THE EFFECT OF SELECTED DRUGS ON THE VASCULAR RESPONSES OF THE RAT TO LOCALIZED COLD

by

George Edward Singer

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ABSTRACT

Of Thesis for Master of Science Degree

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Cold injury of both the dry and the moist types are of the utmost importance in military operations, and are likely to assume even greater importance in the event of polar warfare. The modern literature on frostbite began with Napoleon's retreat from Moscow in 1812 when his surgeon, Baron Larrey recorded the disastrous event in his memoirs. Since then it has been the various wars of the western world that has provided the main stimulus for investigation into the cold problem.

The experimental investigation has effected little positive benefit by way of management of an acute cold injury, but it has served to break down many time honoured doctrines, especially the theory that slow thawing of a frozen limb provided the best treatment. The significance of the various events that occur in the tissues during a freezing reaction are not agreed on by all workers, but the course of the reaction and especially the danger of the secondary effects during thawing are well known.

The present investigation concerned an attempt to observe microscopically the vascular changes in the rat mesoappendix according to a technique of Zweifach. A cold point apparatus described by Hass and Taylor was utilized

for exact freezing of a capillary bed. The influence of ten selected drugs on the reactions of the vascular bed after freezing was tested alternately with control rats. Procaine, priscoline, benadryl, etamon, hydergine, apresoline, chlor-tripolon, rutin, ascorbic acid, and histamine were tested. The criteria for a drug effect included delay in onset of vascular stasis, lessening the rate and extent of the stasis, and resumption of circulation in static vessels. No significant difference was observed between the treated and the control animals.

In an effort to confirm this impression grossly, the hind legs of rats were frozen in a carbon dioxide and ether mixture at -20°C. for twenty seconds. The changes observed grossly following thawing were described numerically and the arbitrary concept of an Injury Index was utilized which could be expressed graphically on a day by day basis. Of the same ten drugs tested, procaine, priscoline, benadryl, etamon, hydergine, and ascorbic acid were found to have no significant effect. Chlor-Tripolon and histamine were found to have an adverse effect, and apresoline, and especially rutin, were found to have a probably significant beneficial effect. The value of rapid thawing in water at 42°C. was consistently confirmed throughout all gross experiments.

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Mr. D.L. Warner, a senior student in the Faculty of Medicine provided much help in two respects. It was he who introduced me to the technique of observing microscopically the vasculature of the rat mesoappendix and he guided me away from many pitfalls during the early stages of the project. He also gave up his Christmas vacation to perform several of the experiments as acknowledged later.

Appreciation is here expressed for the supplies of Hydergine and Apresoline received from the Sandoz Corporation and the Ciba Company.

G.E. Singer,

April 1, 1954.

THE EFFECT OF SELECTED DRUGS ON THE VASCULAR RESPONSES OF THE RAT TO LOCALIZED COLD

THE COLD PROBLEM

Frostbite and cold injuries generally continue to present a challenge to our armed forces battling in the colder regions of the earth. Orr and Fainer (1) have recently reported on the terrific toll in Korea among the United States forces during the winter campaign of 1950-1951 when there were sixty thousand frostbite casualties with six thousand amputations resulting. This definitely puts this problem into the category of a major battle hazard. The possibility, and even the probability of polar warfare in the near future forces greater attention to be paid to the problem of cold injury than has been paid. The matter of acclimatization is being investigated as a preventive measure. Orr and Fainer outline several factors, more or less obvious, that will help the soldier in the prevention of actual freezing injuries. In addition to the various physical agents, we would like to have some medication that could be administered to protect the combatant and civilian alike against both the actual

freezing injury and also against the resultant immediate and chronic sequellae of frostbite once it is established. At the moment no such medication is known, and all drugs suggested fail through being administered following the freezing, either immediately or later, often after the thawing has been completed. Webster and Bigelow (2) writing for the recent special Civil Defence issue of the Canadian Medical Association Journal summarize the conflicting current theories regarding pathogenesis and treatment without actually stressing any special regimen. They do emphasize, as we all must, the value and necessity for continued experimental work both in the laboratory and in controlled clinical studies, and they seem to feel that the knowledge obtained so far is barely laying the ground work for the great advances possible in this field. The Josiah Macy Jr. Foundation has even started a Conference on cold injury (3). The best summary of the experimental work to date lies in the article prepared by Shumacker and Lempke (4) which gives a resumé of the literature from as far back as 1917 right up to articles still in press in 1951. This is a rather extensive review and it forms the basis for the experimental work carried out for this thesis.

There are very few articles more recent that are not clinical, and since most of the experimental work now being performed is financed through military research grants of one kind or another, reports are very guarded as highly secret.

