8. Mota, I., Beraldo, W. T., Junqueira, C. U., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 455.

9. Ottoson, R., Glick, D., *Exp. Cell Res.*, 1958, in press.

10. Benditt, E. P., Wong, R. L., Arase, M., Roeper, E., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 303.

11. Thomas, L., *Ann. Rev. Physiol.*, 1954, v16, 467.

12. Compton, A. S., *Am. J. Anat.*, 1952, v91, 301.

13. Hill, M., *Experientia*, 1957, v13, 395.

14. Morris, A. L., Krikos, G. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 527.

15. Velican, C., Velican, D., *Acta haematol.*, 1958, in press.

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Influence of Cooling and CO_2 Content of Blood on Bleeding Time.* (24076)

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Application of a physiological method using hind-limb preparation for studies of bleeding time(1), revealed that certain procedures, such as shorter or larger aeration or more careful and longer refrigeration of blood samples, had an effect on this phenomenon. A systematic study of these effects is here presented.

Methods. As details of the method employing hind-leg of dog for study of bleeding time are in press, including hematocrit, hemoglobin, platelet and clot retraction determinations, we present here a summary of this method. *Hind leg preparation*: Incision of approximately 12 cm on inner side of thigh, beginning at crural arch and following inner edge of sartorial muscle. Dissection of artery, vein and femoral nerve. Isolation of the saphenous branch of the artery and femoral vein, tying off collaterals. Attach 2 threads proximal and distal, to the saphenous branch. Intravenous injection of Liquémine (325 UI Heparin), 0.65 ml/kg of body weight. Plastic tubes were inserted into femoral artery and vein of the heparinized dog and the leg isolated by tourniquet at base of thigh, leaving free the artery, vein and femoral nerve. Arterial blood (citrated) to be tested was forced from a flask by air under variable pressure into the arterial inflow tube and collected as it dripped from the venous outflow. Flow rate was kept constant and controlled by varying flask pressure to maintain an average drop rate of 50 ± 10/minute. Bleeding time was determined on shaved thigh by making 2 shallow razor cuts below incision and wiping away the blood continuously with absorbent paper until bleeding stopped. Blood gas determinations were made with manometric apparatus of Van Slyke(2). For pH determination a direct pH reading meter was used (Electronic Instruments, England). Tests for accuracy were made several times during each experiment with appropriate buffer solution. Blood was aerated by shaking in open flask for half hour. Blood samples previously aerated were restored to normal CO_2 content by agitation 3 hours in flasks containing air plus 9% CO_2. When not in use blood samples were under petroleum-jelly. Blood was refrigerated in environment of water and ice cubes and kept between 1° and 2°C, with occasional stirring, in refrigerator for a half hour; reheating was gradual, leaving blood at room temperature for about half hour, then in water-bath at 37°C, 3 hours. Refrigeration of blood from heparinized dog (1593) and reinjection of this blood after previous rewarming was done as follows: 100 ml of blood was taken from the carotid and refrigerated under petroleum jelly at 1° to 2°C for 15 minutes, then reinjected into saphenous vein after passing a glass coil immersed in water-bath at 40°C. This procedure was repeated every 15 minutes until approximately a whole blood volume of the dog was thus treated.

Results. Cooling Experiments. Normal citrated blood samples from 11 dogs (normal

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TABLE I. Infinitely Prolonged Bleeding Time of Arterial Blood after Aeration.

Aeration time, min.	Blood gases (vol %) Under petroleum jelly CO_2	Under petroleum jelly O_2	After aeration CO_2	After aeration O_2	pH Under petroleum jelly	pH After aeration	Bleeding time (min.) Under petroleum jelly	Bleeding time (min.) After aeration
5	23.0	11.6	23.0	13.1	7.55	7.55	9	11
10			16.9	13.3	7.55	7.75	6	+30
30			6.6		7.55	8.15	8	+30
30	19.7	15.9	5.6	18.0			8	+30
30	22.3	15.4	14.7	16.8			7	+30
30	31.9	11.6	18.6	14.8	7.10	7.25	9	+30
30	30.0	10.2	10.6	14.2	7.23	7.66	7	+30
30	30.8	13.5	7.2	16.2	7.25	7.56	9	+30
30							9	+30

values of platelets, hematocrit, hemoglobin and after addition of calcium chloride coagulation and clot retraction time) when cooled for 10 or more minutes at 1° to 2°C lose their previous ability to control experimental skin bleeding in isolated dog's hind-leg preparation. The above phenomenon was confirmed in whole animal when by successive bleedings and cooling of blood samples and reinjection of these samples after previous heating at 37°C, the bleeding time was progressively prolonged. 140 minutes after beginning of the test, the bleeding time was higher than 30 minutes and remained so until end of experiment (4 hours).

Experiments on blood aeration. Table I shows that blood samples from 7 dogs after prolonged aeration, show increase of pH and O_2 content, decrease of CO_2 content and loss of previous ability to control bleeding. If aeration is slight and the blood gas content is not much modified, the loss of hemostatic property is not manifest. If prolonged aeration is followed by adequate exposure to air plus 9% CO_2 (Table II) the pH decreases slightly, blood gas content remains about normal and retains ability to control bleeding. If CO_2 content does not increase to near normal level, after CO_2 exposure, as in one sample shown in Table II, ability to control bleeding is not recovered.

Confirmation that exposure of blood to air and consequent loss of bleeding control is due to a mechanism of liberation of CO_2 is found when blood samples from 12 dogs kept under petroleum jelly or taken into recipient with air plus 9% CO_2 showed a similar behavior, with bleeding times between 5 and 12 minutes when tested in hind-limb preparation.

Discussion. Two alternatives may explain the infinitely prolonged bleeding time after refrigeration. It may be the result of precipitation of an active protein, a substance of the type identified in certain clinical forms as crioglobulins(3), refrigeration being an agent which subtracts from the blood a principle active in haemostasis. An alternative is that low temperature may constitute an exciting factor, causing liberation of substances of antihemostatic character, by elements in blood (probably platelets). The mechanism of this phenomenon is being studied. A different in-

TABLE II. Normal Bleeding Time Recuperation of Aerated Arterial Blood following Suitable Time Exposure to Air plus 9% CO_2.

Time of exposure to air + 9% CO_2 (min.)	Blood gases (vol %) Under petroleum jelly CO_2	Under petroleum jelly O_2	After CO_2 exposure CO_2	After CO_2 exposure O_2	pH Under petroleum jelly	pH After CO_2 exposure	Bleeding time (min.) Under petroleum jelly	Bleeding time (min.) After CO_2 exposure
30	22.5	15.4	14.7	16.8			7	+30
110							7	8
120	26.7	20.0	28.4	17.9	7.20	6.10	6	8
After aeration			7.2	16.2		7.56	9	+30
180	30.8	13.5	32.5	13.5	7.25	7.13	6	8

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.

1. Cruz, W. O., Oliveira, A. C., *J. Appl. Physiol.*
2. Van Slyke, D. D., Neill, J. M., *Biol. Chem.*, 1924, v61, 523.
3. Lerner, A. B., Watson, C. J., *Am. J. Med. Sci.*, 1947, v214, 410.

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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.).