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Research Article

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 [1]

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Prolonged immersion of the leg of a rabbit in cold water produced pathological and functional changes (1, 2) including muscle fibrosis. The cold rather than the water is responsible (1, 2).

The oxygen tension of the muscle of the rabbit's leg is usually reduced during the immersion of the leg in water at a temperature of 3°C., and inhalation of high concentrations of oxygen increases the oxygen tension to or above the pre-immersion level (3). Thinking that this might be beneficial, and that by the administration of cortisone lessening of fibrosis or some other advantage might be gained, this series of experiments was designed to test separately the effects of oxygen and of cortisone upon the functional and pathological changes of the rabbit leg exposed to cold water.

METHOD

The left hind legs of 107 rabbits were exposed to water at 3°C. by a modification (3) of the method used by Lange, Weiner, and Boyd (1). Female rabbits, averaging three kilograms in weight were used. The left hind legs were depilated two days before their exposure. The first 23 rabbits were fed oats only, the remainder were fed desiccated rabbit ration which appeared to be a much healthier diet. An adequate intake of food and water was maintained throughout the entire exposure period. Rabbits were weighed daily. All surviving animals were sacrificed thirty days after exposure.

Oxygen was administered as described previously (3), in such a manner that, throughout the period of immersion of the left hind leg, 25 rabbits breathed a mixture of 80 to 90 per cent oxygen. This was accomplished by allowing the oxygen to escape into the stalls at a rate of two to three liters per minute and to be recirculated through soda lime to eliminate the carbon dioxide.

Cortisone was administered to 16 rabbits by subcutaneous injection of 10 mg. daily for two weeks, starting on the first day prior to immersion of the left hind leg.

Functional tests were applied to both hind legs on the day prior to exposure, and on each day or each two days subsequently, to test the animal's ability to: 1. Spread the toes; 2. dorsiflex the foot; 3. hop; and 4. to bear weight. We also noted, 5, the extent of edema formation.

When a normal rabbit is held by the scruff of the neck and lowered toward the floor it spreads its toes and dorsiflexes its foot at the ankle. Loss of these functions and any tendency to drag the left hind leg while hopping was considered abnormal. Weight-bearing was tested by lowering the rabbit upon a spring scale in such a manner that the rabbit was forced to bear as much weight as possible upon the leg in question. Edema was graded as slight, moderate, or marked.

Histological studies were made of the muscle of both hind legs, regardless of the length of time each animal survived after exposure. On the 30 ± 1 day after completing exposure, all surviving animals were chloroformed. The hind legs were removed immediately after death, and placed in formalin for future study. Later a muscle specimen was removed from the extensor muscles of the foot 2 inches below the knee (3 inches below the level of immersion) for microscopic study. All staining of microscopic slides was with hemotoxylin and eosin.

Toxicity to oxygen has been reported in rabbits, featuring widespread pulmonary lesions (4). For this reason, the heart and lungs were examined in 9 of the rabbits receiving oxygen, in 2 of the rabbits receiving cortisone, and in 12 of the control series.

Because of the fact that renal lesions and glycosuria (5) have been reported in rabbits receiving cortisone, daily qualitative tests for glucose were performed on the urine of the 12 rabbits receiving this preparation. The kidneys of these rabbits were also subjected to gross and microscopic study at the time of death.

Thirteen of the 107 animals were discarded because of mechanical failure of the cooling apparatus, escape of the animal, death during exposure, or obvious systemic illness at the time of, or shortly after, removal from the stall. Of the 94 rabbits remaining, 27 died within the first 30 days after exposure. The other 67 were studied for a 30-day period by the functional tests. Pathological studies were made on the first 56 of the 94 animals.

An additional 4 rabbits were confined within the apparatus for a period of 50 hours with legs dependent but not exposed to cold water. All 4 of these control ani-

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TABLE I

Effect of cortisone and oxygen inhalation on functional recovery of cold-exposed legs of 107 rabbits at end of 30-day period

Hours of exposure	Total no. (Controls and treated) studied	Expired before 30 days (excluded from study)	Rabbits included in study	No treatment		Cortisone		Oxygen†		Totals	
				Returned to normal*	Did not return to normal	Returned to normal	Did not return to normal	Returned to normal	Did not return to normal	Returned to normal	Did not return to normal
64	4	2	2	0	2	—	—	—	—	0	2
56	4	4	0	—	—	—	—	—	—	—	—
42–45	36	12	24	6	7	4	2	5	0	15	9
30–31	26	6	20	5	3	3	3	4	2	12	8
20–24	19	2	17	6	4	—	—	6	1	12	5
16	3	1	2	1	0	—	—	1	0	2	0
8	2	0	2	2	0	—	—	—	—	2	0
Totals	94‡	27	67	20	16	7	5	16	3	43	24

* Criteria for return to normal was full ability to spread exposed (left) hind toes, dorsiflex the foot, and have normal appearance of hopping. Weight bearing on left and right legs was not considered in this analysis.