THE COLD PROBLEM ASSESSED

The acute variety of cold injury may be divided into three broad categories. Firstly might be mentioned general hypothermia, which is included only for completeness since it uncommon, and survival from it even more rare (5), although it is being investigated for anaesthetic uses in congenital heart surgery. Secondly is the moist cold lesion, the trench foot or immersion foot syndrome. Trench foot was peculiarly a problem of the first World War, while immersion foot was the second World War variety of the same condition, being the result of a prolonged vertical posture with an ambient temperature above freezing. An apparently necessary requirement is the need for moist cold, which conducts heat away from the exposed limb faster than does dry cold. The effect of the dependency of the limb is of course to impede the blood circulation by delaying venous return. Thirdly is the big problem of frostbite. This is the condition result-

ing from exposure to cold of sufficient severity and duration to produce ice in the affected parts. This too had a counterpart in the second World War in the condition known as high altitude frostbite, characterized by a very rapid freezing at very low temperatures, such as the tail gunner of a fighter plane received when he took off his glove to adjust his gun, with his fingers immediately sticking to the metal and being deeply frozen.

The frequent appearance in this brief classification of such terms as trench foot, and high altitude frostbite suggests that cold injury is for all practical purposes a disease peculiarly associated with military operations. The modern literature on the subject begins with Baron Larey's account (6) of Napoleon's casualties on the tragic retreat from Moscow in 1812. However, it was not until the first World War that much effort or interest was put into trying to solve the frostbite problem. Sir Thomas Lewis was one of the main investigators of freezing injury at the time, and several papers proceded from his laboratory on such ideas as supercooling of tissues and the concept that it was the formation of ice crystals intracellularly which caused the damage of freezing.

Experimental work during the second World War was centered mainly in trying to understand the physiology and the pathology of the frostbite lesion. Many are the papers discussing which was the more important of the various processes more or less going on simultaneously. The whole process from initial exposure to cold right through to the development of the late sequellae is well discussed by Shumacker (4) and there seems to be little value in repeating it all here. The important aspect, not of course originated by Shumacker, is the vascular component. In a freezing injury, naturally, cells and tissues will be killed by the cold or by the anoxia accompanying the ischemia produced by the vasoconstriction. But the extent of the freezing influence does not directly determine the extent of the damage resulting. It is the secondary changes following thawing that are the gravest sequels to frostbite. After thawing (to quote from Shumacker) there develops a reactive hyperemia which may represent the reaction to slightly diffusible abnormal cell products resulting from direct cold injury or ischaemic injury. With the return of blood flow, oedema begins to form as protein-rich fluid escapes through the capillary walls rendered

hyperpermeable by the effects of the cold or anoxia. The oedema progressively increases and may reach the maximal volume allowed by the extensile limit of the encompassing skin. Coincidentally with the formation of oedema, stasis develops in the true capillaries. The formed elements of the blood are seen progressively to fill the small vessels, bringing to a halt the circulation through them. This stasis is reversible, at the beginning at least, since mechanical pressure or changes in blood flow in adjacent vessels will break up the red cell masses. There is some controversy as to the cause of the stasis. The view most widely held is that the capillary walls, rendered hyperpermeable by the injury merely filter the cells out of the blood stream as the serum enters the interstitial spaces. The second suggestion is that stasis must be the result of some colloidal chemical alteration in blood unrelated to the clotting mechanism. The third possibility offered connects stasis with sludging where there is precipitated on the cells a sticky coat which causes them to adhere to one another and to the walls of the vessels. Shumacker gives the arguments for and against these views, which need not be mentioned here.

This summary from Shumacker's discussion picks out the course of events in frostbite that occur at the

capillary level. Since studies were already in progress at this University on the observation of the capillary responses in the rat mesoappendix (Warner, D.L., 7) and since Hass and Taylor had described an instrument (8) for applying a quantitative hypothermal injury, it seemed very logical to study the behavior of the blood vessels in the rat mesoappendix after freezing as influenced by various medications applied both topically and parenterally. Except for the technique of studying capillary responses to a local freezing injury as influenced by various drugs, which I have not found described in the literature, there is nothing very original in the testing of the various drugs selected. Most of them have been used previously for gross freezing experiments. Ten were selected: procaine, Priscoline, Etamon, Hydergine, Apresoline, Rutin, Ascorbic acid, Benadryl, Chlor-Tripolon, and histamine. In view of the fact that some of these preparations are called by their trade names, a further discussion of the drugs is in order.

Drugs Tested in Cold Injury Experiments

Procaine

This is believed to paralyze peripheral con-

strictor fibers upon peripheral contact with them. The drug was tested both topically and parenterally. Topically there is no difficulty with dosage, a 0.5% to 2.0% solution being generally adequate as is demonstrated clinically all the time. There is some trouble however, in estimating the effective parenteral dose. The procaine is detoxified principally in the liver, and this is rapid enough to necessitate frequent or better, continuous injections of a solution of appropriate concentration. Griffith (9) gives 200 mg. intraperitoneally as a suitable dose to an adult rat for therapeutic or topical action. 1600 mg. is the M.L.D. and 2100 mg. is the $L.D._{50}$. 45 to 55 mg. given intravenously will kill off the majority of the animals. On this basis the dosage subsequently used was calculated. - Note that procaine is hydrolyzed and detoxified by an enzyme in the blood to give para-amino benzoic acid and diethylaminoethano which may have in turn an indirect action on the vessel endothelium (may act in competition with histamine).

