Report

UPON

AN INVESTIGATION INTO THE EFFECTS
OF COLD UPON THE BODY.*By NORMAN C. LAKE, M.D., M.S., D.Sc.LOND.,
F.R.C.S. ENG.,ASSISTANT SURGEON, QUEEN'S HOSPITAL FOR CHILDREN;
TEMPORARY CAPTAIN, R.A.M.C.

DURING investigations into the growth of tissues *in vitro* upon the lines of Carrel's¹ well-known work it became necessary to investigate the effects of temperature changes. In particular the effect of cold upon the tissues was studied, and, as will be seen from the experiments detailed, led to the conclusion that cold, down to -6°C ., brought about a state of suspended animation in which for considerable periods the tissue could be preserved intact and its potential vitality maintained at the normal standard. This rather heterodox conclusion necessitated a re-investigation of "frost-bite," for if the effect of "chilling" of the tissues is to produce a state of suspended animation, then it will be illogical to accept theories which assume a direct effect of a degenerative and destructive nature. The term "frost-bite" is here used in its widest sense, and includes such conditions as "trench foot." Later on we shall attempt to separate these into two entities having as their basis different pathological processes.

In the more recent of the writings upon the subject the pathology of the condition has been explained in various ways. Lorrain Smith, Ritchie and Dawson,² after a series of very excellent investigations both from the clinical and experimental points of view, came to the conclusion that the pathology of "trench foot" was the same as that of true frost-bite, although of much less intensity, and that both are primarily due to the direct effect of cold upon the tissues, although other factors, such as pressure, had a determining effect. Many other writers agree with them. In several recent articles, written chiefly from the clinical standpoint, it has been suggested that vaso-motor disturbances play an important part; these, again, may be divided up into many smaller groups. Some explain the effects by assuming a lack of oxygen to the part, others by lack of nutritive plasma, and others again by special effects upon the endothelium of the vessel walls. That other factors than cold play a part in the production of "trench foot" is undoubted, but in all probability these factors must be considered as secondary and entirely subsidiary to the temperature variation. Rideal and Waller³ have, for instance, investigated the effects of water and saline solutions upon the feet, but failed in any of their experiments to produce conditions which resembled "trench foot," and it is doubtful whether the slight disturbances of sensibility which they produced in any way correspond to the paræsthesiæ which occur when the additional factor of cold is brought into play. These questions will be dealt with, however, more fully later on.

In considering the pathology of frost-bite, it is logical, at least, to say that the effects are either purely local or are reflex, with the possible combination of both, and an endeavour has been made to bear this constantly in mind.

The details of the work which follow are divided up into five groups of experiments:—

1. Investigation into the effects of cold upon the individual tissues when detached from all extraneous influences, whether of the circulatory or nervous system; such tissues may conveniently be called isolated tissues.

2. Investigation into the effects of cold upon the blood.

3. Investigation into the effects of cold upon the tissues in normal relationship to the circulatory and nervous systems.

4. Investigation into the effects of cold upon the tissues in abnormal relationship to the circulatory and nervous systems.

5. Finally, the conclusions drawn from these investigations both with regard to pathology and treatment.

* Research conducted under a grant from the Medical Research Committee at the Pathological Laboratories, Charing Cross Hospital Medical School. Thesis accepted for the degree of D.Sc. Lond.

(1) *Investigation into the Effects of Cold upon the Individual Tissues when Isolated.*

The method of growing tissues *in vitro* devised by Burrows⁴ and improved by Carrel⁵ lends itself very excellently to this work. It consists, in brief, of explanting small portions of tissue into media, the chief basis of which is plasma. The portion of tissue used is small and, having been placed on a coverslip, is surrounded by the medium, and when this is coagulated or set the whole is inverted on to a celled slide, sealed with paraffin, and incubated to 37.5°C . After a varying number of days the tissue can, if necessary, be transplanted into fresh medium, after washing in Ringer solution, and in this way kept alive and actively growing for an indefinite period. If these growths are not transplanted they sooner or later die from the accumulation of products of metabolism in the media. The effects of cold may be investigated either by exposing the tissue *en masse* before explantation or by placing the slide with the explanted growing tissue into the required temperature.

In all between 500 and 600 experiments have been made in which the tissue has been exposed for some period to a greater or less degree of cold, and the individual experiments quoted here have been repeated several times, in some cases over 100 times, with practically constant results. The tissues used were obtained from rabbits, mice, frogs, and in many cases from human beings during operations.

Practically every tissue in the body has been explanted, including heart, endothelium, connective tissue, skin, thymus, thyroid, kidney, liver, spleen, testicle, ovary, brain, nerve, pituitary, bone, mucosa, pancreas, &c. The media used included Ringer's solution, Ringer's solution and agar, serum and serum agar, plasma, hypotonic plasma, and plasma with the addition of various glandular and other extracts. As far as the effects of cold go, however, the medium appears to make very little difference, and so for the most part hypotonic plasma was used in order to make the growths uniform. Homologous plasma was generally used, but occasionally heterologous plasma (e.g., rabbit tissue on human plasma, &c.).

The criteria of activity in these explants may be divided into two main divisions—namely, growth and function. Growth is very easily observed and often exceedingly vigorous, function is much more difficult to test; in the case of the embryo and young adult heart, however, pulsation of the small explanted piece occurs with a fair degree of regularity and forms a delicate and easily observed test of functional activity. For this reason the great majority of the growths used for this research were young or embryo heart tissues. The staining of the surrounding medium by the excreted bile may be used as a test of activity in the liver growths, although this is rather crude, and hence has only been used occasionally. The functional activity of other tissues, and particularly of nerve tissues, it is almost impossible to test in these growths, and in this case the exact morphological reproduction of the tissue cells may be fairly logically taken to imply that such tissues are functionally active. It may be argued that this jump from vegetative to functional activity is not justified, and that the two are quite separate entities. Against this is the fact that among hundreds of heart growths it has been observed that there is a close relationship between the two; the more vigorous the growth the more active the pulsation. A heart growth pulsating but not growing (functionally but not vegetatively active) was not observed among the whole series.

