planted appropriately in homologous hosts (12). These preliminary experiments also suggest that the latent period may be predetermined within reasonable limits by the number of tumor cells contained in the inoculum.

Summary. 1. The KB and HeLa strains of human epidermoid carcinoma have been established in the cheek pouches of golden hamsters by means of quantitated suspensions prepared from tissue cultures in a medium providing the specific amino acid and vitamin requirements of these cell lines. 2. Strain KB, isolated directly from a biopsy specimen in these media, has been grown in LAF_1 mice bearing homologous ACTH secreting pituitary tumors. This strain also has been established in the cheek pouch of non-conditioned hamsters but thus far has failed to grow in non-conditioned mice. 3. The use of quantitated tissue culture inocula for the production of tumors in the hamster cheek pouch for chemotherapeutic studies is suggested.

1. Eagle, H., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 362.
2. ———, *ibid.*, 1955, v89, 96.
3. ———, *J. Biol. Chem.*, 1955, v214, 839.
4. ———, *J. Exp. Med.*, 1955, v102, 1955.
5. ———, *Science*, 1955, v122, 501.
6. Gey, G. O., Coffman, W. D., and Kubicek, N. T., *Cancer Res.*, 1952, v12, 264.
7. Lutz, B. R., Fulton, G. P., Patt, D. I., Handler, A. H., and Stevens, D. F., *ibid.*, 1951, v11, 64.
8. Toolan, H. W., *ibid.*, 1954, v14, 660.
9. Furth, J., Gadsden, E. L., and Upton, A. C., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 253.
10. Handler, A. H., *Proc. Amer. Assoc. Cancer Res*, 1955, v2, 22.
11. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
12. Handler, A. H., and Foley, G. E., to be published.

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Effects of Temperature and Work on Metabolism and Heat Loss in Man.*† (22225)

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Muscular exercise in a cold environment by either man(1) or animals(2,3) requires a greater oxygen consumption than the same activity in a warm environment. In man, the added energy requirement for work in the cold is not entirely the added hampering effect of clothing(4). Hart(2) concluded that the extent to which heat produced by activity is used to maintain body temperature depends upon the effect of activity on over-all physiological body insulation. The following experiments were devised to disclose changes of insulation in man exercising in warm and cool environments.

Methods. Skin temperature, heat loss and oxygen consumption were measured in 4 male subjects clad in shorts and exercising in cooling and cool environments. The experiments were begun at an ambient temperature of 20°C. The temperature was then steadily decreased to 10°C over ½ hour, and maintained at 10°C during the remainder of the test period (30 to 60 minutes). The subject reported shivering on a 4-point scale. Exercise on a bicycle ergometer was performed at a rate of 150 kg m/min or 70 kg m/min. Exercise was initiated with the start of cooling in some experiments, and after shivering had begun as a result of cooling in others. Skin temperature was measured with copper-constantan thermocouples recorded by a Brown

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potentiometer with a –0.5 to 2.5 mV range. Thermocouples were placed according to the recommendations of Hardy and Du Bois(5), and the thermocouples were paralleled to give an average reading. Heat loss was recorded with Hatfield-Turner discs(6), 4 in series on the thorax and 4 in series on the thigh and leg. Oxygen consumption was measured with a laminar flowmeter(7) with a proportionate sample analyzed in a Pauling oxygen meter, range 110 to 160 mm Hg.

Results. The time course of the parameters measured is given in Fig. 1 and 2, and summarized in Fig. 3. When exercise was initiated during cooling (Exercise During Cooling, Item I, Table I), the skin temperature dropped at a faster rate over the initial 30 minute period than it did during cooling at rest (Item 2, Table I), although the final average skin temperature was slightly higher. The heat debt, calculated on the basis that ⅓ of the body weight was shell, was less when the subject exercised during cooling. Exercise during cooling was accomplished with an oxygen consumption comparable to that required when exercise was performed at 20°C (Item 4, Table I). Rectal temperature re-

