EFFECTS OF COOLING ON EXPERIMENTALLY INFECTED TISSUES*

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THE EFFECTS OF REDUCED TEMPERATURES on tissue survival under anoxia and on tissue resistance to infection have been studied in several recent investigations. Allen,[1] and Brooks and Duncan,[2] showed that cooling greatly prolongs the life of tissues deprived of their blood supply. The experiments of Brooks and Duncan[3] also have demonstrated, conclusively, that the local application of cold without interference with the blood supply prevents the development of the normal tissue response to bacterial organisms (*"Staphylococcus aureus"*) and to chemical irritants (turpentine) injected intracutaneously, and that the appearance of the classical signs of inflammation rapidly follows the return of the affected part to a normal environmental temperature.

In this communication are reported the results of an investigation to determine the effects of reduced temperatures on both the local tissue response and the organisms responsible for it, and to determine the eventual course of a local infection as altered by a temporary period of cooling.

EXPERIMENTAL PROCEDURE

A concentrated suspension of *"Streptococcus hemolyticus"* in broth was used to produce a localized infection. The suspension was obtained from 18-hour cultures, and bacterial counts showed its concentration to vary within the range of 10^8 to 10^9 in all specimens.

Mongrel dogs weighing from four to eight kilos were used as experimental animals. After the hair was removed from one of the forelegs, 0.5 cc. of the suspension was injected into the wrist joint or into the subcutaneous tissues above the wrist. The inoculated limbs were then cooled to six degrees C. for various lengths of time. Cooling was effected by immersing the limb to a level well above the elbow in a constant temperature bath provided by a commercial cooler. The dogs were immobilized in canvas hammocks and the affected limb in the cooler was made secure by a tie placed well below the wrist. This insured the uninterrupted cooling of the limb and did not interfere with the blood supply to the inoculated area. No protective substances were applied to the skin, and food and water were not withheld from the animals during the course of the experiment. The control animals

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were injected in a similar fashion and were placed in cages at room temperature for equivalent periods of time, no effort being made to immobilize the inoculated limb. These experiments were carried out for periods ranging from 24 to 96 hours. Each experiment was performed on two groups of six dogs. In the first group, the gross and microscopic structure of the lesions was studied, and bacterial counts were made from samples of the exudate. This method of securing material for counts was found to be in-

