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Research Article

EXPERIMENTAL IMMERSION FOOT. I. THE EFFECTS OF PROLONGED EXPOSURE TO WATER AT 3° C. ON THE OXYGEN TENSION AND TEMPERATURE OF THE RABBIT LEG[1]

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Prolonged exposure of a limb to cold water causes the disease known as immersion foot. The widespread literature has been reviewed elsewhere (1). Neuromuscular dysfunction and histopathological changes in nerve, muscle, and other tissues are readily demonstrated after such exposure. The question of how cold short of freezing damages the tissues has not been settled. Goldschmidt and Light (2) and Lewis and Love (3) suggested that the poor dissociation of oxygen from cold hemoglobin may make the tissues anoxic. It is true that the supply of oxygen by cold hemoglobin is meager (4) but it is also true that the utilization of oxygen by tissues is conspicuously lowered at such reduced temperature (5). We have demonstrated in short term experiments a reduced oxygen tension of the skin of man exposed to cold (6). The present experiments were carried out in order to learn to what extent, if any, prolonged exposure to cold lowers the oxygen tension of muscle and of the subcutaneous space, and whether oxygen inhalation will increase the oxygen tensions. Preliminary experiments on the effect of cold on the skin of human extremities were abandoned because of the danger involved. Rabbits were chosen because there has been some experience in producing immersion foot in that animal (7). Parallel studies were made of the temperatures of the muscle and of the subcutaneous tissue in order to learn the degree of chilling of tissues and in order to get some idea of the rate of blood flow through the tissues during prolonged chilling.

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METHOD

The open tip platinum electrode (8) for estimating oxygen tension was utilized as previously described (6) except for several modifications. The electrode consists of the sharpened tip of a 0.2 mm. platinum wire, the rest of the wire electrically insulated. At voltage 0.6V. the current measured is linearly proportional to the rate of diffusion of oxygen to the electrode. For greater sensitivity to lower oxygen tension in these studies, electronic amplification was substituted for the galvanometer used in previous studies. The circuit was a modification of that used by Moody for ionization chamber work (9). The electrodes and their wire connections were insulated to minimize electrical leakage in the special condition of underwater work. In spite of this, electrical leakage was not abolished in all experiments. When it occurred the data had to be discarded as were data from an occasional unresponsive electrode. The electrodes were placed in subcutaneous areas rather than in skin because rabbit skin is so thin as perhaps to allow its oxygen tension to be influenced by oxygen of the surrounding water and of the subcutaneous space. All data were corrected for direct temperature effect on electrical current as previously found for these electrodes (6). Measurements were made without calibrating (6) the electrodes, and the data are presented as relative (Figure 3) since the absolute values of oxygen tension are not known. We were especially interested in the changes in oxygen tension in muscle because of a parallel study we made of the neuromuscular dysfunction and histopathological changes resulting in the muscles of the legs of another series of rabbits similarly exposed (10).

Copper-constantan thermocouples were used in conjunction with the Brown potentiometer to record all temperatures at three-minute intervals.

The apparatus for holding the rabbit and exposing the left hind limb to cold water was a modification of that used by Lange, Weiner, and Boyd (7). Each rabbit-holding stall was constructed in the following manner. A vertical plexiglass tube having a wall thickness of one-eighth inch, an outer diameter of three inches, and a length of fourteen inches was fitted flush up into a hole in a horizontally held board having a thickness of five-eighths inch. The plexiglass tube was furnished with a water inlet at its lower end and an outlet tube near its

TABLE I

Average temperatures, and fluctuations of temperatures, of water, of muscle, and of subcutaneous space within the following time periods *

Rabbit No.		Temperature °C.							
		0–1 hrs.	1–6 hrs.	6–12 hrs.	12–24 hrs.	24–36 hrs.	36–48 hrs.	48–60 hrs.	60–72 hrs.
	Water	2	2	2	2	2	2	2	2
1	Muscle		8±2	6	5	5	6	7±1	5±1
	Subcut.		6±1	6	4±1	3	4±1	4	3
	Water	3	3	3	4±1	3	2	2	2
2	Muscle		9±3	8	7±1	5±1	5±1	4+1	4±1
	Subcut.		4	4	4±1	3	3	3	3
	Water	1	2±1	2	1	1	2±1	1	2±1
3	Muscle		3±1	3±1	2	2	2±1	1	2±1
	Subcut.		3±1	2	2	1	2±1	1	2±1
	Water	2	3	3	3	3	2	2	
4	Muscle		6	5	4±1	4±1	2	2	
	Subcut.		4	3	3	3	2	2	
	Water	2	3	3	3	3	2	2	
5	Muscle		5±1	4±1	5±1	5±1	4	3±1	
	Subcut.		4	3	3	4±1	3	3	
	Water		3±1	2	2	2	3±1	2	
6	Muscle		6±1	7±1	6±2	12±8	10±6	5	
	Subcut.		4±1	3	3	5±3	5±3	2	
	Water	2	3	3	3	2	2	2	2
Approx. averages	Muscle		6	6	5	6	5	4	3
	Subcut.		4	4	3	3	3	3	3