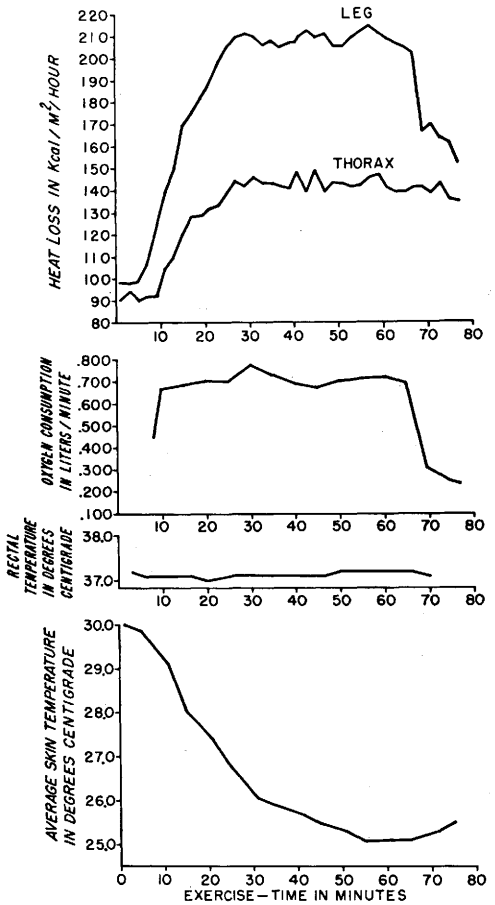

FIG. 1. Change in rectal temp., avg skin temp., oxygen consumption and heat loss with time. Cooling started at 5 min. Temperature changed from 20°C to 10°C over a ½ hr period and maintained at 10°C the remainder of the test period. Exercise started at 5 min. In the discussion this experiment is referred to as exercise during cooling. Avg of 4 subjects.

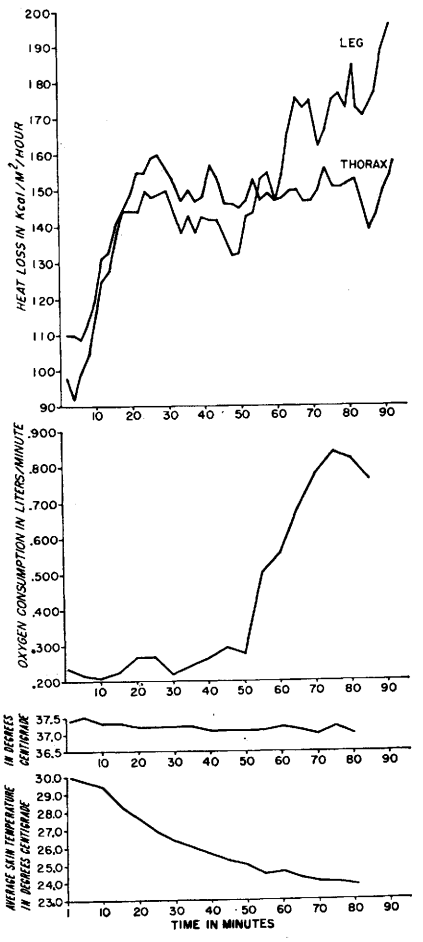

FIG. 2. Change in rectal temp., avg skin temp., oxygen consumption and heat loss with time. Cooling started at 5 min. Temperature changed from 20°C to 10°C over a ½ hr period and was maintained at 10°C for remainder of test period. Exercise was initiated at 50 min. In the discussion this experiment is referred to as rest during cooling and exercise after cooling. Avg of 4 subjects.

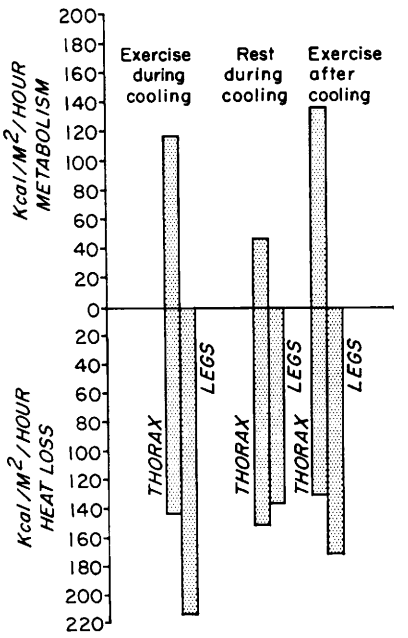

FIG. 3. Avg values for heat production plotted above horizontal line, and heat loss from the thorax and legs plotted below horizontal line. Heat loss from legs with exercise during cooling is greater than with exercise after cooling, and energy requirement when exercise is initiated after cooling is greater than when exercise is accomplished during cooling.

mained unchanged during the experiment when there was exercise during cooling, but fell slightly during cooling at rest.