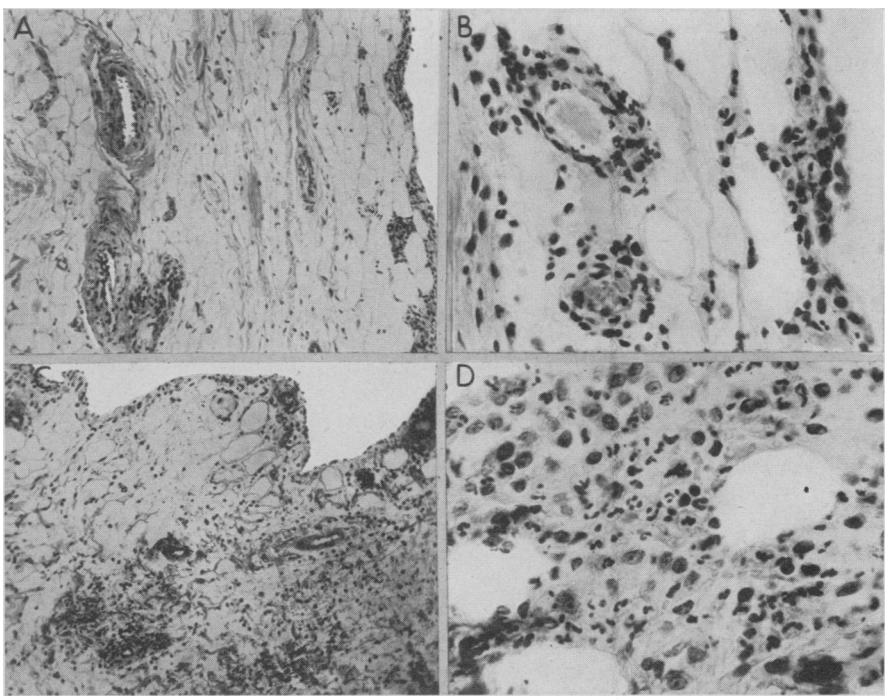

PLATE I.—A. Photomicrograph (× 135) of the synovial membrane of a dog's wrist joint inoculated with *"Streptococcus hemolyticus"* and cooled at 6°C. for 48 hours.
B. Photomicrograph (× 425). Same as A, to show more clearly the vascular congestion and minimal degree of leukocytic infiltration.
C. Photomicrograph (× 135) of the synovial membrane of a dog's wrist joint inoculated with *"Streptococcus hemolyticus"* and maintained at room temperature for 48 hours.
D. Photomicrograph (× 570). Same as C, to show more clearly the slight degree of acute inflammatory response exhibited in the joint synovial membrane of the control dog.

accurate—so that a second group of animals was prepared in which at the completion of the experimental cooling, the tissues at the site of inoculation were excised *"en bloc,"* care being taken that the excision extend well out into normal tissue. These specimens were then homogenized and the total number of bacteria per sample determined. The controls were carried out similarly in two groups of six animals for each experiment.

All bacterial counts were determined by dilutions in broth poured over blood agar plates and are expressed in number of bacteria per cubic centimeter. Contamination was very infrequent, and the organisms recovered were invariably identified as *"Streptococcus hemolyticus."*

EXPERIMENTAL RESULTS

The effect of local cooling on infected subcutaneous tissues was studied for periods of time varying from 24 to 96 hours.

In a first experiment dogs were inoculated subcutaneously with 0.5 cc. of the bacterial suspension. In one group the limbs were cooled for 48 hours immediately following the injection, and the animals were then sacrificed. The other group was left at room temperature for the same period of time before being studied.

