Role of Rat Liver in Nonshivering Thermogenesis.* (24919)

AIKOH KAWAHATA AND LOREN D. CARLSON
Dept. of Physiology and Biophysics, University of Washington School of Medicine, Seattle

Hart(1) has recently reviewed evidence indicating that, when exposed to cold, cold-adapted rats increase their heat production without shivering. The sympathetic nervous system and adrenal medullary hormone are important in mediation of this thermogenesis (2), but the origin of increased heat production remains unidentified. Visceral tissues as a whole seem to have been excluded as important sources of chemical heat production(1); however, elevated oxygen consumption(3-7) and increased succinoxidase activity(3), as well as higher temperature(8,9), of the liver in cold-adapted rats have been reported. Our experiments were designed to determine whether liver is the primary organ in chemical heat production or whether metabolic activity of liver is secondary and supportive to increased metabolism of other tissues.

Methods. Oxygen consumption, rectal and liver temperatures, relative oxygen tension of the liver, and liver blood flow were measured in 26 male Sprague-Dawley rats weighing 318-437 g. Sixteen animals were cold-adapted (10). Experimental procedures were the same as used by Cottle and Carlson(10) with addition of measurements of oxygen tension and liver blood flow. Production of heat through muscular activity was prevented by curarization. A thermocouple of No. 30 copper-constantan wire was placed between liver lobules to measure temperature of the liver. Oxygen tension was observed by a platinum electrode inserted into liver tissue; a microammeter and a calomel half cell were included in the circuit. The technic was similar to that described by Montgomery(11). Liver blood flow was determined from clearance curve of radioactive chromic phosphate injected into the external jugular vein(12). Chromic phosphate suspended in normal saline solution was injected in amounts of .1-.3 ml, determined on basis of radioactivity. Blood was obtained by snipping off the tail. Samples (0.0078-0.0130 ml) were taken every minute for first 10 minutes after injection and every 5 minutes for following 30 minutes. Blood was transferred to gilt metal dishes, 2.5 cm in diameter, and mixed well with tap water, poured into the dishes beforehand. After complete drying of blood, radioactivity was determined with a Nuclear Instrument DS5-7 scintillation probe. Measurements were corrected for background radioactivity, and calculated in terms of activity/0.013 ml of blood. This activity was plotted as logarithm against time. The slow components were subtracted, and the half time for the fast component was taken directly from the graph. This disappearance constant (k) was calculated from the half time ($T^{1/2}$) by the formula, $k = 0.693/T^{1/2}$(12). Minimum values for liver blood flow were calculated from the disappearance constant and the blood flow. The results were expressed as blood flow/unit volume of tissue/minute. Blood volume was estimated to be 55.6 ml/g body weight(13). Two series of experiments were performed. In the control series, the room temperature was about 30°C, and radioactive chromic phosphate was injected 60 minutes after test began. Five warm-adapted and 7 cold-adapted rats were used. In the experimental series, room temperature was kept at about 30°C for the initial 60 minutes of test, then lowered to 5°C for 70 minutes and held at that temperature. The active chromic phosphate was injected 30 minutes after cooling was started. Five warm-adapted and 9 cold-adapted rats were used.

Results. Liver blood flow. In the uncooled series, liver blood flow in warm-adapted and cold-adapted rats did not differ significantly (Table I). In the cooled series, however, liver blood flow of the cold-adapted rats was significantly greater (nearly at 1% level) than that of warm-adapted ones. In other words,

* This study was supported in part by funds from Dept. of H.E.W.; Public Health Service, N.I.H., Bethesda, Md.

TABLE I. Values of Liver Blood Flow Related to Oxygen Consumption and Rectal Temperature.