Heat input increased when exercise was initiated after cooling, and the heat loss from the legs was not as great in this circumstance as when there was exercise during cooling. The increase in heat loss from the legs with exercise reflects the change in convective losses as well as the increased leg skin temperature. If the air insulation, Ia, is calculated for thorax and thigh plus leg according to the equation $Ia = \frac{Ts - Ta}{H}(8)$ this value for the thorax in the 2 experimental conditions is 0.12°C cal/hr/m². The value of Ia for the legs is 0.105 during shivering and 0.067 to 0.081 during exercise.

Fall in rectal and skin temperature was arrested during exercise, but skin temperature did not rise during 20 min. of exercise. Increased heat loss via respiration is estimated to be of the order of 20% greater when exercise was initiated after shivering (29 kg cal/hr vs. 24 kg cal/hr).

Even after 90 min of shivering (Item 3, Table I), a steady state was not reached, and the heat input was approximately doubled. The skin and rectal temperatures were still slowly declining. The heat output did not balance with the heat input. At this time, the metabolism was 48 kg cal/hr/m² above the resting level at 20°C. Exercise, which at 20°C required a heat input of 45 kg cal/hr/m², required an additional 76 kg cal/hr/m² and then did not stop shivering in 30 min.

The drop in skin temperature may be used to calculate the heat debt on the basis that ⅓ of the body weight was cooling at this rate with a specific heat of 0.83. If the skin temperature decline is divided into 2 phases, the initial rapid decline and the subsequent slower fall, the heat debts may be compared. During cooling at rest heat debt was initially incurred at the rate of 75 kg cal/hr/m², and during cooling with exercise, at 93 kg cal/hr/m². In the second phase, skin temperature dropped an average of 3.9°C an hour at rest, and only 2.1°C an hour during exercise. Calculated on the same basis, this represents a rate of heat loss of 34 kg cal/hr/m² at rest and 18 kg cal/hr/m² during exercise. The area represented by the Hatfield-Turner discs on the thorax was assumed to be 0.35 body surface, and the area represented by the discs on the leg was assumed to be 0.32 body surface; Table II was then constructed. Considering the perils of the assumptions, the agreement of values obtained during exercise is particularly good.

Discussion. It appears from these data that the energy cost of exercise is greater after than during cooling. The reason is not demonstrated. Shivering usually continues during the exercise. The over-all direct heat loss, as indicated by the Hatfield-Turner discs, was less when exercise was performed after cooling. It would appear that the control of peripheral circulation exerted by exercise is modified by prior cooling. There may be an increased work related to the changes in temperature and motility that occur in muscles

TABLE I. Metabolic Changes with Exercise at Low Temperature.

	Rate of cooling, °C/min.	Time to shiver, min.	Skin temp., °C Initial	Skin temp., °C Final	Heat debt*	Heat input,† rest, 20°C	Heat input, shivering	Heat input, exercise	Heat loss, shivering	Heat loss, exercise	Heat loss, shivering	Heat loss, exercise
					kg cal/hr/m²							
Item 1												
Exercise during cooling	.16	—	30.2	25.5	49			122		145		223
	.13	—	29.8	24.5	57			112		149		183
	.14	—	29.8	24.7	53			110		146		224
	.17	—	30.5	25.5	64			120		132		206
Avg	.15	—	30.1	25.0	56	36		117		143		214
Item 2												
Cooling at rest; exercise after	.16	13	30.8	24.7	63		48	140	151	147	136	174
	.11	49	30.0	23.9	74		37	133	126	130	122	147
	.12	34	29.9	24.2	66		60	132	143	149	142	185
	.10	66	29.7	23.3	83	36	37	135	181	160	149	179
Avg	.12	48	30.1	24.3	72		46	135	151	147	137	171
Item 3												
Same as 1 & 2 (see ‡)	.18	33–48	31.5	25.0	67			83‡		182		223
	.15	25	30.7	25.0	60	35	79	111‡	170	168	134	189
Item 4												
Exercise at 20°C	0							107				
	0							80‡				