Priscoline

This is benzazoline hydrochloride, Ciba. It acts as a vasodilator of peripheral arteries and arterioles. It is an adrenolytic agent, blocking the

pressor without disturbing the depressor effect of epinephrine. The action is peripheral and there may also be some direct dilating effect on the vessel walls themselves (Beckman, 10). Dosage level based on approximate adult human dose.

Etamon chloride

Tetraethylammonium chloride, Parke-Davis, is a depressant of both sympathetic and parasympathetic peripheral ganglia, with possibility of some locally exerted vasodilator action. Used at dosage level similar to reports tabulated by Shumacker (4).

Hydergine

Hydergine is not mentioned in Shumacker's review, probably because the first report by Hurley (11) appeared just when the larger review was received for publication. The use of the dihydrogenated ergot alkaloids stemmed from basic pharmacological findings that these derivatives had a sympathicolytic action on the peripheral circulation. Bluntschli and Goetz (12) showed that dihydroergocornine produced no constriction in the sympathectomized limb, and gave vasodilatation in the normally innervated limb, this being the first known ergot derivative acting purely as a sympathicolytic and also acting over higher sympt

sympathetic centres rather than peripherally at the myoneural sympathetic junction, and they showed that the compound acted on the vascular centres in the medulla and or the hypothalamus. Rothlin of Basle a year earlier showed (13) all four of the dihydrogenated derivatives of ergot had this sympathicoadrenolytic activity, and Hurley used a preparation called CCK-179 containing equal parts of dihydroergocornine, dihydroergocristine, and dihydroergokryptine, now put out by Sandoz as Hydergine. Hurley felt vasoconstriction (due to local response, local central nervous system reflex, and hypothalamic vasoconstriction due to cooled blood) could contribute greatly to tissue anoxia following frostbite, and that release of this could be of prime importance in the therapy of frostbite. He found, following exposure of sixty Sprague-Dawley and Wistar rats (distal seven centimetres of the tails to -15°C for sixty seconds) that had received varying dosages of Hydergine, that there was statistically significant improvement of each treated group over the controls. Hurley suggested that these dihydrogenated ergot alkaloids might prove to be of value in the treatment of high altitude frostbite. The dosage of Hydergine used in the present experiments was nearly that used by Hurley.

Apresoline

Hydralazine hydrochloride, Ciba, has an action claimed by the manufacturer to be largely central on the midbrain, serving to lower both the systolic and diastolic blood pressures, especially in hypertension, yet permitting increased blood flow through the kidneys. It has adrenergic blocking effects, adrenolytic and sympatholytic, against the pressor effects of epinephrine and nor-epinephrine. This drug has not yet, to my knowledge, been reported in frostbite experiments. LD_{50} for white rats is 34 mg. per kilogram body weight. The dosage level used was on the range of 2 mg. per animal.

Rutin and Ascorbic acid

It is logical to try agents that reportedly reduce capillary permeability as well as fragility. Rutin is one of the vitamin P substances, and ascorbic acid is of course vitamin C. The dosage selected for rutin was that reported in Shumacker's review. In the case of ascorbic acid, it is regarded as impossible to make rats deficient in this vitamin since they manufacture their own, hence excessively large doses were used, which had the effect to be noted later in the gross experiments.

Benadryl

This antihistaminic agent has been used frequently before in frostbite (4) and the dosage level

supposedly effective in rats is known. 0.43 mg. per kilogram body weight was used by Shumacker.

Chlor-Tripolon

This is an alternate antihistaminic, very powerful, and not previously reported in frostbite lesions experimentally. The dosage level selected was rather empirical at 5 mg. per animal.

Histamine

It was decided to attempt to assess the influence of histamine by virtue of the questionable findings with the antihistaminics. No stated effective dosage level could be found, and two values were selected for trial, 10 mg. and 50 mg. per kilogram body weight.

Need for Confirmatory Gross Experiments

As mentioned, it was decided it would be logcial to carry on with microscopic observation of the blood vessels in the rat mesoappendix. The vessels at this location were selected primarily for convenience, since very beautiful preparations can be obtained with a minimum of effort, and with the equipment to be described, such preparations can be maintained in a healthy condition for up to four hours. However, in cold injuries, the mesoappendix is seldom frozen,

and there is no reason to expect that capillaries that functioned one way in the protected environment of the peritoneal cavity would function in the same way in the hind leg of the same animal. Accordingly, the series of gross experiments were performed as described later in an effort to correlate the findings in the two areas.

Microscopic Observation of the Rat Mesoappendix

The Rat Mesoappendix

There are numerous areas in various species of animals where the fine details of the blood circulation can be observed clearly. Of these, the mesentery of the rat lying between the caecum and the terminal ileum is one of the most accessible and most convenient to study. All that is needed is an animal sufficiently anaesthetized by an agent such as nembutal, and with a median abdominal incision made with an instrument such as a pair of scissors, the caecum may be exteriorized and draped over a supporting loop with the so-called mesoappendix exposed for view with a low power microscope, and illuminated by transillumination.