The experiments in this group may be divided up as follows:—

(a) In which the tissue *en masse* was stored in Ringer's solution at low temperatures. Portions of the tissue were then explanted and incubated.

(1) Stored at temperature of $+2^{\circ}\text{C}$. (in ice chest) for 48 hours and then "cultured." In practically all these cases growth, rather more vigorous than that in the controls which were immediately explanted, took place.

Typical example.—March 2nd, 1916. Rabbit embryo (14 days) placed in Ringer and stored at $+2^{\circ}\text{C}$. March 4th: Portion of heart explanted into medium of rabbit plasma (No. 448). March 6th: Vigorous pulsation and extensive growth—transplanted. March 7th: Pulsation continues, fresh growth actively occurring.

(2) Stored at temperature of $+2^{\circ}\text{C}$. for 7 days and then cultured. May 31st: Rabbit embryo (10 days) placed in Ringer and stored at $+2^{\circ}\text{C}$. June 6th: Skin explanted into medium of hypotonic rabbit plasma (No. 974). June 8th: Extensive growth of fibroblastic and epithelial cells.

(3) Stored at temperature of -10°C . for 30 minutes and then cultured (May 13th) in hypotonic plasma (859). May 17th: No growth observed.

From this series of experiments the conclusion may be drawn that tissues may be stored at a temperature of $+2^{\circ}\text{C}$. for periods up to at

least 7 days without losing any of their vitality, either functional or vegetative, and, indeed, in some cases appear to have increased in vitality over the controls which were explanted immediately. At the same time, temperatures as low as -10°C . kill the tissues within 30 minutes.

(b) Actively growing tissue in explants exposed to varying degree of cold for certain periods of time and then incubated again.

(1) Stored at temperature of $+2^{\circ}\text{C}$.

March 9th: Small portion of fresh human thyroid (removed at operation) explanted into human plasma. March 10th: Someliquefaction of medium had occurred; placed in ice chest for 24 hours and again incubated. March 13th: Fairly extensive growth of epithelial cells.

(Liquefaction is common in human growths, and there is usually over 24 hours latent period before growth starts.)

(2) Stored at temperature of $+2^{\circ}\text{C}$. for 16 days.

April 24th: Rabbit embryo heart stored at $+2^{\circ}\text{C}$. in Ringer for 24 hours, then explanted in hypotonic plasma. April 26th: Active growth and pulsation. Placed in ice chest for 16 days; incubated again; within 2 hours vigorous pulsation was occurring and later active new growth was noted which continued for several transplants.

(3) Stored at temperature of -5°C . for 30 minutes.

April 24th: Rabbit embryo heart stored at $+2^{\circ}\text{C}$. in Ringer for 24 hours, then explanted into hypotonic rabbit plasma. April 26th: Vigorous pulsation and extensive growth. Kept on ice and salt at -5°C . for 30 minutes. Incubated again. As soon as temperature reached $+30^{\circ}\text{C}$. pulsation started and growth went on in further transplants for several generations.

(4) Stored at temperature of -7°C . for 30 minutes.

May 5th: Rabbit embryo heart stored at $+2^{\circ}\text{C}$. in Ringer for 24 hours, then explanted into hypotonic plasma. May 7th: Vigorous pulsation and active growth. Placed on ice and salt at -7°C . for 30 minutes the plasma froze into an opaque solid material at this temperature. On incubating the fibrin net-work was completely broken up and the medium liquefied; the new grown cells were expanded into circular form, much vacuolated, and rapidly broke up altogether. Pulsation stopped, and although the tissue was transplanted several times no further growth or pulsation occurred.

This experiment repeated with temperatures down to -18°C . gave the same result.

It is necessary here to interpose some further observations upon this last series of experiments. We have already drawn attention to the fact that these growths, if not transplanted, eventually die of toxæmia from accumulation of metabolic products. The onset of this condition can be readily observed. In the first place, the growth becomes much slower and pulsation less active; in the second place, the newly grown cells become circular and much vacuolated. In these particular experiments the controls began to exhibit this condition in about five days and transplantation became necessary. It will be noted that in the chilled specimens growth had already been occurring for two days before they were placed in the ice chest, and upon re-incubating special care was taken to look for the onset of vacuolation in the cells. Contrary to expectation, this did not occur on the third day, but on the fifth or sixth day. The explanation is probably that the metabolic products had diffused during the condition of suspended animation from the immediate proximity of the growth and so had become diluted. The further significance of this observation will be noted later.

Chart showing the approximate periods of survival of tissues preserved at temperatures between -18°C . and $+37^{\circ}\text{C}$.

Chart showing approximate periods of survival of embryonic chick tissues at temperatures between -20°C . and $+33^{\circ}\text{C}$. (R. Lambert).

In this first series of experiments upon isolated tissues results were obtained which may be represented graphically. (Fig. 1.) The curve has been charted from the general

Results obtained upon rabbit tissues and does not pretend to any very great accuracy. Its general form is, however, correct, and it agrees so closely to the curve given by R. Lambert⁶ for chick and rat tissues that it may be taken as approximating to accuracy. (Fig. 2.) The main characteristics of both these curves are the sudden drop in the region of -6°C . and the dip in the curve at $+14^{\circ}$ — 16°C . The deductions to be drawn from these curves will appear in the last section.

(2) Effects of Cold upon the Blood.

In Da Costa's "Surgery," in the section on Frost-bite,⁷ appears the statement that definite changes occur in the blood in the nature of cellular degenerations, &c.

It remains to be seen whether the changes occurring in isolated blood *in vitro* corresponded to those occurring in the other tissues of the body. If the cellular contents of the blood became injured by the cold we should expect two results: (1) The breaking up of the white cells would liberate tissue ferment and so bring about coagulation; (2) the red blood cells would hæmolyse.