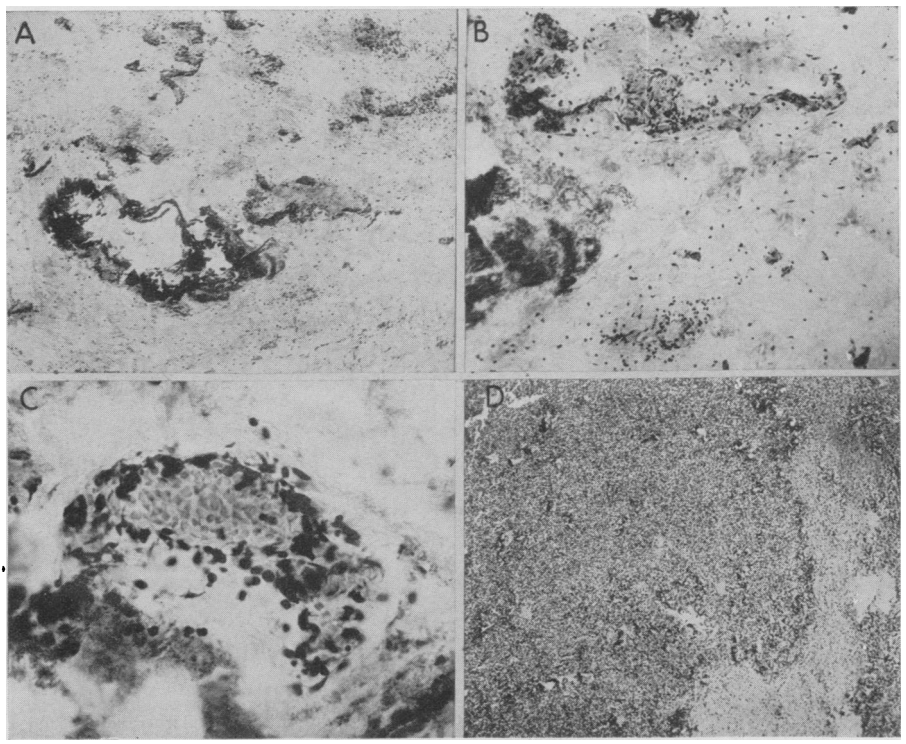

PLATE II.—A. Photomicrograph (× 50) of the subcutaneous tissue of a dog's limb at the site of inoculation after cooling 48 hours at °C. Note the presence of vascular congestion and edema without appreciable leukocytic infiltration around the dark ring of bacteria embedded in the tissues.

B. Photomicrograph (× 135) from the same area as A to show the relatively acellular edema and minimal diapedesis.

C. Photomicrograph (× 570) from the same area as A showing a distended capillary near a cluster of bacteria (lower part of picture). Note the absence of tissue reaction to the bacteria.

D. Photomicrograph (× 50) of the subcutaneous tissues of a dog's leg at the site of inoculation with *"Streptococcus hemolyticus"* after 48 hours at room temperature. Note the marked acute inflammatory reaction.

In the limbs that had been subjected to cooling there was no external evidence of inflammation at the site of inoculation. Local anesthesia and local loss of motor function were noted in all animals. At autopsy, on gross examination, diffuse edema, though of varying degree, was found to be present in all cases throughout the area that had been immersed and to involve mostly the subcutaneous layer. The edematous tissue had a greyish-blue color and a viscid, gelatinous consistency. Around the site of injection, these greyish tissues assumed a faint pinkish color. Closer examination of

the area showed a fine reddish reticulum suggestive of dilated blood vessels. Gross extravasation of blood was not observed.

Even away from the site of inoculation, there was microscopic evidence of marked vascular congestion and edema. Around the congested capillaries one could see a few polymorphonuclear and red blood cells. These findings were also observed in the subcutaneous tissues of animals that had been submitted to cooling without any bacterial inoculation. In the inoculated area the vascular dilatation was more marked and the diapedesis, although minimal, was somewhat more marked, also. Only a few inflammatory cells were observed however, at any distance from the capillaries and the edematous tissue was remarkably free of cellular infiltration. In many sections large clumps of bacteria were seen embedded in the tissues, without any evidence of cellular activity around them (Plate I, A, B and C). The bacterial counts performed on the exudate fluctuated widely but were all considerably lower than the original counts of the bacterial suspension injected.

The control animals, at room temperature, all showed a well-defined external swelling at the site of inoculation with redness of the skin in a few instances. At autopsy, on gross examination, a small cutaneous abscess was found in all animals. The microscopic picture was characteristic of an acute inflammatory reaction with abscess formation (Plate I—D). The purulent material obtained from the abscess yielded bacterial counts that varied widely, but were definitely higher than those obtained from the animals subjected to cooling. On the basis of subsequent evidence obtained in this research, it is clear that the method employed for obtaining the samples of exudate was defective. The difficulty with which only minute amounts of fluid could be obtained from the edematous cooled tissues repeatedly necessitated resorting to washings, and the clumping observed in the microscopic sections suggested that variable counts could probably be obtained from different parts of the same specimen.

To eliminate these objections the experiments were repeated on other animals. The same procedure was followed but after the completion of the experiment the inoculated tissues were excised widely and the counts made from this whole specimen.

This procedure was first carried out over a period of 24 hours. The number of bacteria recovered from the tissues subjected to cooling was only slightly lower than that injected (Table I). The counts obtained from

TABLE I

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER LOCAL COOLING AT 6°C. FOR 24 HOURS

No. of Dog	Bacterial Counts Per Cc. Suspension	Tissue
43A	5.9×10^8	2×10^8
45A	5.9×10^8	2×10^7
11C	4.9×10^8	1×10^8
6C	4.9×10^8	9×10^8
20C	7.8×10^8	4.6×10^8
15C	7.8×10^8	1.8×10^8

the controls though consistently high, were lower than those obtained from the cooled tissues and lower than the original number injected (Table II).