The Circulation of the Mesoappendix

Without discussing the controversies concerning the anatomy of the capillary circulation that began in 1874 with Rouget, and have continued right up to the present, and will be with us long into the future, the capillary bed structure as described by Chambers and Zweifach (22) was the basis for identification and naming of the various parts of the capillary beds observed in the mesoappendix. They built up a veritable anatomy of the capillary bed, and their scheme is here outlined.

They felt the rat mesoappendix was predominantly a nutritive field, with a central channel and true capillaries as side branches. The small arborizing arteries finally become what Chambers and Zweifach term the first division of the capillary bed, the terminal arterioles. These are channels twenty to twenty-five microns in diameter, with a single continuous smooth muscle layer. Here pulsations are related to those in the larger arteries, but are less regular. Secondly in line of blood flow comes thecapillary bed proper. The metarteriole is from eight to fifteen microns in diameter, it has a typical discontinuous muscle cell coat, and it shows vasomotion with slow constrictor-dilator phases. The blood then flows through the precapillary junctions or sphincters. These are usually twisted and are outflowing. Typical muscle cells act as sphincters. Vasomotion here is independent of that in the arterioles. Finally the blood passes into the true capillaries. Here there is passive dilatation and tonic constriction of the endothelial cells. There are more rapid, by-pass channels known as arterio-venous channels or shunts. The proximal segment of an arterio-venous channel apparently has an atypical type of smooth muscle cell that shows no vaso motion and responds only to abnormal stimuli. These lead

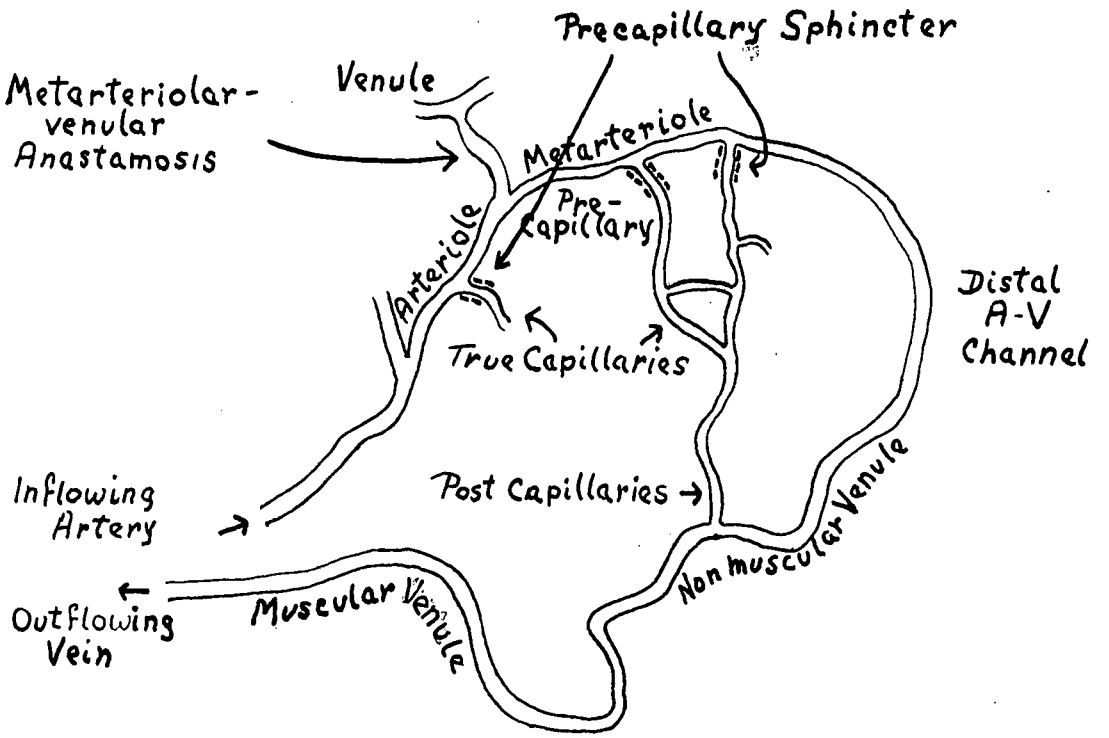
into precapillary junctions, or into true capillary junctions, and hence into the true capillaries. The distal segment of the arterio-venous channel has a connective tissue coating that permits slight passive changes in diameter. The post capillary junctions are inflowing, the same as the capillaries are. Following either the true capillary channels or the arterio-venous channels, the blood enters the nonmuscular venule, which is really nothing more than a fusion of thoroughfare channels. This has a connective tissue covering, and slight passive changes in diameter are permitted. Thirdly, and lastly, in the anatomy of the capillary bed are the muscular venules. These are twenty-five to thirty microns in diameter (the non-muscular venules were only fifteen to twenty-five) and they are covered with both smooth muscle cells and connective tissue. They are highly responsive and show varied contractions.