For the most part human blood was used for these experiments, as it was found to be difficult to keep rabbit's blood longer than 12 hours without coagulation occurring. As far as rabbit's blood was investigated, however, it agreed entirely with the results given below for human blood. It was aseptically drawn off from the superficial veins of the arm through a very large ($\frac{1}{2}$ in. bore) needle evenly coated with paraffin. From this it flowed into paraffined test-tubes contained in a special ice chest, the temperature of which averaged $+2^{\circ}\text{C}$. These tubes were sealed with paraffined corks.

(1) Blood collected quickly in this manner and stored at $+2^{\circ}\text{C}$. can be kept between three and four days without coagulating. If at the end of three days the plasma and corpuscles be mixed by shaking, and the whole centrifuged, the supernatant plasma is practically unchanged in colour, a few specimens showing the slightest darkening of the straw tint which probably indicated hæmolysis. Even at the end of four days coagulation at this temperature is slow and imperfect, for if the exuded fluid, which is apparently serum, be warmed to $+30^{\circ}\text{C}$. and a little tissue extract added coagulation again occurs and a fairly firm clot is formed.

(2) Three specimens of the same blood were collected in three well paraffined tubes—A, B, and C.

A. Immersed in melting ice at temperature 0°C . for 30 minutes, then kept at $+10^{\circ}\text{C}$. for 5 minutes. At the end of this time the blood was quite fluid and slight separation of plasma had occurred. Centrifuged $3\frac{1}{2}$ minutes. Excellent separation of perfectly clear plasma. Plasma and corpuscles were again mixed and centrifuged. Again there was excellent separation of clear plasma.

B. Immersed in ice and salt at temperature -5°C . for 30 minutes, then kept at $+10^{\circ}\text{C}$. for 5 minutes. The blood remained uncoagulated and was centrifuged $3\frac{1}{2}$ minutes. Excellent separation of clear straw plasma. Plasma and corpuscles mixed and centrifuged. The supernatant plasma still remained absolutely clear.

C. Immersed in ice and salt temperature -9°C . for 30 minutes. The blood became thick and gelatinous. Upon warming to $+10^{\circ}\text{C}$. for 5 minutes the blood coagulated, and on centrifuging no separation occurred. The clot was well broken up and the whole again centrifuged. The supernatant serum was stained very dark red, so that it was difficult to distinguish any line of separation.

(3) Three specimens of the same blood in three tubes, D, E, F, were kept at $+2^{\circ}\text{C}$. for $1\frac{1}{2}$ hours. At the end of this time all were uncoagulated and the blood freely mobile.

D. Immersed in melting ice at 0°C . for $1\frac{1}{2}$ hours. Partial separation of plasma occurred. Centrifuged $3\frac{1}{2}$ minutes. Good separation of clear plasma. Mixed and centrifuged again for 2 minutes. Plasma again clear. Warmed to coagulate and kept at room temperature 17°C . for 1 hour. Clot broken up and the whole again centrifuged; supernatant serum absolutely clear.

E. Immersed in ice and salt for $1\frac{1}{2}$ hours. The temperature at start was -5°C . and slowly rose during the immersion to -1°C . Partial separation of plasma by gravity. Centrifuged $3\frac{1}{2}$ minutes. Good separation of absolutely clear plasma. Mixed and centrifuged again, plasma still clear. Warmed to coagulate and kept at room temperature for 1 hour. Clot broken up and the whole again centrifuged; serum absolutely clear.

F. Immersed in ice and salt; temperature -12°C ., rising to -7°C . during course of experiments ($1\frac{1}{2}$ hours). Whole solid; no separation of plasma by gravity. Centrifuged $3\frac{1}{2}$ minutes; no separation, the whole having clotted. Clot broken up; centrifuged again. Serum dark red; difficult to see line of separation. Warmed and kept at room temperature for 1 hour. Mixed and again centrifuged. Serum so red as to be almost opaque to transmitted light.

The conclusions to be drawn from this second group of experiments may be represented by the curves plotted in Figs. 3 and 4. The first point of importance to be noted in regarding these curves is the close similarity between Fig. 3 and Fig. 1, and from it the conclusion may be drawn that the effect of cold upon the actual blood cells, both

white and red, is precisely the same as its effect upon isolated tissue cells. The explanation of the curve in Fig. 4 is not quite so obvious. It must be remembered, however, that, among other things, the presence of tissue extracts containing thrombokinas is essential for the coagulation of the blood. In the case of blood kept at the body temperature the leucocytes, and perhaps the platelets, are in a state of normal activity, and during their metabolism throw off products containing the necessary thrombokinas. As we have already shown, the tissue and blood cells at tempera-

Chart showing the degree of cellular destruction in the blood at varying temperatures. The dotted portion of the chart is not the result of actual experiment, but is the suggested form derived by analogy from Fig. 1.

Chart showing roughly the coagulation time in relationship to varying degrees of temperature.

tures in the region of zero are in a state of suspended animation. All metabolic processes are, therefore, inhibited, hence coagulation only occurs after prolonged periods and then very imperfectly. On the other hand, as soon as temperatures below -6°C . are reached, there is actual physical destruction of the cells liberating the necessary thrombokinas. The blood at this temperature is already frozen (solid), but as soon as the temperature rises clotting must occur. This we consider is a logical explanation of the phenomena recorded in the above paragraphs, and it agrees entirely with the deductions drawn from the first paragraph. As a result we must regard temperatures in the region of -6°C . (the exact temperature varies a little with different tissues) as being critical in nature as far as the function and activity of both tissues and blood cells go.

(3) Investigation of the Effects of Cold upon the Tissues in Normal Relationship to the Circulatory and Nervous Systems.

In this branch a considerable amount of work has already been published. In perusing the literature the first thing that strikes one is the difficulty that all experimenters have experienced in producing any signs of frost-bite unless temperatures below the freezing-point of the tissues (that is, -6°C .) were used. In the recent experiments already quoted by Lorrain Smith, Ritchie, and Dawson, after exposure of rabbit's feet to temperatures just below zero for three days on end, only the slightest reactionary swelling was produced, and, in order to produce anything like marked swelling, it was usually necessary for these observers to apply constricting bands to the legs.