The bacterial counts obtained after 48 hours of cooling were substantially the same as those observed after 24 hours of cooling (Table III). The controls showed a further reduction in the number of bacteria, the

TABLE II

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER 24 HOURS AT ROOM TEMPERATURE

No. of Dog	Bacterial Counts Per Cc. Suspension	Tissue
37A	5.9×10^8	6×10^7
40A	5.9×10^8	1.6×10^7
9C	4.9×10^8	1×10^7
2C	4.9×10^8	2×10^6
22C	7.8×10^8	1.3×10^8
18C	7.8×10^8	2.4×10^7

TABLE III

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER LOCAL COOLING AT 6°C. FOR 48 HOURS

No. of Dog	Bacterial Counts Per Cc. Suspension	Tissue
44A	5.9×10^8	1.7×10^8
41A	5.9×10^8	4×10^7
8C	4.9×10^8	2.5×10^8
7C	4.9×10^8	1.5×10^8
26C	8.9×10^8	2.8×10^8
23D	8.9×10^8	1.6×10^8

TABLE IV

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER 48 HOURS AT ROOM TEMPERATURE

No. of Dog	Bacterial Counts Per Cc. Suspension	Tissue
38A	5.9×10^8	4.5×10^4
42A	5.9×10^8	8×10^6
3C	4.9×10^8	8.1×10^7
1C	4.9×10^8	1.7×10^6
25D	8.9×10^8	1.5×10^6
27D	8.9×10^8	2.4×10^5

counts being lower than those obtained after 24 hours in untreated animals, and markedly lower than the original number of bacteria (Table IV). In both these experiments, the gross appearance of the tissues showed the characteristic changes previously described.

Similar experiments were then carried out with the period of cooling extended to 96 hours. The aspect of the cooled tissues was similar to that found after 48 hours. The amount of subcutaneous edema however, and the vascular congestion were usually greater. In these animals after block excision of the tissues, the number of bacteria recovered approximated roughly the number injected (Table V). In the controls, abscess formation was a constant finding. In many animals, after the required period of time at room temperature, the abscess had ruptured through the skin. A large number of animals had, therefore, to be inoculated before we could obtain six

tissue specimens with an intact skin. The bacterial counts obtained from these tissues were much lower; showing that in this time period the local defense mechanisms had overcome the infecting organisms to a marked degree (Table VI).

TABLE V

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER LOCAL COOLING AT 6°C. FOR 96 HOURS

No. of Dog	Bacterial Counts Per Cc. Suspension	Tissue
9E	8.7×10^8	1.8×10^8
11E	8.7×10^8	1.9×10^8
12E	8.7×10^8	4.3×10^8
14E	$6.6 \quad 10^8$	5.4×10^8
15E	6.6×10^8	6.6×10^8
16E	6.6×10^8	4.2×10^8

TABLE VI

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER 96 HOURS AT ROOM TEMPERATURE

No. of Dog	Bacterial Counts Per Cc. Suspension	Tissue
26C	6.3×10^8	1.0×10^3
48C	6.3×10^8	1.4×10^3
47C	6.3×10^8	3.4×10^5
5E	3.0×10^8	8.0×10^3
6E	3.0×10^8	2.6×10^5
7E	3.0×10^8	1.0×10^3