Vasomotion is a peculiar type of motor activity of the metarteriole and of its precapillaries. These channels show irregularly recurrent series of dilatations and contractions at intervals varying from fifteen seconds to three minutes. During active metarteriolar vasomotion the sphincter-like closure of the precapillaries tends to restrict the capillary blood flow to the central

channels. Therefore, the central arterio-venous channel carries on the basal work of the capillary bed in ischemia. The endothelial wall of the capillary possesses a certain degree of elasticity. The true capillary shows passive changes in diameter as a consequence of the variations of the blood flow through it. When the pressure in the capillaries falls the endothelial cell tends to lose its expanded state, whereupon its nucleus rounds up and creates a bulge into the lumen of the capillary. Note that capillaries of one "bed" also connect with other capillary "beds." According to Chambers (21), the smooth muscle sphincter cells are activated by vasoconstrictor nerves, with there being no evidence for vasodilator nerves.

Also according to Zweifach and Chambers, vasomotion is markedly affected by irritation such as handling, stroking, stabbing, by nerve supply such that vasomotion ceases when the nerve supply is interrupted, and by deep anaesthesia with nembutal or similar agent. It can be increased further by acute haemorrhage, by sympathetic nerve stimulation, and by intravenous injections of such drugs as adrenalin, angiotonin, and adrenal cortical extract. It can be decreased (with some oedema formation) by a temperature rise (37.5 to 41°C), by a temperature fall (10 to 20 °C.), by direct trauma, and by increased

vital activity as muscular exercise or gland secretion. Agents as histamine, adenylic acid, adenosine, kallikrein, and acetyl choline are supposed to increase the capillary circulation without decreasing precapillary vasomotion. In the epinephrine response, the metarteriole and precapillary sphincters constrict. Increasing the concentration gives progressively further constriction along the metarteriole toward the distal central arteriole. In the histamine response there is dilatation of the arterioles, metarterioles, and precapillary sphincters. This dilatation occurs in the capillaries only, if the blood was flowing through them at the time.