In our own case the experiments were carried out upon rabbits and human skin. For the latter series I have to express my deep thanks to the numerous students at Charing Cross Hospital who so readily offered themselves for these investigations.

Experiments upon Rabbits.

A.—Rabbit anaesthetised, left ear immersed in melting ice for 10 minutes and allowed to warm at room temperature. Ear slightly blue, no swelling. 24 hours, both ears normal. 48 hours, both ears normal.

B.—Rabbit anaesthetised, left ear immersed in ice and salt, temperature -3°C . for 10 minutes and immediately transferred to water at $+40^{\circ}\text{C}$. for 15 minutes. Ear slightly more congested than control, no swelling.

C.—Rabbit anaesthetised, left ear immersed in ice and salt, temperature -5°C . for 3 minutes, then transferred to water at $+45^{\circ}\text{C}$. for 2 minutes. This process was repeated four times in succession. 1 hour, slight increased vascularity of ear, no swelling. 24 hours, ears perfectly normal.

D.—Rabbit anaesthetised, ear immersed in ice and salt, temperature -10°C . for five minutes and then exposed to room temperature. 1 hour, slight swelling present. 12 hours, very extensive swelling, some blistering.

E.—Rabbit anaesthetised, ear immersed in ice and salt, temperature -4°C . for 20 minutes, then transferred to water at $+39^{\circ}\text{C}$.

Special attention was paid to the state of the vessels. While chilled, certain medium-sized vessels, apparently from their bright-red colour arteries, stood out very well, and the whole appearance of the ear by transmitted light was a healthy pink. Upon warming, these vessels rapidly diminished in size and practically disappeared, only to be replaced by a very large series of vessels, dark blue in colour, which were obviously veins. By transmitted light the ear became very blue and congested. 2 hours, slight congestion remained, no swelling. 12 hours, ear absolutely normal.

This series of experiments was repeated upon rabbit's feet and gave precisely similar results.

Experiments upon Human Skin.

F.—N. C. L., May 15th. Two areas on skin of forearm were used. One, a small area $\frac{1}{2}$ in. in diameter, was exposed to CO_2 snow for 35 seconds. The second larger area was exposed to ice and salt, temperature -5°C . to -3°C ., in a larger container for 20 minutes. In both cases the arm was transferred immediately to water at $+40^{\circ}\text{C}$. Within 10 minutes the first area was blistered, showing that the exudation had been enormous. The second area was rather red, but otherwise normal. 12 hours, blister has increased in size. The second area now normal. Under cover of the blister a slough formed, which separated after 14 days, leaving behind a well-marked depressed scar.

G.—W. J. C., June 5th. Two areas on forearm. The freezing mixture was applied in a tall glass cylinder, and had to be considerably lower than the actual temperature required, owing to the enormous amount of heat conveyed to the area from the underlying tissues. First area: Temperature of mixture -12°C . to -10°C . Time 12 minutes. Then immersed in water at $+40^{\circ}\text{C}$. The skin did not solidify. Second area: temperature of mixture -18°C . to -16°C . Time 12 minutes. Then immersed in water at $+40^{\circ}\text{C}$. The skin solidified.

In the first area, other than slight temporary hyperaemia, no effects were produced. In the second area there was immediate swelling of the skin and subcutaneous tissues, the effects of which lasted for several days, and, although the actual destruction was not enough to cause sloughing, yet the overlying skin underwent atrophy, and has left behind a slightly depressed smooth area.

This experiment was repeated several times upon different students and always produced the same result—namely, that unless the skin solidified (that is, reached -6°C .), no effect other than transient hyperaemia was produced; but the slightest degree of solidification produced swelling.

As a result, then, of this third series of experiments we draw the conclusion that -6°C . is not only critical in regard to isolated tissues, but also with regard to tissues in normal relationship to the body. At the same time, the transient hyperaemia which invariably occurs in temperatures above -6°C . must not be overlooked, for, as will be seen later, it is of the utmost importance in the elucidation of the effects of "chilling" as opposed to true frost-bite.

(4) Investigation into the Effects of Cold upon the Tissues in Abnormal Relationship to the Circulatory and Nervous Systems.

In the last three groups of experiments we have indicated that below -6°C . there is actual destruction of tissue, and so, in tissues in normal relationship with the body, swelling and other signs of frost-bite were easily produced when this temperature was exceeded. On the other hand, lesser degrees of cold have very little local effect upon the tissues, and correspondingly signs are very difficult to produce experimentally. Smith, Ritchie, and Dawson⁸ have produced swelling of the feet by standing rabbits for several days in cold mud and immersing them in hot water. In other cases, however, the animal was placed on dry mud at temperatures of $+1^{\circ}\text{C}$. to -1°C . for over a week with only the slightest signs of swelling, especially when the warming was slow. In yet other cases the temperature was kept at 0°C . to -5°C . for as long as five days, and even although a constricting band was used there was no swelling when the legs were slowly warmed. The greater number of their experiments were conducted with constricting bands, which brings in an additional factor. On the whole, the impression derived from their article agrees with the writer's experience—namely, that it is difficult to produce any pathological change at temperatures above -6°C . This being so, it will be difficult to estimate the effect of placing the part in abnormal relationship to blood and nerves. In the following series of experiments special attention was therefore paid to the condition of the vessels in those cases where temperatures above -6°C . were used, while in those below this temperature

the swelling and blistering of the parts formed obvious indications. As in the last group, the experiments were conducted upon rabbits' ears and legs and upon human skin.

Experiments upon Rabbits.

A.—All the nerves going to the left ear were "blocked" at the base with $\frac{1}{2}$ per cent. cocaine. Around the base of the opposite ear (control) a corresponding amount of Ringer's solution was injected. Under general anaesthesia both ears were exposed to freezing solution at -20°C . for 10 minutes and then immersed in water at $+40^{\circ}\text{C}$. for a further 10 minutes. The vascular changes during this experiment were exactly the same as those given in the corresponding experiment in the last group. No swelling or other signs of frost-bite occurred, the general congestion of both ears having passed off in 12 hours.