† Discounting the 64-hour exposure group a significantly larger number of rabbits returned to normal statistically among those receiving oxygen than among the control (or no treatment) group. (Chi square = 4.1, $P < 5\%$.)

‡ The first 56 of these rabbits were used in the pathological study. The data on 13 rabbits of the original 107 were discarded for reasons given in "Method."

mals were subjected to the functional tests but none was included in the pathological study.

The number of rabbits used in each of the studies is given in the tables.

RESULTS

Functional

1. Ability to spread the toes of all left hind legs was lost when they were examined immediately after exposure, but was restored in an average of 5.5 days in the group which was exposed for 24 hours, of 8.2 days in the group exposed for 30 hours, and of 10.9 days in the group exposed for 45 hours.

2. Ability to dorsiflex the foot of the left hind leg was totally lost for an average of 18 days in rabbits whose legs were exposed to cold for 45 hours or more. In those exposed less than 30 hours this ability was almost invariably restored in 15 days or less. Two rabbits with leg exposure for 64 hours had not regained this ability when sacrificed 30 days later.

3. The return of a rabbit's ability to hop normally 30 days after exposure of one leg returned in inverse proportion to the duration of exposure.

4. The ability to bear weight was most impaired immediately after exposure, was much more impaired in the left (exposed) leg than in the right, and varied inversely as the time of exposure. Weight-bearing had returned to normal 30 days after exposure in 96 per cent of the rabbits' legs exposed 45 hours or less.

5. The rabbits lost about 0.5 Kg. in weight during exposure in spite of the leg edema. This weight was regained by the end of thirty days in all animals except one-third of those receiving cortisone. Marked edema was always observed in both hind legs immediately following exposure. It was more pronounced in the exposed leg, and almost invariably cleared up within forty-eight hours after exposure,

The group treated with oxygen differed in two ways. The per cent of limbs returning to normal at the end of thirty days by the criteria given (Table I) was maintained significantly higher. Second, in rabbits with legs exposed for 30 hours,

TABLE II

Days required to recover any ability to spread toes in rabbits breathing 85 per cent O_2 and air. Rabbits were exposed to water at 3°C. for varying periods of time

Hours of exposure	Average number of days required to regain ability to spread toes (left)		Standard deviation of average number of days		Number of rabbits	
	Control	Oxygen	Control	Oxygen	Control	Oxygen
24	7.0	4.5	1.2	1.8	8	7
30	11.0	6.5	1.0	1.3	8	6
45	12.0	11.0	2.1	1.6	13	5

TABLE III

Frequency and degree of histopathological findings in muscle

Hours exposure to cold	No. of cases	Basophilia		Cellular infiltration		Giant cells		Edema		Fragmentation	
		No. involved	Degree invlmt.*	No. involved	Degree invlmt.	No. involved	Degree invlmt.	No. involved	Degree invlmt.	No. involved	Degree invlmt.
50–60 (exp. L leg)	8	3	S	3	M	0	—	3	M	6	C
(unexp. R leg)	8	1	S	1	S	0	—	1	S	1	S
42–45 (exp. L leg)	19	7	M	8	M	0	—	7	M	13	M
(unexp. R leg)	19	2	S	2	S	0	—	2	S	2	S
30–31 (exp. L leg)	13	1	M	0	—	1	M	1	S	5	M
(unexp. R leg)	13	0	—	0	—	0	—	0	—	1	S
20–25 (exp. L leg)	16	2	S	0	—	0	—	1	S	3	M
(unexp. R leg)	16	0	—	0	—	0	—	0	—	1	S

* S = slight, M = moderate, C = considerable.