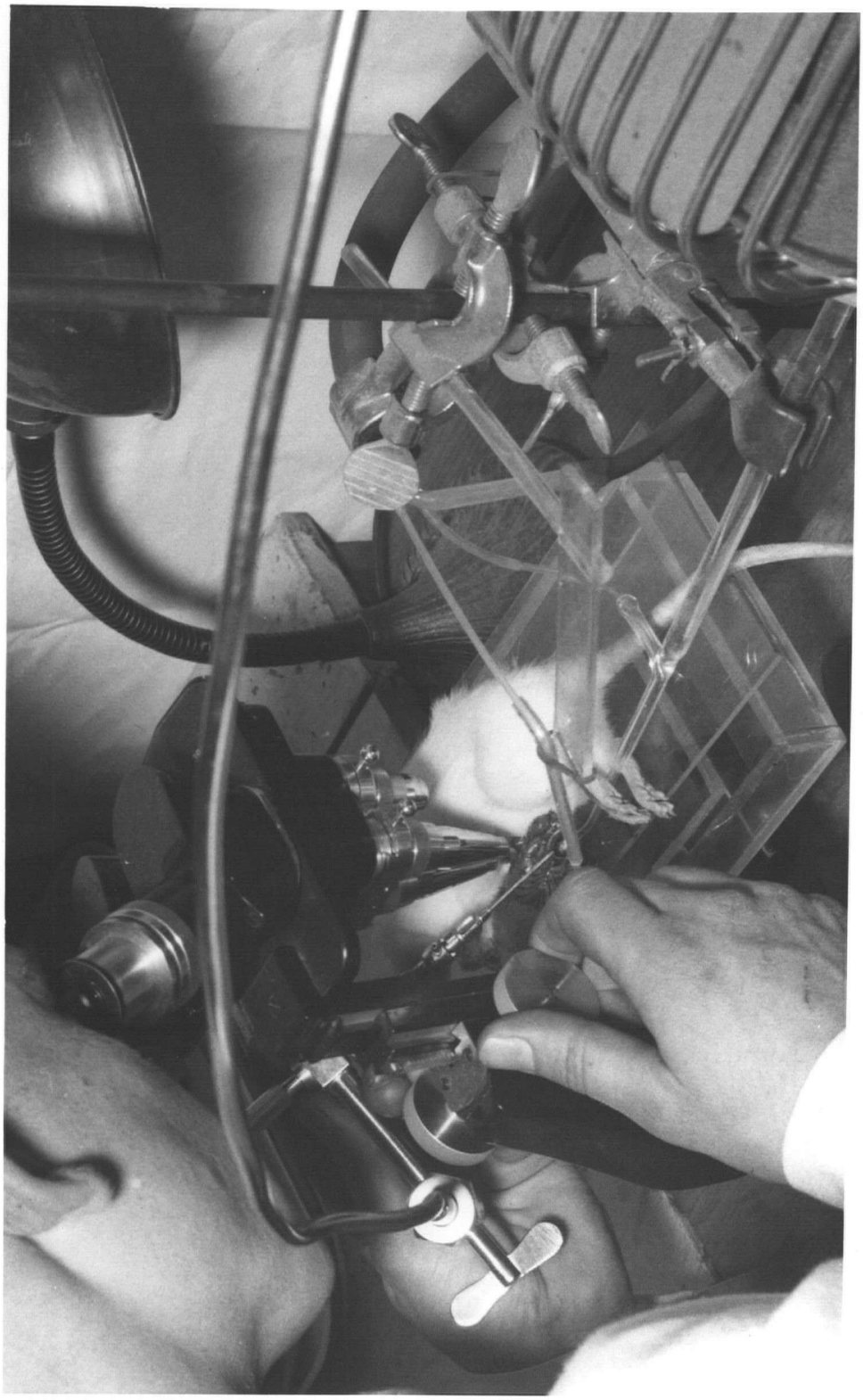
Capillary Bed

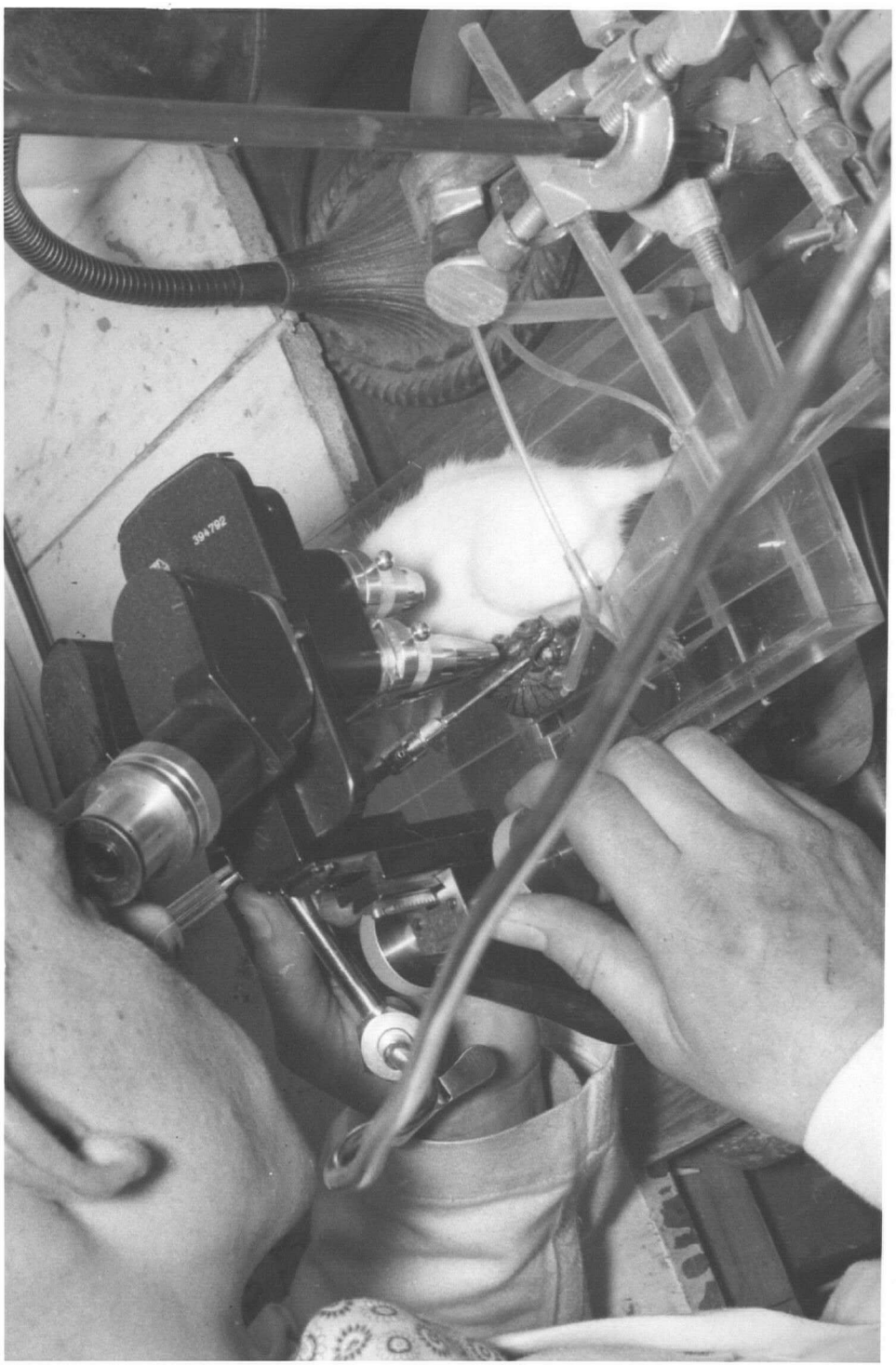
Whether all that Chambers and Zweifach describe is true or not, they have mapped out very well the various functioning units of the capillary bed, and their manner of action, and such an understanding was very helpful for a beginner to find his way around in the mesoappendix, for the parts labelled by Chambers and Zweifach were readily identified, and frequently observed to function as they said they should.

The Observation of the Rat Mesoappendix

As mentioned near the beginning of this thesis, D.L. Warner (7) was observing capillary changes in rats rendered hypertensive with compound F, and the same apparatus he used was taken over for the present work. Much of the following description of the equipment is taken from his unpublished thesis.