Rat No.	Liver blood flow (cc/g/min.)	O_2 consumption (cc)	$\triangle$T (rectum), °C	Liver blood flow (cc/g/min.)	O_2 consumption (cc)	$\triangle$T (rectum), °C
			Series tested at 30°C			
		Warm-adapted			Cold-adapted	
1	.58	6.3	−1.9	.64	13.0	− .6
2	.65	5.0	−1.1	.71	10.7	− .8
3	.72	5.5	−2.5	.95	11.7	− .4
4	.84	6.3	−2.5	.97	8.5	− .2
5	.99	7.7	− .6	1.04	11.1	− .6
6				1.05	11.5	− .5
7				1.06	11.3	−1.3
8						
9						
Mean	.76 ± .14	6.1	−1.7	.92 ± .15	11.1	− .6
			Cooled series			
		Warm-adapted			Cold-adapted	
1	.45	5.9	8.8	.65	16.5	−5.0
2	.58	6.0	7.1	.67	15.2	−1.9
3	.62	5.3	7.1	.78	19.5	−3.6
4	.64	7.1	−9.7	.86	15.0	− .4
5	.66	6.3	8.2	.89	13.5	−1.7
6				.98	15.6	−1.5
7				1.04	19.0	−2.1
8				1.24	15.0	−2.1
9				1.90	15.5	−3.4
Mean	.59 ± .08	6.1	−8.2	1.01 ± .38	16.1	−2.4

$\triangle$T = Difference in temperature readings at 1 hr and end of 2 hr test.

greater liver blood flow in cold-adapted rats was observed only when rats were in a cold environment. A representative example from each cooled group is shown in Fig. 1 and 2.

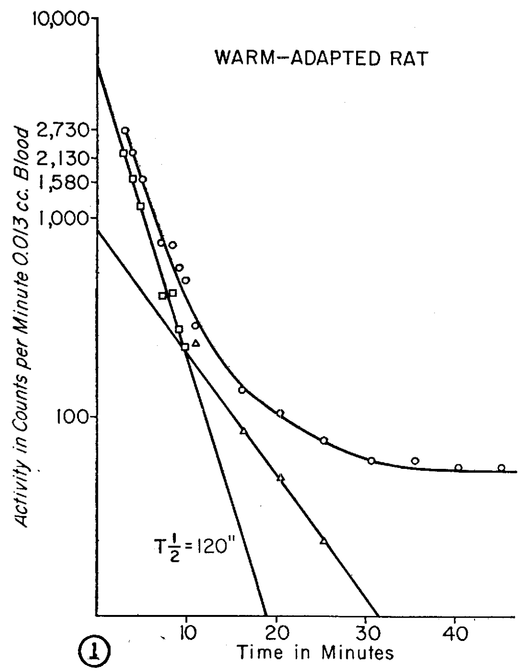
Oxygen consumption. Both warm-adapted and cold-adapted rats in the uncooled series showed a slightly lower oxygen consumption during the latter 60 minutes of test than during initial 60 minutes. In the cooled series, the warm-adapted rats fail to increase their oxygen consumption as cold-adapted animals did, as previously reported(10).

No significant differences between liver and rectal temperatures were observed in any one test. However, both liver and rectal temperatures decreased towards the end of the test, and this lowering was more pronounced in warm-adapted rats, especially those which had been cooled.

Oxygen tension in liver. No significant differences in O_2 electrode current readings either between warm-adapted and cold-adapted rats or between individual animals were seen. However, the value could be decreased by asphyxia and increased by inspiration of pure O_2.

Discussion. While there is no question that capability for heat production is enhanced in cold-adapted rats, the site of thermogenesis cannot be partitioned between various tissues. The greater liver blood flow in cold-adapted rats when they were exposed to cold, is undoubtedly linked to their greater metabolism, but the mechanism of the linkage cannot be determined from these experiments. That liver blood flow in cold-adapted animals increased approximately 10% while metabolism increased approximately 50% would indicate that if the liver were supplying the heat it would require a large oxygen extraction from blood and an increase in temperature. As previously indicated(14), liver metabolism would have to increase 6-fold to provide this heat. It seems impossible that the liver produces all of the additional heat.

