terpretation must be sought for the prolonged bleeding time when blood is aerated to the point of losing an appreciable amount of its CO_2 content. Since these samples recover their normal bleeding time after shaking in air containing sufficient CO_2, seems to indicate that a particular pH is necessary for the substances concerned in hemostasis.

Observations here reported may guide the research worker, and perhaps guide transfusionist and clinician on the manner of treating blood before employing it as a therapeutic means. In fact, according to experience of workers on platelets, refrigeration of blood as soon as it leaves the donor is essential for good preservation of these elements. However, no particular care is necessary for longer or shorter contact of blood with air. The question of content of CO_2 in blood to be transfused is unimportant, since it will attain its normal gas content in the first passage through tissue capillaries and lungs, although the significance of excessive cooling is very different, since it has been verified that loss of hemostatic property is irretrievable and not recoverable by heating. This could have some practical significance in transfusions to patients with hemorrhagic manifestations.

Summary. Employing the dog's hind-leg which permits a controlled study of bleeding time, it has been determined that refrigeration of blood for 10 minutes at 1-2°C causes it to lose its normal hemostatic properties, not recoverable by heating to 37°C. Furthermore violent aeration of blood in air, by liberating CO_2 and driving the pH to the alkaline side, also renders blood non-hemostatic, although by returning the liberated CO_2 to blood, by shaking in atmospheric air containing 9% CO_2, hemostatic action may be recovered. The mechanism of these phenomena, their application as routine methods in the laboratory, and their implication in technics of blood transfusion are discussed.

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Influence of Environmental Temperature and Chlorpromazine on Biochemical Changes Following Bowel Shock. (24077)

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Several groups of investigators have demonstrated the beneficial action of chlorpromazine* (2-chloro-10-(3-dimethylaminopropyl)-phenothiazine) on survival following hemorrhagic(1,2), traumatic(3), and bowel obstruction(4) shock. Its ability to abolish temperature regulation in animals has been described (5). Evidence for a specific effect upon thermoregulatory centers of the central nervous system is inconclusive; however the apparently complete poikilothermia suggests a primary interference with thermoregulatory centers. The influence of environmental temperature upon survival of normothermic rats following traumatic shock has been evaluated by Erickson, Smith and D'Amour(6).

This report is concerned with the possibility that the beneficial action of chlorpromazine in shock is a result of its effect on thermoregulation, leading in ordinary environmental temperatures to hypothermia and diminished metabolism.

Procedure. White female Holtzman strain rats, weighing approximately 275 g were used. The method for inducing a standard bowel shock was that described by Wendel *et al.*(4). For maximum measurable effects length of obstruction time was 4 hours. Treated animals

* Thorazine hydrochloride (Smith, Kline and French Labs.).

TABLE I. Mean Changes in Rectal Temperatures before and during Bowel Shock Procedure.

	Room temp.	Operation: Before	Operation: After	Release of obstruction	Hours following release of obstruction: 2	4	6	8
Control group	30°	37.0°	36.3°	35.6°	34.4°	33.0°	32.2°	30.6°
	24	36.7	35.5	33.4	30.4	28.2	26.1	25.0
	14	36.8	35.9	33.9	26.2	22.4	20.5	19.0
Chlorpromazine-treated group	30	37.0	35.4	38.2	38.6	38.5	38.7	38.5
	24	37.1	36.5	25.9	25.8	25.3	24.9	24.2
	14	36.8	36.0	16.8	15.8	15.0	14.8	14.6

Each temperature group contained 5 animals.

were administered chlorpromazine intraperitoneally, 12 hours prior to occlusion of the bowel segment, at dose level of 20 mg/kg body weight. Animals from both groups were placed in constant temperature environments of 14, 24, and 30°C immediately following obstruction of the bowel, following release of obstruction, and for 8 hours thereafter. Rectal temperatures were taken prior to and immediately following obstruction of the bowel, at time of release, and every 2 hours thereafter. Heparinized blood samples were obtained 4 and 8 hours following release of obstruction. Blood ammonia was determined by the microdiffusion method of Conway(7); plasma protein by the method of Lowry *et al.* (8): plasma urea nitrogen by the method of Genzkow(9): and plasma glutamic-oxalacetic transaminase by the method of Karmen(10).