* Six rabbits. Temperatures °C. are shown of the water to which each rabbit's left hind leg was exposed, of the muscle of the exposed leg, and of the neighboring subcutaneous space. The temperatures throughout each time period are analyzed in detail. Temperatures tabulated to nearest 1°C. Fluctuations less than ±1°C. not tabulated. Fluctuations of ±1°C. to ±1.4°C. as ±1°C., etc.

upper end, just below the board. A heavy-duty electrically driven centrifugal pump was inserted in a half-inch rubber tubing connecting a large-volume thermostatically controlled water cooling tank with each inflow tube. Starting the pump raised the water through the vertical tube, and the water overflowed through the outlet tube back into the cooling tank. This served to maintain a water surface level well above the knee of the left hind leg. The water in the vertical tube could be withdrawn, and be replaced by air, by turning off the pump.

The rabbit was placed on the board with the left hind leg in the vertical tube, the right hind leg in a similar tube filled with air, and the body gently held by straps. To complete each stall there was a nearly airtight housing over the board for use when the temperature of the air surrounding the rabbit was decreased, or when oxygen was administered.

Air temperature could be decreased by pumping cold air, from above the surface of the water in the cooling tank, into the stall. When oxygen was used it was administered from an oxygen tank by flow at 10 liters per minute directly into the stall. This rate of flow, with a rabbit in the stall, presented a 640 to 760 mm. oxygen tension as measured by a calibrated Pauling meter.

PROCEDURE

The experimental animals were divided into two groups. Numbers 1 through 8 were used to study changes in temperature of muscle and of the nearby subcutaneous area in response to exposure of a limb to cool water (averaging 2.3° C.). In the case of rabbits numbers 1 through 6 the exposure was continuous, in numbers 7 and 8 intermittent. Numbers 9 through 13 were used to study the changes in oxygen tension under the same conditions.

Female rabbits averaging 3 kilograms in weight were selected in order to fit the rather exacting dimensions of the stalls. Throughout the experiment they were fed a desiccated rabbit ration and water ad lib. The left hind leg was depilated two days before using the rabbit in an experiment in order to allow any inflammatory response to subside. With the exception of rabbit No. 12, none of the limbs had been previously exposed to cold.

The thermostat of the water cooling device remained at the same setting throughout all experiments.

Thermocouples and electrodes were inserted under ether-atropine anesthesia, except in rabbit No. 12, in which the necessary muscle dissection was performed under the narcotizing effect of two days of cold exposure.

In the first eight rabbits thermocouples were tied deep in the extensor group of muscles half-way between the

TABLE II

Ranges of air temperatures and of body temperatures of same rabbits as shown in Table I *

	Maximum and minimum temperatures °C.									
		Body								
Rabbit No.	Air	0–1 hrs.	1–6 hrs.	6–12 hrs.	12–24 hrs.	24–36 hrs.	36–48 hrs.	48–60 hrs.	60–72 hrs.	Mean
1	22.0	36.8	36.8	38.3	38.5	38.5	39.0	39.2	38.8	38.2
	26.0	36.8	38.2	38.7	37.4	37.4	38.7	38.3	38.1	
2	13.0	36.8	37.5	38.5	37.7	38.8	38.5	38.4	38.5	38.2
	17.0	37.5	38.5	37.5	38.9	38.2	37.8	39.0	39.5	
3	12.0		37.5	38.0	37.0	37.3	37.0	37.0	37.3	37.3
	16.0		38.0	37.3	37.8	37.5	38.0	37.3	36.5	
4	16.0	37.0	37.0	37.7	37.5	37.9	38.0	37.5		37.8
	18.0	37.0	37.7	37.7	37.9	38.3	38.6	39.5		
5	24.0	37.2	37.2	38.0	38.0	38.0	38.2	38.7		37.9
	26.0	37.2	38.0	38.0	38.0	37.2	38.5	38.3		
6	21.0	35.2	36.0	37.9	37.8	38.7	38.6			37.6
	26.0	36.0	38.0	38.1	38.8	37.8	38.0			