Generally Knisely's technique (18) for trans-illumination of living structures was used. A light source from a dismantled lantern slide projector with a four hundred watt bulb was used. This may be seen in the lower right hand corner of the first photograph. One end of the projector was sealed off by a circular piece of one inch board five inches across. A hole was made in its centre for the end of the fused quartz rod (the glass-like rod may be clearly seen in the first

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photograph going across from the projector towards, and actually between the hind feet of the rat). This rod transmitted and "bent" the focused beam of light right to the desired location. This may be seen quite clearly in both photographs as a highlight on the mesoappendix approximately 1 cm. under the microscope nose.

The quartz tube used was modelled after the improved tube described by Knisely (16). It was twelve inches long, and the first ten inches were eleven mm. in diameter. The last two inches tapered down to about three mm. The last half-inch was curved upward at an angle of ninety degrees. The last two inches were hollow, with a one mm. channel down the shaft from a side arm. A Lietz dissecting microscope was used, binocular as illustrated and it provided for magnifications of 12.5x, 50x, and 100 x. The latter, 100 x, was the customary system used. These lenses were mounted on a moveable nose-piece, which was used primarily for observing various areas of the mesoappendix (and not for changing lens systems as designed) instead of moving the animal mounting.

The irrigation fluid was maintained in a four litre brain jar container covered in a water bath which was thermostatically controlled for about 43°C. The solution was siphoned off and passed along two poly-ethylene tubes, one connecting to the side arm of the

quartz tube, the other ending in a fine glass tube that was suspended over the mesoappendix preparation in a moveable fashion. All this is clearly visible in the illustrations. The polyethylene tubes will be seen emerging from a thick rubber tube about one inch across. This was devised to keep the drip solution warm until it was delivered to the mesoappendix, and hot water bath water was circulated through this rubber casing by means of a simple rotary pump. The drip solution was delivered at the mesoappendix at a temperature of approximately 37°C.

The anaesthetized animal would be given a median abdominal incision and laid on its right side on a little shelf of lucite that was attached on the top of a lucite box one inch deep to catch the expended drip solution. The caecum would be carefully exteriorized, and the mesoappendix would then be draped over a little lucite loop two cm. across attached at a suitable height above the shelf to avoid tension on the mesoappendix. Warm saline soaked cotton packs (not shown in the illustration) would then be packed around the loops of bowel to keep them warm and moist, and also to support further the caecum. The warm drip solution was then started, which flowed over the mesoappendix from above. Solution also flowed out the channel in the quartz tube, but in as much as this tube did not usually touch the mesoappendix, this fluid

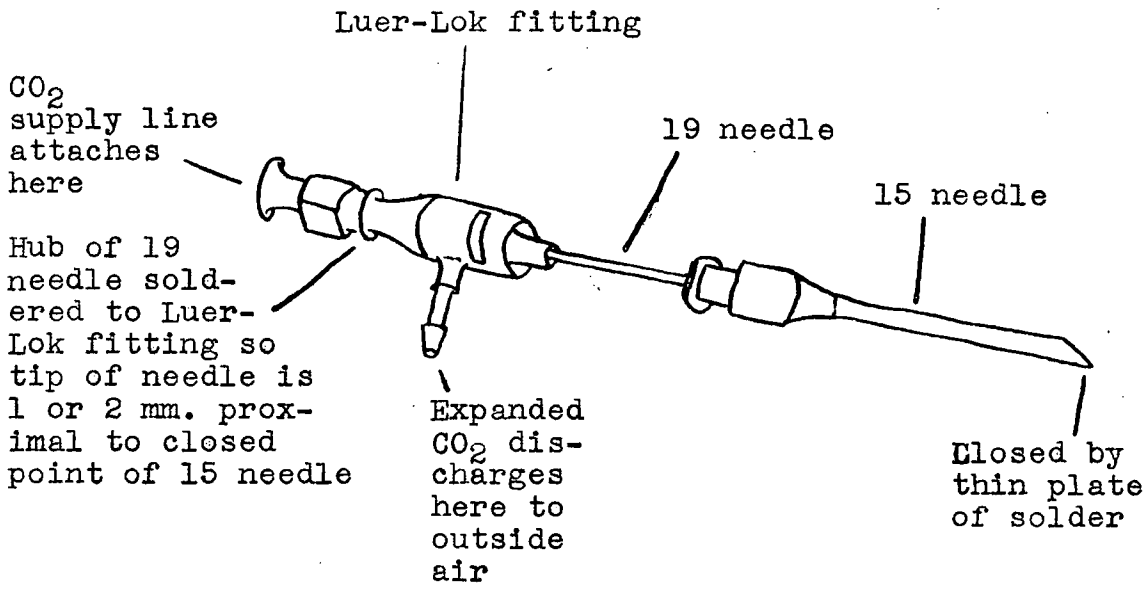
served little more than to keep the end of the tube from gathering blood, etc., that would obstruct the beam of light. Warner had perfected the apparatus and little adjusting was necessary. The photographs illustrate the equipment exceedingly well, and all points mentioned can be picked out. The second picture shows very clearly the loop of caecum draped on the observer's side of the loop (also visible, but not too well demarcated, with the highlight from the quartz tube showing at its centre). The highlight is produced not by the end of the quartz tube alone, but mainly by the light shining on the translucent mesoappendix.

