from 20% ethanol only upon addition of RNA which can effect the precipitation of six times its weight of CHO. Denatured fractions of RNA, soluble under these conditions, also precipitate in the presence of CHO. Despite the interaction of these macromolecules in solution, the unusual solubility properties of phenol extracted RNA seem to be a consequence of its native state rather than the presence of CHO.

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Effect of Hyperthermia on Blood Platelets in Male Rats. (32454)

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Previous reports on the effect of various types of stressors on platelets have indicated that the trauma of surgery(1) and hemorrhage(2) as well as hypothermia(3) have increased the number of platelets in the peripheral blood of experimental animals. Though exposure to cold has caused thrombocytosis (3) as well as thrombocytopenia(4), information on the effect of heat stress on platelet counts has been lacking. This investigation was undertaken to determine whether short term exposure of hyperthermia could alter the platelet counts of rats.

Methods and materials. Forty male Wistar rats (150-300 g) were divided into 2 equal groups and maintained before use in an air-conditioned animal room (26°C) with 14 hours of artificial lighting and were fed Purina laboratory chow and water *ad libitum*. On the day of exposure to heat, body weights and platelet counts of all animals were recorded (initial values) before placing them in individual glass cages. Experimental animals were placed in an incubator with an ambient temperature of 55°-57°C for 15 minutes while control animals remained at room temperature in the laboratory. After the 15 minute period (0 hour), the body weights and platelet counts were again determined in both groups. The animals were kept in the individual cages in the laboratory without access to food or water for the next 4 hours, and body weights and platelet counts were determined hourly (day 1). They were subsequently returned to the colony cages in the animal room and weights and platelet counts were recorded daily for the next 7 days (through day 8). Data were analyzed for changes from the animals' own initial (control) values as well as for differences between the average changes for both groups.

Tail vein blood was collected in Trenner automatic red blood cell pipettes (1:200) and diluted with 1% ammonium oxalate solution which had been regularly checked for contamination. A collodion seal permitted repeated collection of blood from the same incision. Pipettes were shaken for 5 minutes in

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TABLE I. Effect of 15 Minutes of Hyperthermia (55°-57°C) on Platelet Counts, (1×10^5/mm^3) in Male Rats.

Time, day	Controls N = 20				Experimental N = 20				Differences§	
		Mean	Mean change‡ ± S.E.M.	P	Mean	Mean change‡ ± S.E.M.	P	Diff. ± S.E.M.	P
1	Initial*	9.09 ± .41			9.23 ± .64				
"	0 hr†	8.97	− .12 ± .26	N.S.	10.17	+ .94 ± .27	<.005>.001	+1.06 ± .37	<.01 >.005
"	1 "	9.06	− .03 ± .37	"	10.95	+1.72 ± .37	<.001	+1.75 ± .52	<.005>.001
"	2 "	9.38	+ .29 ± .34	"	9.19	− .04 ± .75	N.S.	− .33 ± .82	N.S.
"	3 "	9.79	+ .70 ± .36	"	11.28	+2.05 ± .24	<.001	+1.35 ± .43	<.005>.001
"	4 "	9.56	+ .47 ± .34	"	12.15	+2.92 ± .33	"	+2.45 ± .48	<.001
2		9.15	+ .06 ± .27	"	10.45	+1.22 ± .40	<.005>.001	+1.16 ± .49	<.025>.02
3		9.23	+ .14 ± .31	"	8.59	− .64 ± .36	N.S.	− .78 ± .48	"
4		8.88	− .21 ± .44	"	8.81	− .42 ± .30	"	− .21 ± .67	"
5		8.90	− .19 ± .37	"	8.57	− .66 ± .61	"	− .47 ± .72	"
6		9.18	+ .09 ± .32	"	9.11	− .12 ± .55	"	− .21 ± .64	"
7		9.81	+ .72 ± .29	<.02>.01	9.32	+ .09 ± .40	"	− .63 ± .50	"
8		9.72	+ .63 ± .14	<.001	8.38	− .85 ± .27	<.005>.001	−1.48 ± .30	<.001

* Initial—before start of experiment.
‡ Mean change—differences from animals' initial values.
† 0 hr—immediately after 15 min period of hyperthermia.
§ Difference—mean change of exp minus control.

a Burton automatic shaker. Samples were then transferred to both sides of certified Neubauer counting chambers. To permit settling of platelets without evaporation, the chambers were kept in covered Petri dishes with moistened filter paper for 20 minutes. Platelets in all 25 squares on both sides of the chambers were counted under high power with a high intensity illuminator and the average counts of both sides used.