B.—The internal and external popliteal nerves (left) were blocked with 1 per cent. cocaine in the popliteal space. Control injection of Ringer into the right popliteal space. Both immersed in freezing solution temperature -6°C . for 10 minutes. The legs did not solidify, transferred to water at $+40^{\circ}\text{C}$. Slight transient congestion of both feet, no other swelling or other signs of frost-bite occurred.

C.—Injections similar to those in B. Immersed in freezing solution, temperature -16°C . for 10 minutes and then in water at $+40^{\circ}\text{C}$. 2 hours later right foot slightly swollen, left not swollen. 12 hours later there was extensive swelling of the right foot and slight swelling of the left.

D.—Three minims of novocaine and glucose solution injected, probably extracutaneously, into the spinal column between the second and third sacral vertebrae. Right leg immersed in ice and salt at -15°C . for 12 minutes, and then in water at $+40^{\circ}\text{C}$. Upon recovery from the general anaesthetic the animal was paralysed in both hind legs. This passed off in the course of two to three hours. 12 hours later there was extensive swelling of the right foot, which lasted several days.

Note.—Spinal injections are difficult in rabbits owing to anatomical arrangements of the dura and cord. In two other cases this experiment was attempted, but in each case a permanent paralysis of one leg occurred. The animals were killed, and at post-mortem examination a small area of oedematous tissue was found surrounding the cord in the region of the injection. These experiments are of interest, however, in view of the fact that in one case, despite the exposure of the feet to freezing mixture at -12°C . for 10 minutes, followed by water at $+40^{\circ}\text{C}$., no signs of frost-bite occurred. In the third case slight swelling of one foot occurred after 12 hours.

E.—The perivascular tissues around the femoral vessels on the left side were infiltrated at two points with 1 per cent. cocaine. Both legs immersed in freezing mixture at -15°C . for 10 minutes, and then in water at $+40^{\circ}\text{C}$. One hour later there was slight swelling of the right foot. In 12 hours both legs were extensively swollen, the right rather more than the left.

F.—Dorsum of left foot infiltrated with 1 per cent. cocaine $\frac{1}{2}$ c.c. Dorsum of right foot infiltrated with adrenalin 1 in 10,000 $\frac{1}{2}$ c.c. Both immersed in freezing mixture at -15°C . for 8 minutes, and then in water at $+40^{\circ}\text{C}$. 1 hour, left foot slightly swollen and hot; right foot no swelling, cold. 12 hours, left foot extensive swelling; right foot no swelling. 36 hours, left foot less swollen; right foot now swollen. 72 hours, left foot normal again; right foot extensive swelling.

G.—Both feet immersed in freezing mixture at -3°C . for 20 minutes, and then in water at $+40^{\circ}\text{C}$. The left leg had an elastic constricting band above the ankle. While chilled the right was bright pink in colour, the left cyanotic. Upon warming the right foot became blue, and the left very congested and cyanotic. 2 hours, very slight swelling of the left foot; right apparently normal. 24 hours, both feet normal.

Human Experiments.

H.—N. C. L., May 15th. Three areas on the forearm were exposed to CO_2 snow for 30 seconds. The highest was used as a control; the centre was completely surrounded by a ring of novocaine and adrenalin, with deeper injections into the fascia to complete the "blocking." The lower area was rendered anæmic by a piece of plate glass strapped tightly on to the arm. In all cases the arm was immersed in water at $+40^{\circ}\text{C}$. at the end of the experiment. The control area blistered within 10 minutes and eventually left behind a depressed scar. The middle area showed slight signs of congestion. After two or three days there was slight scaling of the cornified layer. There was absolutely no swelling, no blistering, and no scar remained. The lowest area blistered even more quickly than the control, and a slough having separated has left behind a depressed scar.

The experiment when repeated did not give absolutely constant results. This depends in all probability upon the time of exposure and the type of skin. For instance, in two cases (W. E. P. and N. S.) the upper and lower areas blistered quickly, as in Experiment H. The centre area raised a small and imperfect blister after two to three days' delay.

I.—M. A. O., June 10th. Two areas, the first injected with 1 per cent. cocaine, the second with Ringer's solution. Exposed to ice and salt at -16°C . for 12 minutes. Within 10 minutes the second area showed marked swelling; the first, although reddened, was not swollen.

J.—W. J. C. June 10th. Two areas, one infiltrated with adrenalin, in 10,000, the second with 1 per cent. cocaine in Ringer. CO_2 snow then applied to each for 15 seconds. After 15 minutes there was swelling of the second area, the first, although reddened, was not swollen. 12 hours, second area covered by a large blister; first area, except for discolouration, was normal. 30 hours, the first area had started to blister. 1 week, both areas showed sloughing of equal extent.

The conclusions drawn from this group of experiments are that in true frost-bite, produced by temperatures below -6°C ., there is actual tissue destruction, due directly to the cold, the effects of which it is, therefore, impossible to prevent entirely by modifying either the blood or nerve supply to the part. In the case of chilling alone, produced by temperatures above -6°C ., the normal congestion of the parts is not prevented by nerve blocking, although it may be less severe; it is, however, prevented by the local injection of adrenalin, and to some extent also by local injection of cocaine. The results obtained in the case of human skin agree very closely with those obtained in the limbs of rabbits.

(5) *Conclusions Drawn from these Experiments with Regard to the Pathology and Treatment of the Effects of Cold.*

In the short conclusions given at the end of each group of experiments we have drawn attention to the fact that cold below -6°C . has a very different effect from cold above that temperature, and have suggested that this temperature must be considered as being decidedly critical in nature. Constant results were obtained in all varieties of experiments and upon all tissues. Many writers would appear to suggest that the effects of cold are slowly progressive and vary concurrently with the extent of drop in the temperature. The curves given above show that this theory cannot be held. The sudden drop at -6°C . implies that at this temperature some additional factor occurs, probably in the nature of a physical change which destroys absolutely the protoplasmic content of the cell. The changes occurring at temperatures above this point are changes of degree and of a chemical nature; once the freezing-point of the protoplasm is reached, however, fundamental changes occur in the cells of a gross physical character. The gradual depression in the curve which occurs in the region of $+15^{\circ}\text{C}$. fits in entirely with the above suggestion of chemical changes, and is probably explained in the following manner.