* Six rabbits (same animals as in Table I). Body temperatures obtained by thermocouple under belly, skin upon dry wood floor. Extremes of body temperature tabulated for each time period.

knee and the ankle of the left leg and in the nearby subcutaneous space. Interference with blood supply was avoided as far as possible when placing ligatures. After the rabbit was placed in the holding stall, body temperature was recorded from a thermocouple that had been inserted into the subcutaneous space of the belly wall, the skin of which presented against the dry wood floor of the stall. A fourth thermocouple recorded the temperature of the water (or air) surrounding the left hind leg. A fifth recorded air temperature. In this group of animals left hind legs were exposed continuously to cold for from 66 to 130 hours.

In the second series (rabbits 9 through 13) platinum electrodes for the measurement of oxygen tension were inserted. Simultaneous measurements of oxygen tension and of limb temperature were avoided because of the complexity of such experiments and in order to minimize current leakage through water between the two electrical systems. In most instances only one electrode was tied into the muscle, and one in the nearby subcutaneous space, in the left hind leg. In rabbit No. 11 two electrodes were used subcutaneously.

As soon as the electrodes were in place, the rabbit was placed in its stall. All rabbits except No. 12 were recovering gradually from the anesthesia, and their left hind legs were at first in an air-filled tube. The duration of experiments ranged from two hours to ten hours and forty-five minutes. In general, the first exposure of a rabbit leg was to air at room temperature, and the rabbit was given air, oxygen, and air to inhale. Then cold water was allowed to flow around the leg, and the rabbit again inhaled the gases in the same order. Some experiments were carried further, as shown in Table III and in Figure 3.

Unlike the others, the leg of rabbit No. 12 was studied after chilling for two days, and again was studied on the third day. The electrodes remained in place between the two periods of study. A comparison of the results of these studies on successive days, and observations with paired electrodes (rabbits No. 11 and No. 13) afford an understanding of the variability of oxygen tension data obtained in the difficult condition of underwater electrical work (Figure 3).

In the oxygen tension experiments occasional measurements of the temperature of the water (average 2° C.), and of the surrounding air (average 25° C.) demonstrated no regular difference from these conditions in the first experimental group.

RESULTS

During the prolonged experimental period the rabbits consumed food and water, and eliminated urine and feces normally. The rabbits remained alert, and appeared healthy throughout all experiments. On removal of the thermocouples and electrodes the tissues were found to be clean and grossly uninfected.

A. Changes in temperature of muscle and of the nearby subcutaneous area in response to exposure to cool water

Eight rabbits (No. 1 through No. 8) were studied. The temperature of the water averaged 2.3° C., and fluctuated less than 1° C. except as