Previous experiments(2) showed metabolic response to cold in the cold-adapted rat to be mediated *via* the sympathetic nervous system and indeed the metabolic response to noradrenaline changes markedly(15). There is no elevation of blood sugar or increase in blood lactic acid during thermogenesis in re-



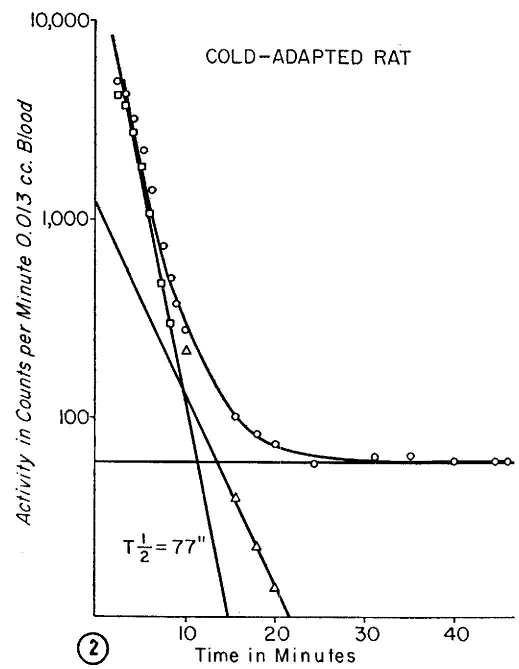


FIG. 1. Disappearance curve for chromic phosphate inj. intrav. ○ = actual experimental data; △ = difference between experimental data and slowest component, and □ = difference between experimental data and 2 slow components.

FIG. 2. Disappearance curve of chromic phosphate inj. intrav. ○, △, and □ have same meaning as in Fig. 1.

sponse to cold(15). If the response described by Cottle and Carlson(10) results from muscle metabolism, as suggested by Depocas(16), the mediation *via* sympathetic nervous system is an unexplored, though attractive, mechanism. Adrenaline will increase muscle metabolism, but this increase is accompanied by production of lactic acid(17). Freund and Jansen directed attention to the role of the sympathetics in chemical thermogenesis in muscle, although the manner of mediation was not specified(18). Donhoffer *et al.*(19) observed that the metabolism of 7 narcotized rats cooling at approximately 20°C fluctuated with a periodicity of 15-20 minutes. The authors show that the liver and intestine temperatures follow this fluctuation while muscles continue to cool.

Summary. Comparative studies on liver blood flow, oxygen consumption, liver and rectal temperatures, oxygen of liver were conducted on cold-adapted and warm-adapted rats. Muscular activity of all rats was blocked by curare. Liver blood flow was measured by blood clearance curve of radioactive chromic phosphate injected intravenously No difference in temperature between rectum and liver was found in a given rat in both groups. Liver blood flow in cold-adapted rats did not differ from that of warm-adapted ones so long as tests were performed in a warm room (30°C). However, liver blood flow in cold-adapted rats was considerably greater than that of warm-adapted ones when animals were cooled, though not sufficient to account for the excess oxygen consumption.

1. Hart, J. S., *Rev. Canad. Biol.*, 1957, v16, 133.

2. Hsieh, A. C. L., Carlson, L. D., Gray, G., *Am. J. Physiol.*, 1957, v190, 247.

3. You, R. W., Sellers, E. A., *Endocrinology*, 1951, v49, 374.

4. Desmarais, A., *Rev. Canad. Biol.*, 1953, v12, 99.

5. Weiss, A. K., *Am. J. Physiol.*, 1957, v188, 430.

6. ———, *ibid.*, 1954, v177, 201.