The experimental method was then modified to determine the effects of a return to a normal environment after a preceding period of cooling. Groups of animals were inoculated according to the same technic, and cooling applied to the limb for a period of 48 hours. The animal was then removed from the cooler and left at room temperature for an additional period of 48 to 96 hours. The limbs warmed up very rapidly following removal from the cold bath. Within 48 hours after discontinuing the application of cold, the edema of the limb would disappear, leaving a localized swelling at the site of injection. The results of the examination of this area, in the gross, were very similar to those observed in animals left at room temperature for 48 hours after inoculation. But in most of the animals which had been subjected to a period of cooling the area of inflammatory changes was slightly greater than in the controls. In none, had the abscess broken out through the skin but in some instances, superficial necrotic lesions of the skin were noted. Microscopically, there was a typical acute inflammation that could not be differentiated from that observed in the controls except for the subjective impression that the amount of edema was slightly greater. The bacterial counts obtained from the excised tissues showed a decrease from the original number of bacteria injected and approximated those observed in animals after 48 hours at room temperature without previous cooling (Table VII).

When 96 hours were allowed to elapse after cooling had been discontinued, the individual variations in the gross appearance of the lesion

were greater than in any of the previous experiments. A localized abscess was observed in all cases. While in some instances, the area of inflammation was slightly smaller than that observed after 48 hours, in others it was larger than any observed in the controls after 96 hours. This wide variation was also noted in the bacterial counts obtained from the excised tissues (11 dogs). In some animals the number of bacteria recovered showed only a slight decrease or no change at all; in others, the counts showed a marked decrease over the number of bacteria originally injected (Table VIII).

TABLE VII

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER LOCAL COOLING FOR 48 HOURS FOLLOWED BY 48 ADDITIONAL HOURS AT ROOM TEMPERATURE

	Bacterial Counts Per Cc.	
No. of Dog	Suspension	Tissue
27C	6.3×10^8	5.8×10^7
23C	6.3×10^8	1.4×10^5
29C	6.3×10^8	1.8×10^8
4D	5.1×10^8	3.9×10^6
3E	3.0×10^8	4.8×10^4
4E	3.0×10^8	4.3×10^5

TABLE VIII

NUMBER OF BACTERIA IN THE SUSPENSION INJECTED AND IN THE EXCISED TISSUES AFTER LOCAL COOLING FOR 48 HOURS FOLLOWED BY 96 ADDITIONAL HOURS AT ROOM TEMPERATURE

	Bacterial Counts Per Cc.	
No. of Dog	Suspension	Tissue
1F	8.0×10^8	3.4×10^5
2F	8.0×10^8	1.1×10^5
3F	8.0×10^8	8.3×10^8
4F	8.0×10^8	3.7×10^8
5F	7.2×10^8	3.7×10^4
7F	7.2×10^8	2.6×10^3
8F	7.2×10^8	3.9×10^3
23G	2.1×10^9	4.2×10^6
24G	2.1×10^9	4.8×10^6
28G	2.1×10^9	1.6×10^9
29G	2.1×10^9	1.0×10^9

Finally, another series of experiments was carried out with the site of inoculation at a greater distance from the surface. In spite of the small bulk of the limb, the wrist joint seemed best suited to this purpose. The joint was inoculated with 0.5 cc. of the bacterial suspension. One group of animals was treated by local cooling and the other left at room temperature for periods of 24 and 48 hours.

The control animals did not show, grossly, as severe an acute pyogenic arthritis as had been expected. There was some congestion of the synovia and minute amounts of turbid fluid in the joint. The recovery of "*Streptococcus hemolyticus*" from this fluid, however, established the fact that the joint had been inoculated. Microscopic examination of the capsule showed an acute inflammatory reaction but not of the intensity of that observed in the subcutaneous infections (Plate II, C and D).

In the animals which had been refrigerated the gross appearance of the

limbs was not greatly different. The microscopic sections showed only minimal reactive changes (Fig. II, A and B). The bacterial counts obtained from the joint exudate showed the same trend as those reported after subcutaneous injections.