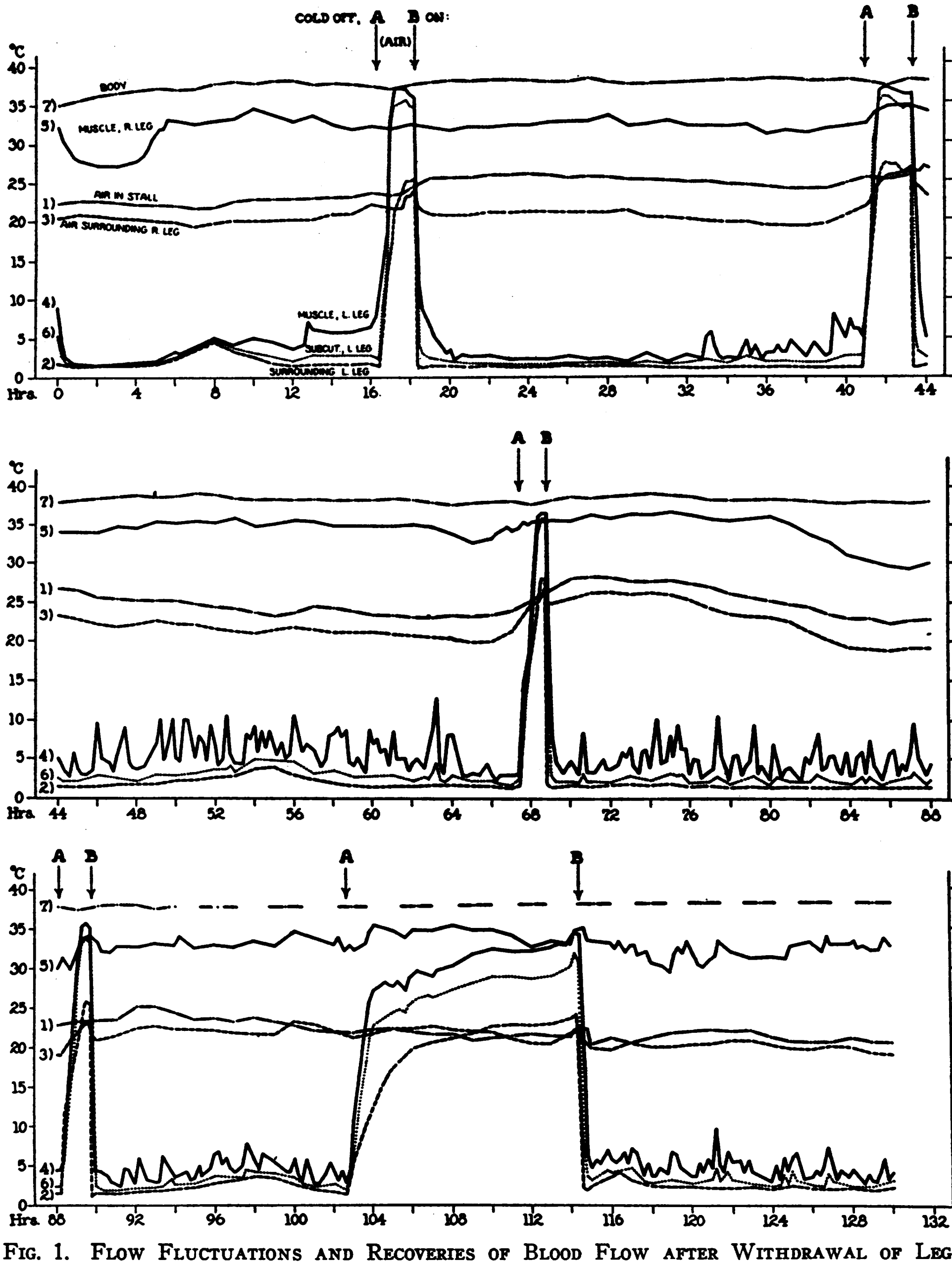

FIG. 1. FLOW FLUCTUATIONS AND RECOVERIES OF BLOOD FLOW AFTER WITHDRAWAL OF LEG FROM COLD WATER

Rabbit No. 7. Left hind leg in cool water for 130 hours except for temporary quick withdrawal of the water at 16 hours, 41 hours, 67 hours, 88 hours, and slow withdrawal at 102 to 114 hours. Right hind leg in air. Time in hours shown in abscissae, temperature in °C. in ordinates. Graph shows temperatures of the following:

1 = T °C. of the air surrounding the rabbit.
2 = T °C. of the water (or air) surrounding the exposed (left) leg.
3 = T °C. of the air surrounding the unexposed (right) leg.
4 = T °C. of the muscle of the exposed (left) leg.
5 = T °C. of the muscle of the unexposed (right) leg.
6 = T °C. of the subcutaneous area of the exposed (left) leg.
7 = T °C. of the body (subcutaneous, belly against dry wood).

shown in Table I. Air temperatures and body temperatures are given in Table II. The visible responses of the leg to cold were much as reported by Lange, Weiner, and Boyd (7). The leg became pink and remained so throughout the experiment. Muscle temperature decreased rapidly, and even more profoundly than in their experiments (Table I). Subcutaneous temperatures averaged a little lower than muscle temperature. After twenty-four hours there was considerable swelling of the exposed leg and some edema of the contralateral, unexposed, equally dependent leg. In subsequent days edema, predominating in the exposed leg, became severe. Occasional motions of the exposed leg appeared to result solely from the action of muscles which were above the level of the cold water. Muscle action caused transient increases of temperature of the submerged muscle. Since the animals were sacrificed at the termination of each experiment, and recovery following withdrawal from cold was not studied, the usual rapid loss of edema, and the persistent neuromuscular dysfunction, were not observed. Fluctuations in temperature of muscle and of the neighboring subcutaneous spaces were slight, with the exceptions of rabbit No. 6 (Table I) and rabbit No. 7 (Figure 1).