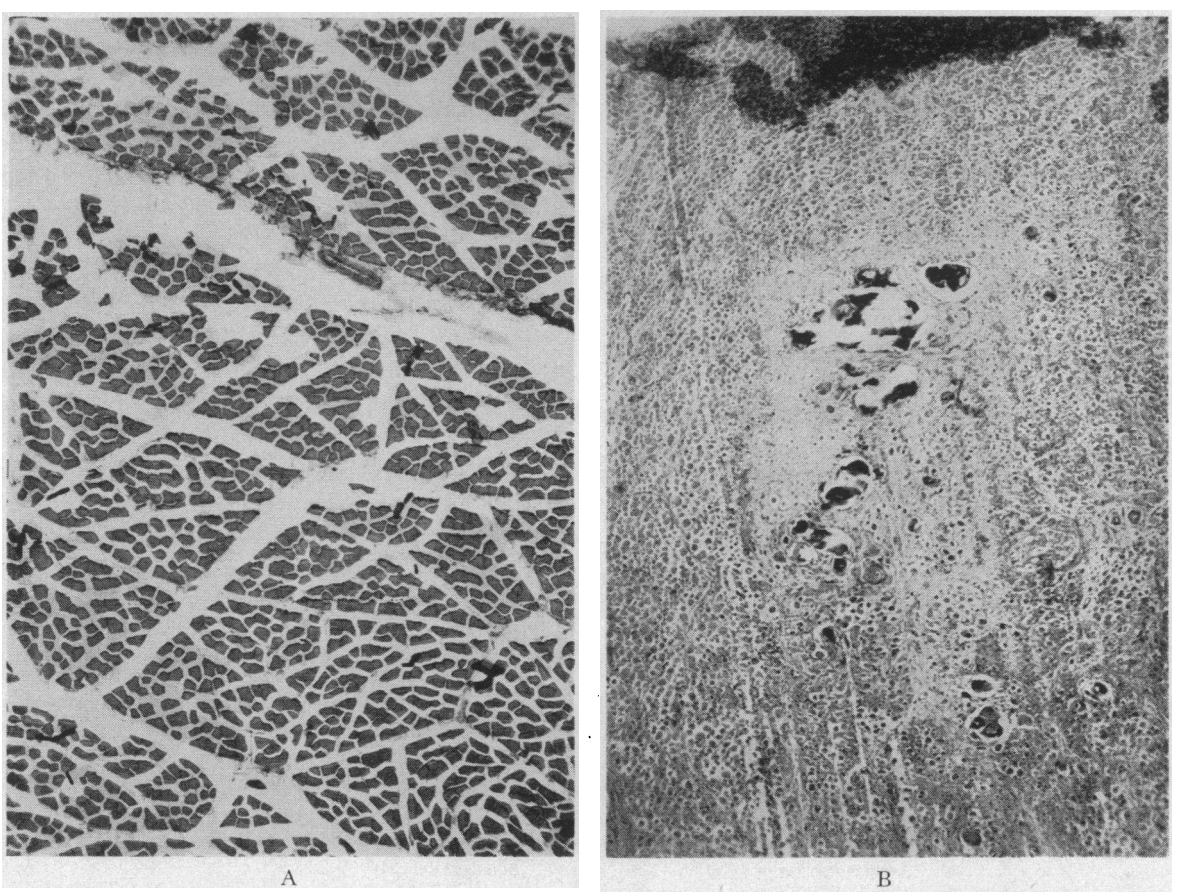

FIG. 1. MUSCLE DAMAGE BY COLD

A. Section of muscle of right (unexposed) leg of rabbit whose left leg was immersed in water at 3°C. for 64 hours. Section taken at necropsy 30 days after exposure (×80).