7. Hannon, J. P., *ibid.*, 1958, v192, 253.

8. Federov, N. A., Sur, E., *ibid.*, 1942, v137, 30.

9. Grayson, J., Mendel, D., *J. Physiol.*, 1956, v133, 334.

10. Cottle, W. H., Carlson, L. D., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 845.

11. Montgomery, H., *Circulation*, 1957, v15, 646.

12. Dobson, E. L., Jones, H. B., *Acta Med. Scand.*, 1951, v144, Suppl. 273.

13. Everett, N. B., Simmons, B., Lasher, E. P., *Circulation Res.*, 1956, v4, 419.

14. Hsieh, A. C. L., Carlson, L. D., *Am. J. Physiol.*, 1957, v188, 40.

15. Carlson, L. D., Hsieh, A. C. L., *ibid.*, 1957, v190, 243.

16. Depocas, F., *Canad. J. Biochem. Physiol.*, 1958, v361, 691.

17. Lundholm, L., *Acta Physiol. Scand.*, 1956, v39, Suppl. 133.

18. Freund, H., Jansen, S., *Pflüger's Arch. Physiol.*, 1923, v200, 96.

19. Donhoffer, Sz., Szeguari, Gy., Varga-Nagy, I., Jarai, I., *Pflüger's Arch. Physiol.*, 1957, v265, 104.

Received March 16, 1959. P.S.E.B.M., 1959, v101.

Renal Excretion of Calcium and Phosphate in the Mouse as Influenced by Parathyroids.* (24920)

G. D. BUCHANAN,† F. W. KRAINTZ AND ROY V. TALMAGE
Dept. of Biology, Rice Inst., Houston, Texas

Over recent years, influence of the hormone of parathyroids on bone metabolism has been reemphasized. McLean(1) described parathyroid effect on bone as a "feedback" mechanism for maintaining serum calcium levels. Our reports(2,3) have also emphasized importance of the action of parathyroids on bone. However, for complete picture of parathyroid action, influence of the hormone on kidney function cannot be disregarded. Greenwald's classic observation(4) that parathyroids directly influence renal phosphate excretion has been amply confirmed by many workers, and formed the basis for widely held theory of parathyroid function(5). That parathyroids might also exert a direct influence on renal calcium excretion has been for the most part ignored. Nevertheless Jahan and Pitts(6) reported increased tubular reabsorption of calcium following parathyroid administration in dogs; Talbot *et al.*(7) suggested that parathyroid extract raised renal calcium excretion thresholds. Talmage *et al.* (8,9) studying influence of parathyroidectomy on parathyroid extract administration on calcium excretion interpreted their data as changes in renal threshold for calcium, which caused altered excretion rates until plasma levels equilibrated with new thresholds established by changes in circulating parathyroid hormone concentrations. The work here presented was undertaken to study this effect in yet another species with hope of further emphasizing the overall role of parathyroids on renal excretion.

Methods and materials. Approximately 240 Rockland mice weighing 20-25 g were used. In most experiments, mice were placed on calcium and phosphate free diet with 14 mg Ca/100 ml in drinking water for 2 to 3 days prior to use. In a few parathyroid extract experiments, animals were maintained on stock diet. Parathyroids were removed individually under Nembutal anesthesia, using jewelers' forceps and dissecting microscope. A midline incision was made just posterior to larynx and thyroids exposed. The single pair of parathyroids lie on lateral border of thyroids, usually midway of length of the gland. Occasionally glands are slightly dorsal to thyroids. Parathyroid extract when given was injected subcutaneously in 6 doses at 1 hour intervals. Radioisotopes were injected intraperitoneally. In a typical experiment, animals were given radioisotope and 1 ml water intraperitoneally at beginning of experiment. Mice, in groups of 4 to 6, were placed on metabolism funnel and urine collected for 2 hour periods. Additional doses of 1 ml water were given at start of each collection period. Parathyroidectomy or parathyroid extract administration was performed as indicated in re-

* Aided in part by grant from Atomic Energy Comm.

† Present address: Division of Anatomy, Univ. of Tennessee, Memphis.