Results. Table I presents data showing changes in body temperature of control and chlorpromazine-treated animals kept in the 3 different temperature environments. Table II presents data showing changes in biochemical constituents of blood examined in all groups of animals. Biochemical changes were negligible at lower body temperatures, not entirely because metabolic rate was decreased, but presumably also because of decreased bacterial activity due to considerable cooling of the animal (Table I).

Discussion. Poikilothermic drugs, including chlorpromazine, inhibit in some manner the thermoregulatory mechanism in animals(5) thus requiring the animal to assume the temperature of environment, and to decrease metabolism by 10 to 20%(11). In normothermic animals, survival time following shocking is significantly increased when the animal is kept in environmental temperatures between 17.5 and 25°C(6).

The value of inhibiting metabolic processes of animals in shock has been demonstrated by Kovach *et al.*(12) who administered thiouracil during the shock state and found increased survival time. Conversely, epinephrine stimulation of the metabolic rate reduced survival time. Whether such retardation of metabolic processes is accompanied by a sparing effect on high energy phosphate stores is unknown. On the other hand it has been shown that the adenosine triphosphate content is significantly increased in brain(13), and liver (Wendel and Brophy, to be published) following adminis-

TABLE II. Mean Biochemical Values for Whole Blood and Plasma following Release of Obstruction.

	Room temp.*	NH_3-N, μg/100 ml: 4†	NH_3-N, μg/100 ml: 8	Urea-N, mg/100 ml: 4	Urea-N, mg/100 ml: 8	Protein, g/100 ml: 4	Protein, g/100 ml: 8	PGO-T,‡ units/ml: 8
Control group	30°	422	1349	25.9	5.6	5.69	4.46	193
	24	391	1420	21.6	8.4	5.79	5.20	89
	14	243	277	9.6	10.5	6.56	6.44	50
Chlorpromazine-treated group	30	784	1630	31.8	4.3	5.04	4.81	203
	24	261	454	24.2	31.7	6.11	5.63	46
	14	38	88	12.4	16.8	6.16	6.19	34

* Each temperature group contained 5 animals.
† Hours following release of obstruction.
‡ Plasma glutamic-oxalacetic transaminase.

tration of chlorpromazine. The value of protecting high energy phosphate levels in liver during shock, and its influence on survival have been demonstrated(14).

An increase in plasma urea nitrogen indicates a compensation for increased ammonia production with its subsequent detoxification by the liver, whereas a decrease in urea nitrogen with continued high ammonia levels seemingly indicates liver failure (Krebs-Henseleit cycle). Increased plasma glutamic-oxalacetic transaminase activity indicates liver damage according to Wroblewski and LaDue(15). Decreased plasma protein levels have been observed(16) following induction of shock, thus indicating extensive protein breakdown as a significant factor in aminoacidemia of shock(17).

In the present study, the increases noted in blood ammonia may have been due in part to extrinsic changes in the gastro-intestinal tract, namely increased bacterial activity in the gut. This also was manifested grossly by distention of the bowel segment following obstruction, and by necrosis in bowel wall observed at necropsy.

More interesting, however, is the difference effected by chlorpromazine at 24°C. Here the chlorpromazine-treated animals more quickly assumed the lower environmental temperature, more effectively maintained urea production and blood protein level. At 8 hours after release of bowel obstruction the animals were still actively removing ammonia whereas control animals were not. This lends credence to the suggestion that by this time the controls had already seriously drained the body resources (high energy phosphate?) needed to support this function and that a function of chlorpromazine in the other animals had been to spare these resources by permitting a more rapid development of hypothermia and more rapid reduction in metabolism.