B. Section of muscle of left (exposed) leg of same rabbit (×80).

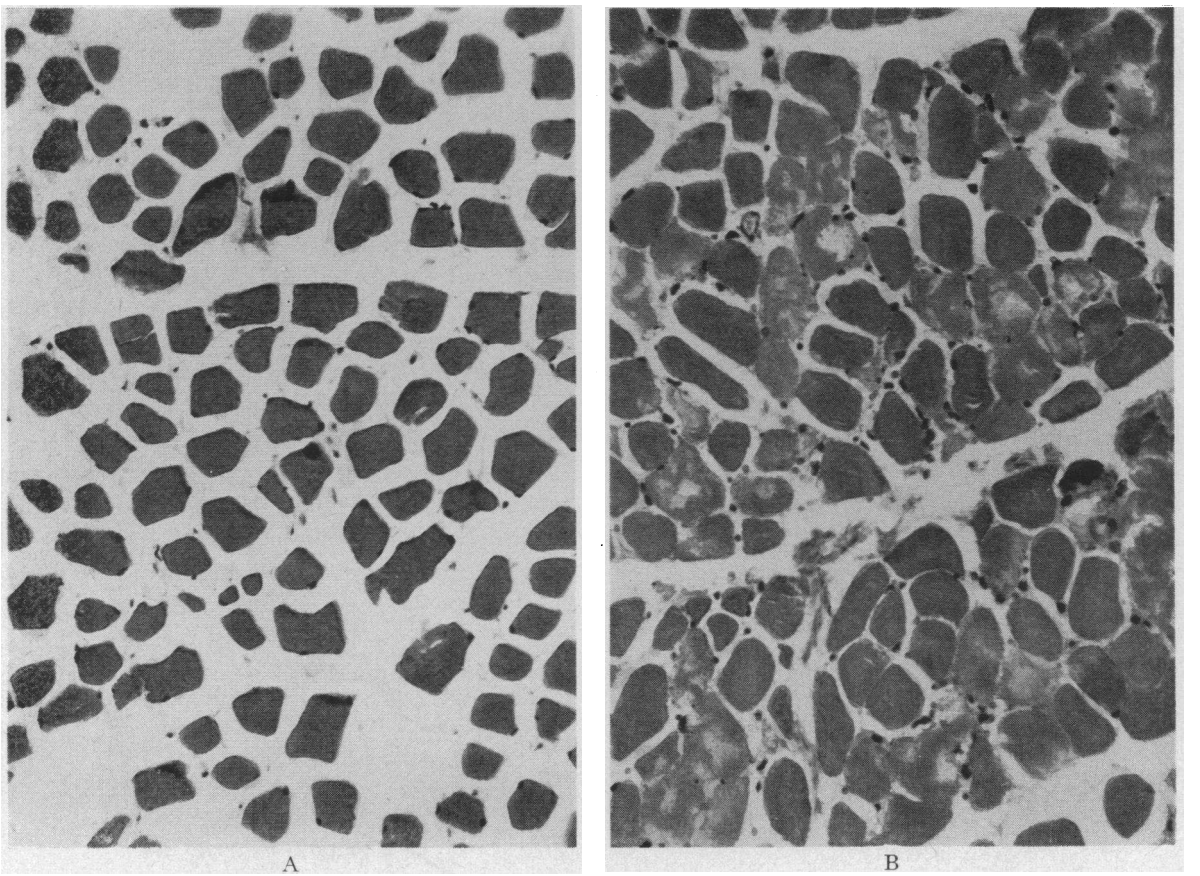

FIG. 2. MUSCLE DAMAGE BY COLD

A. Section of muscle of right (unexposed) leg of rabbit whose left leg was immersed in water at 3°C. for 45 hours. Section taken at necropsy 30 days after exposure (× 360).

B. Section of muscle of left (exposed) leg of same rabbit (× 360).

ability to spread toes was regained in a significantly shorter time by the animals receiving oxygen than by those without oxygen (Table II). No difference was detected between the exposed legs of rabbits breathing oxygen and air when exposures were for 24 or for 45 hours.

No difference in functional changes or recovery was observed between the control animals and those receiving cortisone.

Four rabbits confined in the exposure apparatus with legs dependent but not exposed to cold water developed edema and some diminution of ability to bear weight in both hind legs. The edema disappeared within 24 hours, more rapidly than in legs exposed to cold water. At no time was there any impairment of the ability to spread the toes. All measured functions returned to normal within four days after "exposure".

Room temperatures were kept at 23°C. or above during these experiments and no abnormal drop in body temperature was observed.

Pathological

Limbs of 56 rabbits were studied for pathological changes. Nineteen had received oxygen, 12 cortisone, and 25 were untreated. The time of exposure to cold varied from 20 to 64 hours. Thirty-five rabbits survived for 30 ± 1 days and were sacrificed. Of those exposed for 20 to 25 hours 15 of the 16 survived for 30 days. Of those exposed for 50 to 60 hours only 2 of 8 survived for 30 days. Generally speaking, only in animals dying within 48 hours after exposure was marked tissue edema noted grossly. Ulcers were an inconstant and uncommon finding and were believed, because of their location, to be on a traumatic

rather than ischaemic basis. There was no vesiculation and no gangrene.