COMMENT.—The exposure of inoculated tissues to a temperature of 6° C. for periods up to 96 hours results in a definite inhibition of the usual inflammatory response to "*Streptococcus hemolyticus*" encountered in the control animals. This inhibition is evidenced by the gross and microscopic picture

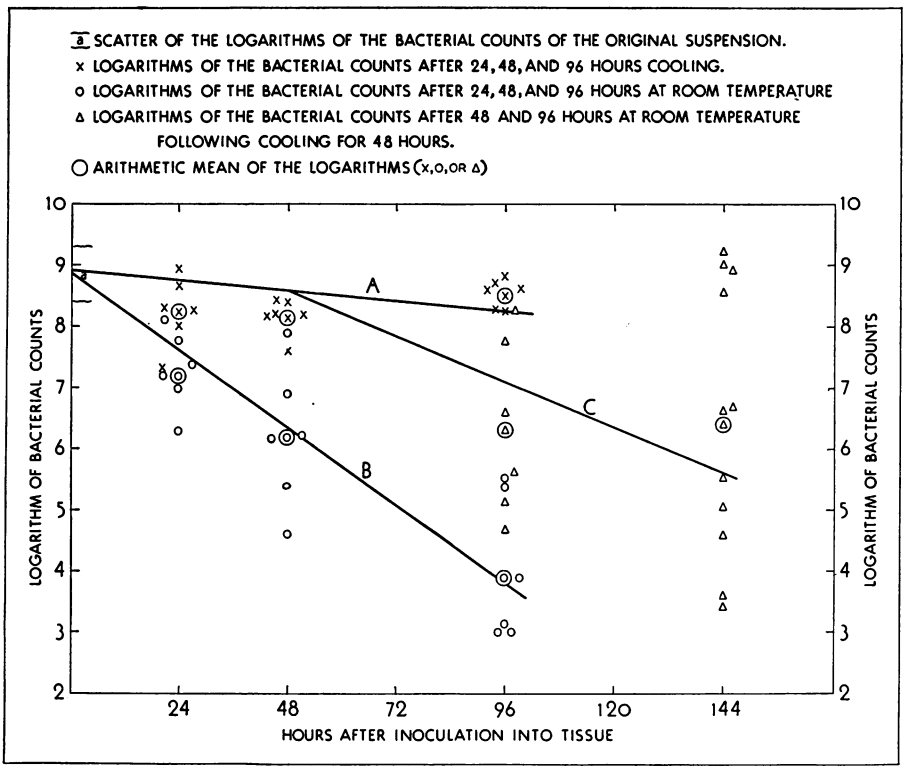

CHART I.—A. Curve showing that during cooling the number of bacteria in the tissues remains relatively unchanged.
B. Curve showing tbe decrease in the number of bacteria in the tissues which occurs at room temperature.
C. Curve indicating the tendency for bacteria to disappear more slowly from the tissues when a period of cooling has preceded the return to a normal environmental temperature.

observed after cooling. It is further substantiated by the fact that no significant decrease in the number of organisms injected is observed under these conditions. At room temperature, the decrease in the number of bacteria follows a logarithmic curve. A comparison of these data (Chart I) indicates that cooling delays markedly the rate of destruction of bacteria in living tissue, in spite of the inhibition of growth which probably takes place at low temperatures *in vivo* as well as *in vitro*. In these experiments no attempt was made to determine the proportion of bacteria that had spread to the regional lym-

phatics or that was carried away by the blood stream. Gelatinous edema, vascular congestion and minimal diapedesis were noted throughout the cooled limb, even at a distance from the site of inoculation. The degree of edema seemed related to the length of the cooling period. Finally, these changes were also observed in limbs that were cooled without any previous inoculation. This would suggest that some of the reactive changes observed in the tissues are due to the effects of cold itself. As the limbs were immobilized in a dependent position, dependency could be regarded as an additional factor, although it alone did not produce this lesion. It is noteworthy that this edema, with temporary anesthesia and loss of motor function and the occasional appearance of necrotic skin lesions after the return of the limb to a normal environment, bears a striking resemblance to the clinical syndrome of immersion foot. At the site of inoculation, the pathologic findings were similar but slightly more marked. At the same depth at which these slight reactive changes were found clusters of bacteria without any inflammatory cells around them could be observed. The short, but inevitable, time-lag necessary to reduce the tissue temperature to an adequate level may also be partly responsible for these findings. The evidence seems to indicate that the application of cold in itself for periods up to four days will not have a beneficial effect on the course of a localized infection. At most, a relative *status quo* is present as long as the reduced temperature is maintained. However, the constant finding of diffuse edema in our experiments argues against regarding this condition as a merely innocuous suspension of bacterial and tissue activity.