The essential vital process of any cell is summed up in the word metabolism, and this consists of a delicate balance between anabolism and catabolism. In the living cells the protoplasmic molecule by means of side chains attaches to itself certain anabolic products of food assimilation. This chemical (query physico-chemical) union will require the absorption of a certain amount of energy. At the same time the protoplasm is being continually broken up (by the total function of the cell) and metabolic products thrown off. During this process a certain amount of energy will, of course, be liberated in various forms (heat, motion, &c.). Of these two processes anabolism being of the nature of a synthesis, and requiring external energy in the form of heat, is the more easily affected by external agencies and may be considered to be the more delicate of the two. During the process of chilling of the tissues, therefore, although both will be affected, we shall expect *a priori* that anabolism will stop before catabolism. At temperatures in the region of $+15^{\circ}\text{C}$. anabolism may be assumed to have stopped entirely, while catabolism continues slowly. When temperatures in the region of zero are reached catabolism also stops, and so it follows that tissues at the temperature of 0°C . can be preserved for longer periods than those at $+15^{\circ}\text{C}$. The question of the effect of autolytic enzymes has been purposely ignored, for their function is so bound up with the metabolism of the cell protoplasm as to be included in the above suggestions. The analogy to some of the high explosive compounds is fairly accurate. These compounds are notoriously unstable; if raised to the explosive temperature rapid combination with oxygen occurs and the explosion results; this corresponds to normal cell metabolism. If stored at rather lower temperatures they fairly rapidly degenerate into simpler and more stable compounds. When stored at low temperatures, however, they retain their potential explosive properties over considerable periods, being meanwhile quite stable. At the risk of repetition, we again state that the effects of cold below and above -6°C . respectively can in no way be compared—the one is chemical, the other physical, and the difference between them is as great as that between a mixture of hydrogen and oxygen gases in a tube and the water which results from their chemical combinations. From this point onwards, therefore, we shall divide these considerations into the effects of true "frost-bite" and the effects of chilling of the tissues.

Effects of True Frost-bite.

Frost-bite produces actual destruction of tissue and, as a result, the pathological effects are numerous. In the minor cases the destruction of the endothelial walls of capillaries allows of a rapid exudation of the fluids and also in some cases of the cells of the blood into the surrounding tissues. In some cases the endothelial and other cells, by their destruction, will set free tissue extracts and so intravascular clotting will occur. This process may extend until the larger vessels are affected and lead to complete cutting off of the blood-supply followed by gangrene. In other cases the actual pressure of the exuded fluids will bring about the same result. In the minor cases the destroyed tissues will be

absorbed slowly by a process of aseptic inflammation and leave as a result a certain amount of fibrosis and scarring. In the worst cases the whole thickness of the part is affected (this takes a considerable exposure, as the deeper tissues freeze slowly) and necrosis or sloughing occurs. In addition to these results, in many cases there will be microbic infection with its concurrent effects. Clinically the results of frost-bite are rarely seen in our temperate climate, but in other countries they are not at all uncommon. In Canada, for instance, a large number of cases occur every year and are often of the utmost severity. It is interesting to note in this connexion that in the *Journal of the Royal Naval Medical Service*⁹ a case of true frost-bite is recorded as occurring during an aeroplane flight at a height of 15,000 feet, the calculated temperature at this height being 34° below zero.

It now remains to be seen whether we can prevent or in any way diminish these effects. Bearing in mind the pathology of the condition as given above, we should *a priori* expect that very little could be hoped for in the way of treatment. The experiments detailed in Group 4 absolutely confirm this prognostication. Whether the nerves to the part were blocked with cocaine or the blood-supply was temporarily cut off, the results were invariably the same. If the vaso-motor nerves accompanying the blood-vessels were blocked by perivascular infiltration with cocaine the results were no better. There is, however, one exceedingly important observation which must not be overlooked—namely, that the local injection of cocaine or, better, of adrenalin in one or two cases entirely prevented the exudation, and in all cases caused considerable delay and probably lessened the amount. This delay in some of the cases amounted to three or four days. The explanation of this phenomenon rests with the vaso-motor system. With regard to the effects of temperature changes upon the calibre of the vessels, too much emphasis has been laid upon the nervous reflex. The above experiments would tend to show that the central mechanism in this case is quite subsidiary to the local one. It is a well-known fact that involuntary muscle fibres react to temperature changes by contracting during falls and relaxing when the temperature is raised. The involuntary muscle of the arterial walls may be expected to form no exception. It must be remembered that the circulatory system may be divided up into four main types of vessels—namely, the larger arteries, containing very little musculature; the arterioles, which are exceedingly muscular, and which, in the normal condition of arterial tone, act as a dam protecting the capillaries, through which alone exudation occurs, from the arterial blood pressure; and finally the veins, with little or no musculature, but capable of great distension. The first effect of cold will be to contract the musculature of the arterioles. This, as proved by some of the experiments in Group 3, occurs in the presence of nerve blocking, and so must be due to the direct effect of cold upon the muscle. The contraction of the arterioles occurs to such an extent as completely to obliterate the vessels. The capillaries and veins will therefore be comparatively empty, while the larger arteries will be distended at full pressure, hence the red appearance of the parts by transmitted light. When the part becomes frost-bitten, the muscles of the arterioles are destroyed. As a result upon thawing the damming effect of the arterioles upon the blood pressure is lost and the capillaries become subject to sufficiently high pressure to cause exudation. If the endothelial wall is also destroyed by the cold, then the exudation will be all the more rapid and extensive. Both cocaine and adrenalin cause a local contraction of the vessels by direct effect upon their walls and so the vessels just beyond the frost-bitten area will remain contracted, even when thawing occurs. This will prevent the blood pressure in capillaries from rising and so no exudation occurs. As the effects of the local injection begin to wear off, however, the capillary pressure will rise, and, should the wall have been seriously damaged by the frost-bite, delayed exudation will now occur. If, on the other hand, the capillary wall is now normal, no exudation will occur. This appears to afford a simple and logical explanation of the phenomena of late exudation and of those cases where no exudation occurs, the injury in these latter cases being submaximal and the capillary walls rapidly restored to the normal condition. In the severe cases, therefore, nothing can be done. In the milder cases, however, it has been