The muscles of both hind limbs of the 56 rabbits were studied histologically. The most consistent changes were basophilia, cellular infiltration, giant cell formation, edema, and fragmentation of muscle cells. Abnormal variation of the size of the muscle bundles was occasionally observed.

The slides were examined without knowledge to the examiner of the time of exposure or as to whether the slide was from tissue of the exposed or unexposed leg. In order to quantitate the histopathological changes, a score was given to each slide with respect to the amount of basophilia, cellular infiltration, giant cell formation, edema, and fragmentation (Table III). Each of these changes was graded 1, 2, 3, and 4 corresponding respectively to slight, moderate, considerable, and marked. The total count for each slide was then calculated, as well as the total and average (Figure 3) pathological change for each exposure group. In general the degree of pathological change varied directly as the number of hours of exposure to cold.

Microscopic changes were minimal in legs exposed to cold for 30 hours or less (Table III, Figure 3). When the data in this table and figure were compiled comparison of the figures for exposed and unexposed muscle almost invariably permitted the pathologist to identify muscle that had been exposed to cold.

In the legs exposed more than 45 hours, definite and marked pathological changes were easily discerned microscopically (Figures 1 and 2).

Giant cells were rarely found in the muscles of rabbits' legs exposed to cold for less than 60 hours.

Basophilia was found only in the leg muscles of rabbits dying within two weeks after exposure to cold. Since these animals all died of some acquired illness and were not sacrificed, systemic disease as well as exposure of the leg to cold must be con-

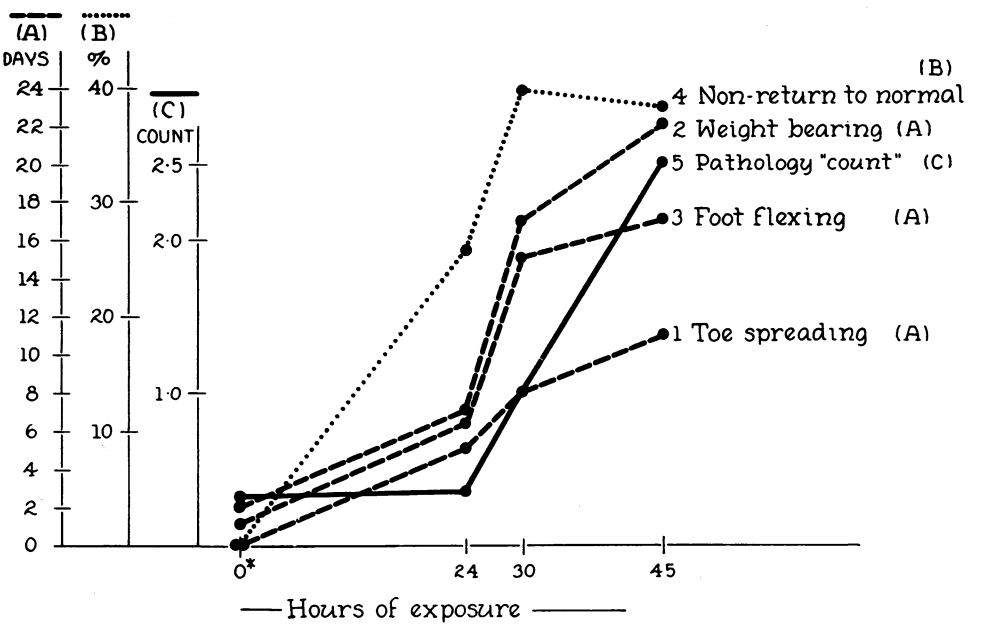

FIG. 3. TIME OF DAMAGE BY COLD

Degrees of damage (functional and pathological) after different times of exposure of legs to 3°C. water. (A) 1. Average number of days necessary to regain ability to spread toes; 2. Average number of days necessary to regain weight-bearing ability; 3. Average number of days necessary to regain ability to flex foot. (B) 4. Per cent of non-return to normal at end of 30 days. (C) 5. Total pathology "count" (see Text).

0* exposure. Functional data for these points were obtained from four rabbits (see Text) subjected to apparatus for 50 hours without exposure of left leg to water at 3°C. Pathological "count" for this point is that of 30-hour exposure rabbits' average on right "unexposed" leg.

sidered in evaluating the basophilia. However, the changes in the exposed leg were always more marked than in the unexposed leg of the same animal.

No damage to blood vessels was detected.