Brooks and Duncan reported that the area of necrosis which appeared after cooling had been discontinued, was larger than if the lesion developed at body temperature. In their investigation, they were able to study both lesions in the same animal. In our experiments a return, after cooling, to a normal environmental temperature resulted, also, in a grossly more severe inflammation, although the microscopic picture was not significantly different from that observed in the controls. As the lesion under study and the control had to be produced in different animals, individual variations made an exact quantitative comparison difficult. Inasmuch, as the rate of destruction of bacteria after a previous period of cooling will give some measurable indirect evidence of the alteration of tissue response by cold, Chart 1 shows that the trend under these conditions is towards a slower rate of destruction than that observed under normal conditions. The scatter of individual variations in the experiments where cooling had been resorted to widens as the elapsed time increases, and precludes the plotting of an accurate curve. Insofar, as is shown by our data, there is reason to believe that the growth restricting action of the tissues is somewhat decreased by cooling. Statistical studies on a very much larger group of animals may be necessary to substantiate this finding.

The inoculation of joints did not measure up to our expectations as an

experimental procedure. Our findings did show, however, that the effects of cooling are manifest even in the deeper structures in spite of their constant perfusion by warm blood. In the human, Bierman[4] reported a drop of 10° to 25° F. one and one-half inches from the surface of the calf when cold had been applied externally for one or two hours. In their experiments Brooks and Duncan noted the appearance of some reactive changes in the subcutaneous tissues while none were observed at the site of the intradermal inoculation. They concluded that this is an indication of the relatively small depth of temperature alteration by external applications. However, their method of cooling differed greatly from that used in these experiments as they applied cold only to the very limited area of injury. This discrepancy between our results and those reported by Brooks and Duncan would seem to lie in the procedure used rather than in the process itself. The temporary anesthesia and loss of motor function which follows cooling also provides supportive evidence that the deeper structures as well as the superficial were affected.

From these experimental data, it seems evident that the clinical application of cold (6° C.) to infected tissue will have no therapeutic value in itself. Under certain circumstances, it may not be harmful to cool an infected limb for a brief period in order to maintain the relative *status quo* observed in these experiments in regard to bacterial activity and to tissue response. However, under our experimental conditions, cooling for longer periods of time (24 to 96 hours) resulted in definite changes in tissue hydration, in marked vasodilatation and in a decrease in the growth restricting action of tissues towards bacterial organisms. Moreover, these changes became more marked as the period of refrigeration was prolonged. While the exact clinical limitations of the procedure remain to be determined, this would contraindicate, except for brief periods, the cooling to levels around 6° C. of infected limbs which one aims to save by conservative measures.

SUMMARY

As long as cooling to 6° C. is maintained, the subcutaneous tissues of the dog fail to show the inflammatory response usually initiated by the innoculation of "*Streptococcus hemolyticus.*" The number of organisms present in the tissues remains remarkably constant and closely approximates the number injected.

The development of extensive subcutaneous edema during cooling and after removal of the cold, the development of a more marked inflammatory reaction, with a decrease in the growth restricting power of the tissues to bacterial organisms, suggest the possibility of harmful effects from prolonged refrigeration.

The effects of cooling are manifest in the deeper as well as in the superficial tissues of the limb of a dog with an intact blood supply.

The clinical implications of the above results are discussed briefly.

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