The data from rabbit No. 7 (Figure 1) are not tabulated with the first six rabbits. As in other experiments the left hind leg of rabbit No. 7 was exposed to the usual cold water but unlike the other experiments the cold water was withdrawn from the leg, and air was substituted for the water each day for a sufficient time to allow the temperatures of the leg to rise to a steady value. In general these temperatures rose to levels close to body temperature, and there was no day-to-day decrease in this effect. Figure 1 shows the extent of this recovery of limb temperature, and therefore of blood flow after various times of exposure to cold water. The average recovery of muscle temperature was to 36° C., and of subcutaneous space to 35° C. The values can be compared with those in the right (unexposed) leg of five of the rabbits in this series, the average muscle temperature of which was 35° C. (11 determinations) and subcutaneous temperatures 34° C. (11 determinations). During leg cooling, rabbit No. 7 most clearly illustrated a conspicuous rhythmic variation in leg temperature, suggesting the "hunting reaction" described for human skin by Lewis (11) and for human muscle by other investigators (12). The fluctuation in temperature in the muscle of this rabbit's leg, at times amounting to 5° C., must represent great increases of blood flow above the relatively low flows in the intervals between the fluctuations. The leg of a last rabbit, No. 8, was exposed in the interrupted way in which the leg of No. 7 was exposed. It failed to show the temperature fluctuations shown by rabbits No. 6 and No. 7, but until the termination of the experiment at one hundred hours, the temperature of the muscle and of the nearby subcutaneous space recovered, when the water was withdrawn, to values near body temperature. In the case of these two rabbits, the circulation of the limbs recovered after prolonged, though interrupted chilling.

An attempt was made to determine whether these temperature fluctuations were truly "spontaneous" and therefore of the nature of the "hunting reaction," or whether they were induced by motion of the leg. Four rabbits were studied. The leg-cooling chamber was made larger in order to allow enough freedom of motion for recording. Legs were cooled for several days and leg motion was continuously recorded simultaneously with temperature. All fluctuations of muscle temperature greater than 1° C. were found to correspond to muscular movement. Motion elicited by faradic stimulation of the skin over the left sciatic notch also caused such increments in muscle temperature. The fact that an arterial tourniquet prevented these muscle temperature rises but did not prevent leg motion indicates that the temperature increases were almost entirely a result

A = cold water withdrawn from around left hind leg.
B = cold water replaced around left hind leg.

All readings of temperatures were made at 3-minute intervals and were begun within 5 minutes after starting cold water exposure to the left leg. At 94 hours the thermocouple recording body temperature was broken, and occasional body temperatures were taken by inserting a mercury thermometer into the rectum.