At 30°C the chlorpromazine abolition of body temperature regulation *lessened* the ability of animals to cope with the effects of bowel shock. Here the chlorpromazine-treated animals developed temperatures higher than those of controls, produced more ammonia, and were depleted more rapidly. Thus there is further support for the idea that hypothermia is the important factor for survival in bowel shock. Chlorpromazine is not indicated as a specific survival factor. It has this effect only indirectly, and only if the environmental temperature is below a critical level, to permit development of hypothermia.

Our study demonstrates that bowel obstruction shock is characterized by progressive increases in protein catabolism, probably by most tissues, once the shock syndrome is established, with the release of ammonia into blood. The liver is flooded with ammonia and presumably with amino acids, which are available for deamination and urea formation. At first, the liver seems capable of meeting the demands and urea production is increased, but later it begins to fail and ammonia leaves the liver.

It is thought that 2 mechanisms participated in the bowel shock syndrome, either alone or in union: (1) Following pre-treatment with chlorpromazine, and a lowering of body temperature to that of a hypothermic level, bacterial activity may have been inhibited, thus preventing formation of lethal toxins, toxic amines, ammonia, and other protein breakdown products generally believed to be implicated in the lethal consequences of the shock syndrome. (2) Diminution of metabolic processes consequent to lowered body temperature may have resulted in reduction in amounts of energy expended, thus enabling the liver to detoxify the products of bacterial activity—a sparing effect.

Summary. (1) The extent of biochemical alteration in experimental bowel obstruction shock was related directly and consistently to actual rectal temperatures developed, in both normothermic control rats and those made poikilothermic by chlorpromazine. (2) The value of hypothermia in protecting rats from bowel shock has been demonstrated. (3) Chlorpromazine was beneficial in shock only when its use led to enhancement of hypothermia. Its use at a higher environmental temperature facilitated development of *hyperthermia,* and aggravated the biochemical syndrome of bowel shock. Chlorpromazine ap-

pears to have no specific protective action against shock. (4) Possible mechanisms are discussed.

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Control Mechanisms for Trauma Induced DLSH Reaction (Decrease in Mouse Liver Non-Protein Sulfhydryl).* (24078)

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The DLSH reaction is a term used to refer to a statistically significant decrease in liver non-protein sulfhydryl (NPSH) or glutathione (GSH) which occurs within a few hours after subjection of animals to certain experimental procedures. This reaction has been found to occur in mice and/or rats in association with severe trauma(1,2,3), severe cold(1,2,4,5), or administration of toxic or pharmacologic amounts of bacterial polysaccharide(2), insulin(6,7) or epinephrine(8). Register and Bartlett(5,8) have postulated that such a reaction occurring in rats subjected to severe cold, is actually due to stressor activation of the sympatho-adrenal system, particularly to release of epinephrine from the adrenal medulla. Our data indicate that in the DLSH reaction induced in mice by tourniquet trauma, both the sympathetic nervous system and the adrenal cortex play essential roles, whereas the adrenal medulla and the islets of Langerhans do not.

Methods. Mice were made diabetic by injection of alloxan, 100 mg/kg, into the tail vein (cf. Waisbren(9)). In bilateral adrenalectomies each kidney and attached adrenal gland was gently exteriorized through incision immediately lateral to backbone; effectiveness of adrenalectomy was checked in each mouse by autopsy at time of sacrifice. Exteriorization of the kidney and attached adrenal gland also proved extremely useful in adrenal demedullations, performed by an adaptation of the method of Evans(10). Effectiveness of adrenal demedullation procedure was checked for each group of mice by chemical estimation of adrenal medullary hormone content of the pooled adrenals. Tourniquet trauma was performed by the double hind leg ligation method of Rosenthal(11). The ligation period was 2 hours, and sacrifice was usually 2 hours after

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