pointed out that, in addition to the actual direct destruction, there are the effects produced by the exudation itself. Indeed, a vicious circle is set up, for the greater the exudation the greater the venous obstruction, and the greater the venous obstruction the greater the rise in the capillary pressure, and hence the greater the exudation. Although, therefore, it will be impossible to prevent or remedy the destructive effects of the cold, it would seem reasonable to attempt to limit the exudation and so to prevent additional damage. This can be accomplished by preventing any considerable rise in capillary pressure, both by damming back the arterial blood and by ensuring quick venous return. The former method has been carried out in this work by the local injection of vaso-constrictors, and although this method is impracticable in the majority of human cases, treatment by the use of intravenous injections of vaso-constrictors is worthy of trial. The disadvantage of all the vaso-constrictors is that it is difficult, and in some cases impossible, to localise their action to the desired area, and, again, in some cases the effect is very transitory and followed by a reactionary dilation which renders the condition worse. By the judicious use of injections of strychnine and ergot it should be possible to keep up a fair degree of vaso-constriction over the first two or three days, and particularly is this necessary during thawing. In discussing this suggested treatment with colleagues, it has frequently been objected that the use of vaso-constrictors in any condition approximating to gangrene is surely not indicated. This objection undoubtedly holds in the case of severe frost-bite, causing necrosis of the part, for all that can be hoped for in such cases is the formation of a line of demarcation and the separation of the dead tissue. In the minor cases of true frost-bite, however, we believe that many of the evil effects are due to the excessive exudation, and that therefore the use of vaso-constrictors to limit the exudation is indicated. We have, unfortunately, not been in a position to give this treatment a trial.

The Effects of Degrees of Cold Above -6° C. upon the Tissues.

We now pass to a consideration of the effects of cold above the critical temperature upon the tissues; this will include one of the essential factors in the production of "trench foot."

S. Delépine¹¹ has suggested the use of the term "frigorism" to denote this condition. This English equivalent of the French "froidure," although not very euphemic, is certainly an improvement upon "water-bite."

In the condition of suspended animation brought about by such temperatures it should theoretically be possible to preserve tissues indefinitely; practically this is not the case. It is certain, however, that tissues may maintain their vitality for periods of from six to eight weeks in this condition. At the end of the first series of experiments we drew attention to the fact that in growths which had been stored for 16 days at +2° C. the onset of the signs of diminished vitality did not occur any sooner than in the controls which were kept at +37.5° C. In both cases the periods averaged five to six days of incubation. It would appear, therefore, that during the 16 days at +2° C. no degenerative change in the tissues worthy of consideration could have occurred. As we should expect also no oxygen is used up in this condition, for the slides are sealed and the supply of oxygen strictly limited and equal in the chilled specimens and in the controls. The bright pink colour of the chilled parts in the third and fourth groups of experiments also indicates that the absorption of oxygen at these temperatures is reduced to a minimum. Theories which assume that the changes are due to ischæmia cannot be accepted, for the chilled tissues need neither nutriment nor oxygen. Clinically, it is found that the actual time of exposure rarely exceeds three or four days, and in this time, in view of the above experiments we can scarcely assume any change in the actual cells of the tissue (from cold alone) likely to bring about the extensive swelling and sometimes gangrene which occur. The essential process would appear to be exudation; the other changes—gangrene, paræsthesiæ, &c.—can be easily explained as being secondary to the exudation and dependent upon it.

The paræsthesiæ appear to have attracted the attention of many authors. Earnsides¹² divides the cases up into those showing objective changes and those with subjective changes

only (paræsthesiæ) and explains the latter as a neurosis or psychical anæsthesia. Most observers (Fearnside¹², Watson and Myers,¹³ Swan¹¹) agree that in the cases with most swelling the hyperæsthesia was least marked. The clinical experiences of the writer agree with this. It is possible to account for all these phenomena by assuming an interneural or even intraneural exudation into the larger nerves. Obviously a slight degree of intraneural exudation will produce more subjective symptoms than a large degree of interneural exudation. The exudation rarely occurs either experimentally or clinically before the part is warmed. Failing a satisfactory explanation from any direct effect of cold, let us examine the vaso-motor conditions during the warming process. At the start the arterioles are contracted practically to obliteration, and pressure in capillaries and veins is at a minimum. As the temperature rises the involuntary muscle fibres, having been in a state of tonic contraction for a considerable period, become relaxed and for the time paralysed. An enormous dilatation of the arterioles, therefore, occurs and the full arterial pressure is transmitted to the capillaries. Whether the endothelial walls of these be damaged or no, the rise of pressure is so great as to cause the fluid contents of the blood to filter through the walls in exactly the same manner as serum passes through a porcelain filter when the pressure is sufficiently raised. This exudation will contain fibrinogen, and hence the deposit of fibrin in the tissues. The exudation will now press upon the veins and so the vicious circle starts. In the same way any artificial obstruction to the venous return will increase the capillary pressure, and therefore the exudation. This probably explains the very marked increase in swelling which the presence of a constricting band produces, either experimentally or clinically, and indicates an important line of prophylaxis. This great vaso-dilation is, we believe, more local than reflex in origin, since experimentally it occurs when all the nerves going to the part are blocked with cocaine.

