—O—O 95 rats, 40% BSA "Standard" burn. Uncontrolled environmental conditions. Group II ●—●—● 85 rats, 40% BSA "Standard" burn. Controlled environmental conditions. Group III O---O---O 85 rats, 40% BSA "Standard" burn. Controlled environmental conditions plus fluid therapy at times indicated by arrows.

FIG. 2. Group III (see Fig. 1) controls, O---O ---O. Group IV 83 rats, same as III plus 1.5 mg chlorpromazine/kg rat, ●—●—●. Group V 50 rats, same as III plus 0.5 mg chlorpromazine/kg rat, O—O—O. Fluid and the drug were administered at times indicated by arrows.

at 48 hours between Groups IV and V are statistically significant, whereas the difference between Groups III and V at 72 hours is not.

Autopsies. Pulmonary effusion was frequent in the early fatalities both in the untreated and in the animals receiving fluid therapy in contradistinction to those which had received chlorpromazine. Spleens were small and dark in the former animals but appeared normal in size and color in the chlorpromazine treated rats. The splanchnic vascular bed in the mesentery and intestinal tract appeared more dilated in treated rats than in controls; the skin did not have as blanched an appearance as the rest of the groups.

Discussion. It is apparent that chlorpromazine afforded a substantial protection from early death, over and above that achieved by fluid therapy alone, during the shock phase following extensive burns. In high doses, chlorpromazine had a striking protective action which in turn was followed by a subsequent deleterious effect on the burned rat. This is in contradistinction to the long range survival usually observed under conditions of hemorrhagic and endotoxin shock once the immediate lethal effects of the noxious stimuli have been counteracted by chlorpromazine.

Rosenthal(13) and others have extracted toxic agents from exudates and tissues of animals injured with hot water. Such extracts have been shown to produce vasodilatation and a fall in systemic arterial blood pressure when injected intravenously(14), an effect analogous to that of hypotensive principles believed to be operative in shock(15). In the present experiments a "hypotensive" agent increased survival in the early shock phase, whereas mortality was not reduced at a later period in which the greatest absorption from the burn wound has been reported to take place. The experiments therefore suggest that hypotensive agents released from the heat denatured tissue do not have important systemic consequences in the immediate postburn period. The question of circulating adrenolytic-type agents inhibiting or impairing vascular smooth muscle contractility remains, however, to be settled(11).

In contrast to the concept of toxins, inhibitors and adrenolytic agents, it is possible that the neurohormones *per se* and similar metabolically active agents are directly involved in creation of the promiscuous condition of burn shock, by "one-sided" overstimulation of the metabolic machinery in the absence of adequate oxygen supply(1). In this context, agents which elicit an immeditate active contractile event in the vascular elements are called vasoactive. Vasotropic refers to those substances which may modify such responses.

As judged from the oxygen consumption in fluid treated burned rats in a previous study (11), aerobic metabolic activity becomes minimal between 24 and 48 hours despite an arterial oxygen saturation of more than 80%.

Whether the effect is the result of an actual inhibition of aerobic metabolism or a confinement of the blood flow to non-nutritional vascular beds is not known. It appears, however, from *in vitro* experiments on smooth muscle, to be reported separately(16), that chlorpromazine in the concentrations used here *in vivo* abolishes completely the contractile response to vasoactive agents after cyanide poisoning of the respiratory enzymes of the muscle, as contrasted with only a lowering of the responsiveness to the same agents in normal smooth muscle treated with the same dose of chlorpromazine.

It is not possible to assess the potential therapeutic value of chlorpromazine for long term survival of extensively burned rats on the basis of the presented data. Apparently dose level and time of administration are of crucial importance and have narrow limits. The possibility exists that much lower doses of the drug at 24 hours after burn might preserve the early beneficial effect and thereby avoid the later deleterious effects. Experiments to test this possibility are in progress.

Summary. 1) The burned rat is extremely sensitive to changes in environmental conditions. 2) Fluid therapy substantially increases survival at 24 and 48 hours, whereas 10 day survival is only moderately increased. 3) The immediately lethal consequence of burn shock is nullified by proper dose levels of chlorpromazine. 4) The same dose of chlorpromazine has a deleterious effect later in the post burn course and increases mortality when given for 48 hours or longer.

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Dissimilarity of Changes Induced in Absorption Spectrum of 2-(4'-Hydroxyphenylazo)-Benzoic Acid by Different Serum Albumins. (28319)

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The pale yellow dye 2-(4'-hydroxyphenylazo)-benzoic acid has been widely employed in quantification of human serum albumin, utilizing the method of Rutstein *et al.*(1). The dye binds selectively to the albumin of human serum, whereupon its spectral absorption increases in the region of 480 mμ, under certain conditions in proportion to the albumin concentration(1,2). Employing this dye method with human albumin standards, we