* Calculated on basis of 1/3 body cooling at skin temperature and 2/3 at rect. temperature.
† " " " " caloric value of liter of $O_2 = 4.825$.
‡ Exercise at 70 kg M/min., others at 150 kg M/min.

TABLE II. Heat Balance Calculations.

Item 1	Heat loss, kg cal/hr		Heat input, kg cal/hr					
	Thorax	94	Metabolism	221				
	Legs	128	Skin temp.	42				
	Remainder	41	Rectal	0				
		263		263				
Item 2	Heat loss (shivering), kg cal/hr		Heat input (shivering), kg cal/hr		Heat loss (exercise), kg cal/hr		Heat input (exercise), kg cal/hr	
	Thorax	100	Metabolism	87	Thorax	97	Metabolism	255
	Legs	82	Skin temp.	79	Legs	105	Skin temp.	0
	Remainder	0	Rectal	16	Remainder	49	Rectal	−4
		182		182		251		251

and joints(9,10). In these experiments, the steady state was not reached during the first 2 or 3 hours of cooling. Metabolism was eventually doubled at 10°C. In these experiments skin temperature dropped at a rate comparable to that reported by Horvath *et al.* (11), whose subjects were well-clothed individuals exposed to −40°C with a heat input of 74 kg cal/hr/m^2 at 2 hours' and 85 kg cal/hr/m^2 at 3 hours' exposure. The increase in heat input with exercise is of the same order of magnitude as that reported by Horvath for clothed men.

Summary. Exercise after cooling is accomplished at a greater energy cost than exercise during cooling. Over-all direct heat loss is less during exercise after cooling.

1. Horvath, S. M., Freedman, A., and Golden, H., *Am. J. Physiol.,* 1947, v50, 99.

2. Hart, J. S., *Canad. J. Zool.,* 1952, v30, 90.

3. Hart, J. S., and Heroux, O., *Canad. J. Biochem. & Physiol.,* 1955, v33, 428.

4. Horvath, S. M., and Golden, H., *J. Clin. Invest.,* 1947, v26, 311.

5. Hardy, J. D., and Du Bois, E. F., *J. Nutrition,* 1938, v15, 477.

6. Hatfield, H. S., *J. Sci. Instruments,* 1950, v27, 1.

7. Carlson, L. D., *Methods in Med. Res.,* 1954, v6, 60.

8. Gagge, A. P., Burton, A. C., and Basett, H. C., *Science,* 1941, v94, 428.

9. Hunter, J., and Whillans, M. G., *Canad. J. Med. Sci.,* 1951, v29, 255.

10. Hunter, J., Kerr, E. H., and Whillans, M. G., *ibid.,* 1952, v30, 367.

11. Horvath, S. M., Golden, H., and Wager, J., *J. Clin. Invest.,* 1946, v25, 709.

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Phosphatase Activity of Hematopoietic Tissues of Nitrogen Mustard-Treated Animals.* (22226)

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Previous studies in this laboratory(1) have shown that whole body x-irradiation markedly inhibits citric acid synthesis *in vivo* by the spleens and thymus glands of rats and mice. Treatment of animals with nitrogen mustards caused a similar inhibitory effect on citric acid formation by hematopoietic tissues (2). More recently we found(3) that sublethal and lethal doses of x-irradiation produce marked increases in the adenosine triphosphatase and 5-nucleotidase activity of the spleens and thymus glands. In view of the known similarities between x-irradiation and the nitrogen mustards in some of their actions on mammalian tissues we were interested in ascertaining whether these chemical agents produced changes in phosphatase activity similar to those which

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