Results and discussion. Platelet counts were significantly elevated at 0, 1, 3, 4, and 24 hours (day 2) after hyperthermia (Table I). Both groups of animals had significant weight losses ($P<.001$) during the 0-4 hour period of food and water deprivation. The weight loss of the heat stressed animals was not significantly different from that of the control animals at 1 to 4 hours after hyperthermia. However, upon removal of heat (0 hour), the weight loss of heat stressed animals (22.9 ± .4 g) was significantly greater ($P<.01>.005$) than that of control animals (1.5 ± .3 g). The initial thrombocytosis at the 0 hour reached a peak at 1 hour and returned to normal by 2 hours after hyperthermia (Table I). The lack of correlation between the weight loss of dehydration and thrombocytosis indicates that hemoconcentration was probably not the causative factor. Since epinephrine(5) and serotonin(6) can release platelets from the spleen(5,6), this initial rise in platelets may have been due to the immediate release of platelet reserves by either or both of these biogenic amines which may been released during heat stress.

The second period of elevated platelet counts appeared at 3 hours, reached an even higher peak at 4 hours and lasted through 24 hours (day 2) after hyperthermia (Table I). It has been shown that plasma corticosterone in rats reaches a peak at 3 hours and remains elevated for 24 hours after scalding stress, resulting in increased hematocrits due to the shift of water from blood to tissue fluid (7). A similar corticosterone-induced hemoconcentration could have produced the second thrombocytosis. The 3-hour period after stress was sufficient time to reflect peripheral blood changes caused by a pituitary-adrenal stress response since adrenocorticotropic hormone

(ACTH) administration can increase the concentration of red blood cells in the peripheral blood of mice within 3-4 hours after administration(8). Unfortunately, the role of the pituitary-adrenal axis in altering platelet counts is not clear. Though exogenous ACTH produced no changes in platelet counts of rats(9,10), exogenous corticosteroids(11,12) as well as adrenalectomy(10) have caused thrombocytosis.

A delayed thrombocytopenia (Table I) occurred 7 days after hyperthermia (day 8). The platelet counts of control animals which had remained relatively unchanged until days 7 and 8 had significantly increased on these days (Table I). The fact that these increases had not occurred in the experimental animals indicates the suppression of changes in platelet counts in the heat stressed animals. It would seem that the delayed thrombocytopenia was caused by the decreased production and/or release of new platelets into the general circulation, which takes about one week(13).

Conclusions. Exposure of male rats to hyperthermia (55°-57°C) for a 15 minute period caused an immediate thrombocytosis lasting for 1 hour after removal from heat stress, a delayed thrombocytosis starting 3 hours after removal from heat stress and lasting through day 2, and a thrombocytopenia appearing 7 days after heat stress. Speculation as to the different physiological mechanisms which might have caused the thrombocytosis at these 2 time periods points toward an immediate release of platelets from their followed by a hemoconcentration due to the pituitary-adrenal stress response. The delayed thrombocytopenia has been attributed to decreased production and/or release of new platelets.

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Some Metabolic Aspects of Calcium Movement Across the Isolated Avian Shell Gland.* (32455)

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The avian shell gland has as one of its primary functions the deposition of $CaCO_3$ to form the egg shell. Studies of the shell gland in the domestic fowl have shown that Ca distribution in this organ is related to egg position in the oviduct. The calcium level in the shell gland was lowest when an egg shell was forming and highest when the egg was in the magnum and infundibulum sections of the oviduct(1). In fractionation studies of the shell gland muscosa of ^{45}Ca-treated hens, the ^{45}Ca content of mitochondria, which was higher than that of other cell fractions on a nitrogen basis, decreased when a shell was being deposited(2). Oxygen uptake studies of the oviduct revealed that the uptake was highest in the shell gland and maximal in this

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