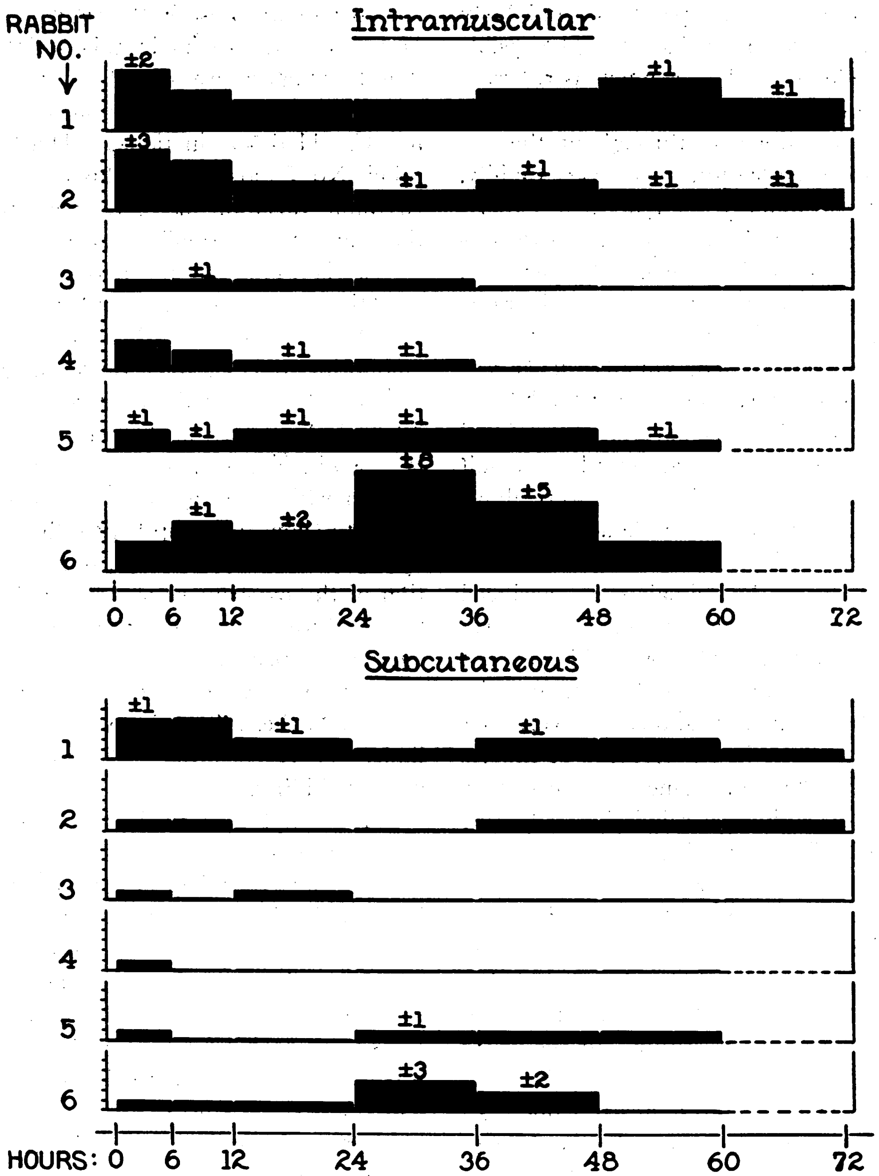

FIG. 2. PLOT OF THE DIFFERENCE BETWEEN WATER TEMPERATURE AND LEG TEMPERATURE, AS AN INDICATION OF BLOOD FLOW

Each division on each ordinate = 1° C. Fluctuations of tissue temperature, in any one time period, show above the value for that time period when fluctuations were as great as ± 1° C. (*i.e.*, 2.0 + °C., between extremes). The dotted lines indicate termination of studies. INTRAMUSCULAR: Difference between muscle temperature and water temperature. SUBCUTANEOUS: Difference between temperature of subcutaneous space and water temperature.

of increase in blood flow, and not of heat produced by the muscle contraction. Leg motion caused increments in the temperature of muscle that appeared to be paralyzed by prolonged exposure to cold. This suggests that the leg motion was induced by the unexposed thigh muscles and the increments in flow so produced carried through to the cold exposed muscle. Further experiments are being carried out to quantitate these responses. They are spoken of here in order that the tem-

TABLE III

Duration of experiments (second series of rabbits) in which oxygen tension of muscle and of subcutaneous space was studied *

Rabbit No.	Leg in air		Leg in water		Leg in air		Total	
	Hr.	*Min.*	*Hr.*	*Min.*	*Hr.*	*Min.*	*Hr.*	*Min.*
9	2	20	8	25			10	45
10	4	40	1	40			6	20
11	1	15	3	55	1	20	6	30
12			2	0			2	0
12	1	45	4	0	1	30	7	15
13	1	55	3	30	2	05	7	30
	1	55	3	30	2	05	7	30

* The leg of rabbit No. 12 was chilled in 2°C. water for two days prior to the studies. After the first study the rabbit was kept in the stall and the leg was kept in air at room temperature overnight. The second study was then made. No other rabbit leg was exposed to cold prior to the beginning of the study.

perature data may properly be related to blood flow and to show that fluctuations in flow were probably a result of leg motion.

Figure 2 gives an idea of the low temperatures, and therefore of the low blood flows in the muscles and subcutaneous spaces of rabbit limbs exposed to cold water. There were unexplained temperature differences in the limbs of different rabbits, and at different times of exposure. Cooled limbs of rabbits (Nos. 2, 3, and 4) whose bodies were exposed to cool air tended to have lower leg temperatures than cooled limbs of rabbits (Nos. 1, 5, and 6) in warmer air.

B. Changes in oxygen tension of muscle and of the nearby subcutaneous area in response to exposure to cool water

Five rabbits were studied. Table III shows the duration of the experiments. Figure 3 summarizes the results.

When the limb was chilled the trend of oxygen tension was downward in both the muscle and the subcutaneous space. This was an inconstant effect both during air inhalation and oxygen inhalation (Figure 3).