Neither daily administration of cortisone nor breathing 85 per cent oxygen during exposure appeared to influence the pathological changes. Under the conditions of these experiments fibrosis was not consistently found in the control animals. It was, therefore, not possible to evaluate the therapeutic effect of cortisone in this respect.

In the 12 rabbits which received cortisone, for 15 days, no glycosuria was noted, nor were there renal lesions such as those found in the animals used by other investigators (5).

Localized areas of edema and congestion were found in the lungs of 9 of the 23 rabbits whose heart and lungs were examined at necropsy. These findings, however, were no more common in the lungs of rabbits that inhaled oxygen than in those which did not. No pathological changes were seen in the myocardia.

DISCUSSION

The data of the preceding paper (3) gave evidence of a reduced blood flow and of a reduced metabolism, since there was a reduced or somewhat variable change in oxygen tension in the muscle and subcutaneous space of the rabbit limb chilled in water at 3°C. Oxygen inhalation restored the oxygen tension of these chilled tissues to or above the pre-cooling level. The present data were obtained in order to learn whether any functional or structural (histological) advantages to the exposed limb could be gained by the administration of oxygen or cortisone. The study resolved itself into measurements of functional and structural damage following various specific periods of exposure. The "treated" animals were exposed just as were the controls, and in each instance the contralateral leg served as further control. In order to avoid any unnecessary damage to any leg in these experiments we omitted the measurements of temperature and of oxygen tension reported in the preceding paper (3). Functional and structural damage rose precipitously after 30 hours of exposure. When oxygen was inhaled throughout a 30-hour exposure its beneficial effect was demonstrable functionally but not histologically. Cold-hypoxia appears to be a causal factor of experimental immersion foot, but not the major cause. Data from this and the immediately preceding paper (3) suggest that of the two variables, *time of exposure* and *tissue oxygen tension,* the former is able to take precedence, but that increased oxygen supply extends the time tolerance of the tissues to the effects of low temperature. The absence of difference between the histological appearance of muscle from control and oxygen-treated animals might mean that the lesion limiting function lies in the nerves, which were not examined.

The functional and pathological changes produced in the rabbits' legs by cold exposure closely resemble clinical immersion foot. The lesion was produced for the most part by prolonged exposure to cold water, rather than by dependency or immobility of the leg, as proven by control observations on unexposed legs.

Our findings agree for the most part with those of Lange, Weiner, and Boyd (1) who reported foot-drop, edema, and lack of toe spread in the rabbit leg so exposed. Neither their nor our pathological sections revealed vascular damage. Lack of fibrosis of muscle tissue in our experiments may have been due to the fact that no pathologic sections were studied after exposure to 3°C. water for as long as 96 hours, the exposure time in some of Lange's cases. The fact that our rabbits failed to show significant decreases of body temperature may have been due to higher environmental temperatures. We did keep two rabbits wet in air temperatures 16°C. and 18°C. (unpublished) and succeeded in lowering their body temperatures to 29.5°C. and 30.7°C. after 64 and 70 hours of exposure, respectively.

Whether or not the fragmentation (or necrosis) of cells (Figures 1 and 2) can be termed "gangrene" as referred to by Lewis and Moen (2) is questionable in view of the lack of slough, of infection, and of intraarteriolar thrombus formation. Our tentative conclusions on the distinction between "immersion foot" and "frostbite" differ somewhat from theirs (6).

The rabbits breathed high concentrations of oxygen for only 45 hours. This probably accounts for the lack of fatal pulmonary lesions described by Binger, Faulkner, and Moore (4).

SUMMARY

1. Immersion foot was produced in 107 rabbits by exposing their left hind legs to water at 3°C. for times varying from 8 to 64 hours.

2. Functional changes resulted. These included inability to spread toes, inability to dorsiflex the foot, inability to hop normally, and decreased ability to bear weight on the left hind leg.

3. Pathological changes resulted in the muscle. These included basophilia, cellular infiltration, giant cell formation, fragmentation, abnormal variation of the size of muscle bundles, and edema.

4. The degree of functional and pathological change varied directly with the time of exposure.

5. There was an abrupt increase in both pathological and functional changes after 30 hours of exposure.

6. There was suggestive evidence that oxygen administration during exposure suppressed the functional changes, particularly of legs exposed to cold water for 30 hours. There was no evidence that oxygen breathing modified the pathological changes in muscle.

7. Cortisone appeared not to influence the functional or pathological changes in rabbit legs so exposed.

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