We should expect this condition to be much more difficult to produce experimentally in animals than in man, for in animals the musculature of the arterioles is normally subject to greater changes than in man, and so is less likely to be rendered paralytic by temperature variations. The difference, then, between the pathology of true frost-bite and of chilling lies essentially in the nature of the local damage. In the first instance the cells are killed by cold, and therefore only in mild cases can any treatment be of much avail. In the second instance the damage is secondary to the excessive exudation, and this to the great rise in the capillary pressure. If, therefore, the capillary pressure can be kept low while the arterioles regain their tone, then the conditions will be amenable to treatment.

Although, therefore, the pathology of the two conditions is different, the treatment of both rests in preventing too great rise of capillary pressure. The suggestions given, therefore, for the treatment of frost-bite will apply equally well to the treatment of chilling. Better than treatment, however, is prophylaxis. Any condition likely to bring about venous congestion (constricting bands, puttees, tight boots, standing too long in one position, &c.) must be avoided. Variations in the temperature will bring about more rapid paralysis of the arterioles than constant cold, and so should, as far as possible, be avoided. This is difficult to accomplish in a limb the blood supply of which is considerably diminished. A thin layer of dry air surrounding the limb will help immediately.

S. Delépine¹¹ has suggested the use of imperishable oiled silk bags to be worn outside the socks as a practical means of attaining this end. Indeed, we believe that the bad effects of water are chiefly brought about by its acting as a good conductor of the varying degrees of cold.

It should be noted here that McNeil¹⁰ calls the condition "water-bite" and considers that it is due entirely to osmosis, the tissue being distended with dilute plasma. It is difficult to believe that any degree of osmotic action could occur through the thick protective skin of the feet; and, indeed, the experiments of Rideal and Waller³ already quoted show that, experimentally, swelling, except of the superficial layers of the skin itself, is not produced even when the exposure is prolonged. "Water-bite" would therefore appear to be an unsatisfactory term, as it fails to indicate the primary factor.

Once the limb has become definitely "chilled" treatment should be immediately started. It would appear better to keep the limb cold by the application of wet cloths until efficient treatment can be given than to allow partial warming to occur. The actual treatment will consist in using any and every means of preventing too great a rise in the capillary pressure. As to whether the use of vaso-constrictors, as suggested above, will be of any avail remains to be seen. They should obviously be given before the part is warmed, and once they are acting, the parts should be warmed not too slowly. If, on the other hand, vaso-constrictors cannot be given, then the part must be very slowly raised to the normal temperature, for this tends to diminish the degree of vasomotor paralysis. Elevation of the limb and gentle massage, by aiding the return of blood and diminishing the capillary pressure, will be of value in after-treatment. They will also be of great use as a means of prophylaxis.

During rest periods the legs should be elevated and not allowed to dangle over the edge of the dug-out. The legs should meanwhile be well massaged, for during the rest period partial thawing is very apt to occur and to start off the train of events. The more powerful of the vaso-constrictors should be given immediately, if at all, and followed by those having gentler and more prolonged action. These must be continued for the first three or four days.

Treatment on these lines has not yet been applied to actual cases of "trench foot," as the opportunity has not arisen. It may well be impracticable to bring about the desired vaso-constriction. We feel sure, however, that it is along these lines that a satisfactory treatment should be sought.

In conclusion, it should be mentioned that at one period it was hoped that blocking of the large nerve trunks or preferably of the cord itself by spinal injections of novocaine would give good results by stopping the reflex nervous dilatation of the vessels. We have shown, however, that for the rabbit at least the dilatation is mainly the result of the local mechanism, whether it be directly muscular or through a local nerve reflex matters not. In transferring the results to man, however, caution must be used, for in this case the local mechanism may play a smaller part in the production of the condition. If this be so, then we may still hope for some success by spinal injection, combined with the action of vaso-constrictors, and certainly in at least one case (exp. 4 D), in which a spinal injection was given, no swelling occurred, although the leg was rendered solid by immersion at -12°C . for 10 minutes.

Summary.

- (1) The temperature -6°C . must be considered critical in relationship to the effects of cold upon the tissues.
- (2) Degrees of cold below this temperature produce true frost-bite and actual damage to tissues.
- (3) Degrees of cold above this temperature only produce effects secondarily by causing vasomotor paralysis.
- (4) Nerve blocking does not prevent either the effects of frost-bite or of chilling.
- (5) The use of vaso-constrictors delays the swelling in cases of true frost-bite, while in cases of chilling the exudation may be entirely prevented.
- (6) Satisfactory treatment of trench foot must be directed towards preventing any appreciable rise in capillary pressure.
- (7) In the production of trench foot cold must be considered the essential factor, other factors being either secondary or subsidiary.
- (8) Treatment by the use of vaso-constrictors, either intravenously or otherwise, would appear logical and worthy of trial.
- (9) Treatment by nerve-blocking, either regional or central, is also suggested, although, in all probability, it cannot be expected to give good results unless used in conjunction with vaso-constrictors.
- (10) Prophylaxis consists in using any and every means of preventing congestion of the legs, and in avoiding, as far as possible, variations in the temperature.
- (11) The question as to whether any surgical procedure, such as temporary occlusion of the arteries, would bring about the desired reduction in capillary pressure is being investigated.

References.—1. Carrel: Studies Rockefeller Institute, xiv. 2. Jour. Path. and Bact., xx., p. 159. 3. Jour. R.A.M.C., xxvi., No. 5. 4. Studies Rockefeller Institute, xiii. 5. Ibid., xiv. 6. Jour. Exp. Med., xviii., No. 4. 7. Da Costa's Surgery, fifth ed., p. 180. 8. Jour. Path. and Bact., xx., p. 172. 9. Jour. Royal Naval Med. Service, ii., No. 1. 10. Brit. Med. Jour., April 24th, 1915. 11. Ibid., Dec. 13th, 1915; THE LANCET, 1915, i., 271. 12. Brit. Med. Jour., Jan. 9th, 1915. 13. Ibid., March 6th, 1915. 14. Proc. Royal Soc. Med., Feb. 12th, 1915.