When the leg was warm, inhalation of oxygen resulted in an increase of oxygen tension of the muscle in seven of the eight instances, and of the subcutaneous space in all ten instances. When the leg was cold, the inhalation of oxygen resulted in an increase of oxygen tension of the muscle in all ten instances, and of the subcutaneous space in eight of ten instances. Before exposing the leg to cold the mean time of oxygen inhalation required to cause the full increment of oxygen tension in the muscle was 21 minutes, and in the subcutaneous area it was also 21 minutes. During exposure of the leg to cold the mean time of oxygen inhalation required to cause the full increment of oxygen tension in the muscle was 40 minutes, in the subcutaneous area 60 minutes.

The studies of oxygen tension were continued during and shortly after sacrificing three of the rabbits. On sacrificing rabbits Nos. 10 and 11 with chloroform and rabbit No. 13 with nitrogen, erratic results were obtained with no one direction of change in values, and no return of the values to zero. Two of the electrodes were shown to have no electrical leak, in spite of agonal muscle action that might be expected to fracture the delicate electrodes. The severe muscle action may, however, have played a leading role in such changes by repeated dislodgment of the electrodes in the muscle.

SUMMARY

Measurements were made of the temperature and oxygen tension of the muscles and subcutaneous spaces of rabbits' legs exposed to water at 3° C. In general, the temperatures decreased to levels characteristic of markedly reduced blood flows. In some instances there were fluctuations in the tissue temperatures, indicating transient periods of increased blood flow. Numerous fluctuations persisted for as long as seven days. In other instances there were no such fluctuations. These fluctuations have been found to be associated with leg motion. Usually the oxygen tension of the tissues was depressed by cold, showing a tendency to a greater reduction in oxygen supply than in oxygen utilization. When the animals were given oxygen by inhalation, while the leg was cold, the oxygen tension of the cold tissues rose to or above the level prior to cold.

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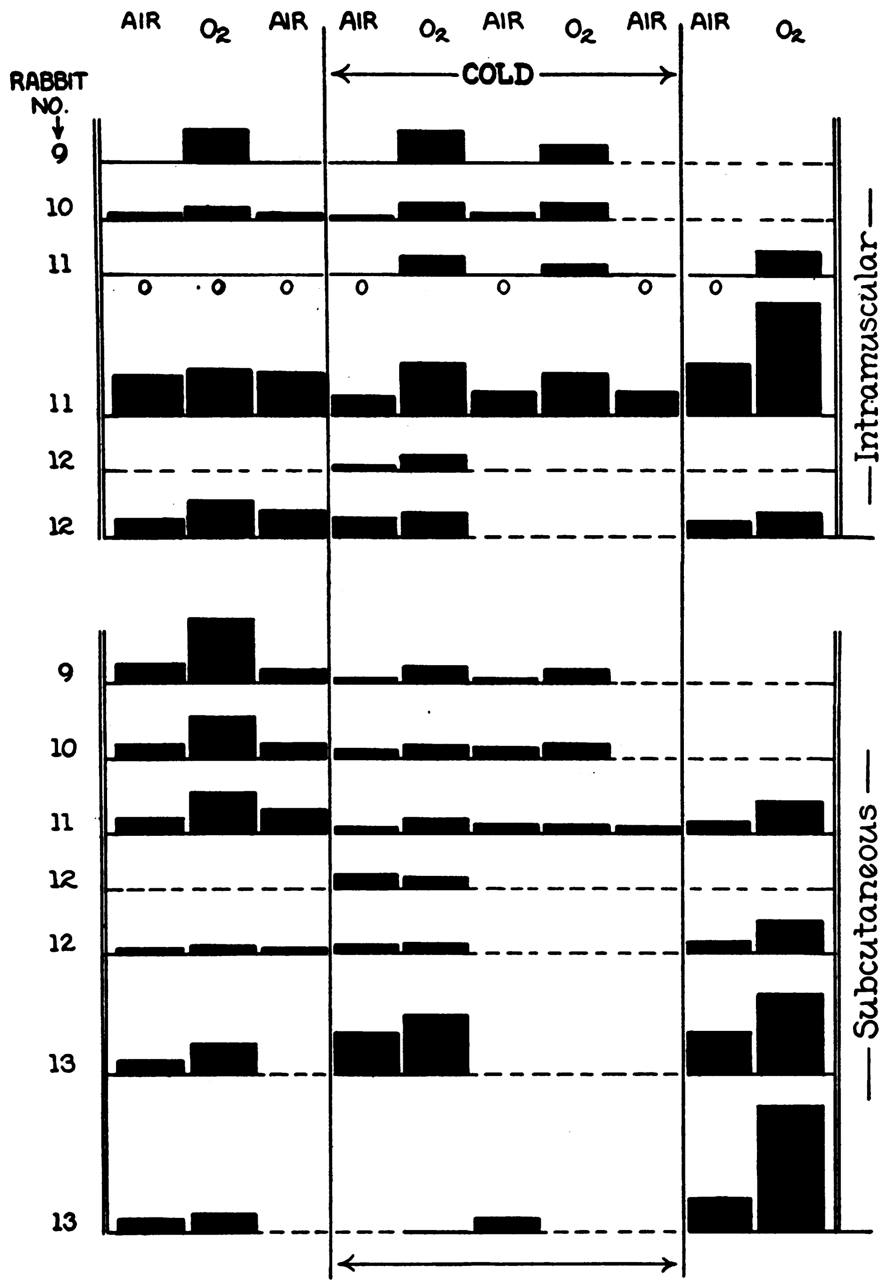

FIG. 3. CHANGES IN OXYGEN TENSION OF MUSCLE AND OF NEARBY SUBCUTANEOUS SPACE OF THE LEFT HIND LIMB OF THE RABBIT

The heights of the blocks vary directly with oxygen tension (see Text).

"Air" denotes air inhalation, "O_2" oxygen inhalation. The results of immersion of the limb in 2° C. water are shown under "COLD," and the times of exposure are shown in Table III. The dotted lines indicate incomplete studies. No data of an electrode were discarded prior to the termination of any experiment, with the exception of those of rabbit No. 9, discontinued early because the galvanometric readings repeatedly swung erratically to zero. A second muscle electrode in the experiment with rabbit No. 10 was completely unresponsive and the data are not included. Rabbit No. 12 was studied on two successive days. Rabbit No. 13 had two subcutaneous electrodes.

REFERENCES

1. Montgomery, H., Experimental immersion foot. Review of the physiopathology. Physiol. Rev., In press.
2. Goldschmidt, S., and Light, A. B., The effect of local temperature upon the peripheral circulation and metabolism of tissues as revealed by the gaseous content of venous blood. Am. J. Physiol, 1925, **73**, 146.
3. Lewis, T., and Love, W. S., Vascular reactions of the skin to injury. Part III. Some effects of freezing, of cooling, and of warming. Heart, 1926, **13**, 27.
4. Brown, W. E. L., and Hill, A. V., The oxygen-dissociation curve of blood, and its thermodynamical basis. Proc. Roy. Soc. London, Series B, 1923, **94**, 297.
5. Crismon, J. M., and Field, J., 2nd, The prevention of gangrene associated with massive edema in frostbite, immersion foot and other injuries. Committee on Med. Res. of the Office of Scientific Research and Development. OEMcmr Contract 476, 1 Aug., 1945.
6. Montgomery, H., and Horwitz, O., Oxygen tension of tissues by the polarographic method. I. Introduction: Oxygen tension and blood flow of the skin of human extremities. J. Clin. Invest., 1950, **29**, 1120.
7. Lange, K., Weiner, D., and Boyd, L. J., The functional pathology of experimental immersion foot. Am. Heart J., 1948, **35**, 238.
8. Davies, P. W., and Brink, F., Jr., Microelectrodes for measuring local oxygen tension in animal tissues. Rev. Scient. Instruments, 1942, **13**, 524.
9. Moody, N. F., An improved dc amplifier for portable ionization chamber instruments. Rev. Scient. Instruments, 1951, **22**, 236.
10. Horwitz, O., Montgomery, H., Sayen, A., and Mescon, H., Experimental immersion foot. II. Functional and histological changes in the rabbit leg exposed to water at 3° C., and therapeutic trial of cortisone and of inhaled oxygen. J. Clin. Invest., 1954, **33**, 370.
11. Lewis, T., Observations upon the reactions of the vessels of the human skin to cold. Heart, 1930, **15**, 177.
12. Miller, H. R., Grundfest, H., Alper, J. M., Korr, I. M., Feitelberg, S., and Klein, D., Changes in muscle temperature of limbs exposed to cold. Engineering memorandum No. 19 CR from the Climatic Research Unit, Fort Monmouth, N.J., 1 Aug., 1944.