Some difficulties with drip solutions were experienced. At first a plain Ringer-Locke solution was prepared (formula in appendix). But according to the prolongation in the normal state through the addition of gelatin as discussed by Zweifach (14), it was decided to add gelatine and glucose both to the Ringer-Locke solution as outlined in the appendix at the end of the thesis. This resulted in much more physiological preparations.

The Freezing Point

Running across both photographs is a winding malleable metal tube leading from a carbon dioxide tank, not shown, to the right of the pictures, across toward

the microscope to end in a control valve apparatus. This apparatus is the operating unit from a Spencer carbon dioxide quick-section microtome. This has been adapted according to Hass and Taylor's directions ( ) by the inclusion of the parts listed in the appendix at the end of the thesis. The freezing plate was removed from the microtome unit to leave essentially the control needle valve. To this was attached a Luer-Lok unit as on the end of Luer-Lok syringes. Then was constructed the unit sketched below which was readily



Hass and Taylor's Cold Point Apparatus

attachable to the rest of the unit (needle valve and carbon dioxide gas supply). The principle of the cold point was quite simple, utilizing the cooling effect of expanding gas, in the same way as carbon dioxide snow is made. The liquid carbon dioxide, or probably highly compressed carbon dioxide was conducted down the shaft of the number nineteen needle at a rate controlled by the needle valve. The tip of the nineteen ended just proximal to the closed tip of the fifteen needle (the cold point, at the end of the apparatus). The gas expanded from the point of the nineteen into the larger shaft of the fifteen. The Luer-Lok fitting with the side-arm had already been affixed by airtight seal to the shaft of the nineteen needle (at an appropriate level so that the point of the nineteen was just immediately proximal to the closed off point of the fifteen when the fifteen needle was locked onto the Luer-Lok fitting) and with the fifteen needle locked onto the fitting, the now expanded gas was able to flow proximally within the fifteen and to escape through the side arm to the outside air. By controlling the rate of gas flow, usually with short, closely spaced "jets", a freezing temperature could be obtained at the closed tip of the fifteen needle. This cold tip could then be applied to a suitable site on the mesoappendix, just as the model in the photographs is

demonstrating. The placing of the cold point could be localized very accurately through the microscope onto just the desired capillary bed. The usual capillary bed just about filled the field shown by the microscope at the 100 power magnification. The cold point could be precisely localized over any certain vessel. When the point was placed, the gas was turned on, and the area at the point allowed to freeze (the warm saline drip was swung out of the way). A freezing condition was indicated by a sudden whitening of the area due to ice formation in the moist film still remaining on the mesoappendix. This involved the whole thickness of the mesoappendix and the area of the mesoappendix affected was always within the field of the microscope at the 100 power magnification. In this way, by centering later at the originally selected area for freezing which was at the centre of the field, the boundaries of the frozen mesoappendix could always be exactly determined. The flow of gas would be turned off at the expiry of approximately twenty-five seconds, and at exactly thirty seconds the warm drip was resumed over the point and the mesoappendix. Thawing was nearly instantaneous, and then, but only then could the needle point be removed.

Epinephrine Response

Zweifach (14) outlines in quite careful detail his

epinephrine response test. He discusses a threshold response to vasotropic substances, and he defines the threshold response as just enough of the substance to give a moderate narrowing of the terminal arterioles and precapillaries just sufficient to slow the flow of blood through the capillaries and tributary venules within fifteen to twenty seconds. The threshold response to epinephrine varies in different arterioles within the same tissue. One part in six million is just below the level to give a response. The warm drip solution should be restored between trials. The epinephrine should not be applied oftener than at three minute intervals, and also repeated applications of epinephrine sensitizes the tissue to the drug. Zweifach lays down certain criteria for considering a preparation in a normal state. It must give the epinephrine response. Blood flowing through the capillary beds should be intermittent. There should be a minimum of leukocytes sticking or diapedesis in the walls of the venules. There should be no capillary stasis or stagnation. He further states no tests can be made more than fifty to sixty seconds after the preparation is mounted. Also, materials with marked side reactions invalidate the mesoappendix test.

These details of the criteria and characteristics of the threshold epinephrine response were of great

value and usefulness in studying the capillary reactions of the rat mesoappendix. As will be seen in the preliminary capillary tests with epinephrine and compound F as recorded in the following section of the thesis, the actual performance of the test was relatively easy.

Localized Cold Injury Experiments

With the cold point apparatus as described, a great number of tests were performed with control animals as recorded in following sections of the thesis. The behavior of the vessels of the mesoappendix to the freezing effects soon fitted into a definite pattern. As mentioned, the cold point would produce solid ice at probably below zero degrees. The onset of the freezing would be too rapid for total vasoconstriction within the affected area, but within twenty to thirty seconds after the thawing any vasoconstriction present would be overcome with a generalized hyperemia within the injured area. Usually within thirty seconds of thawing (called rapid thawing because the warm drip was used to melt the cold point away from the mesoappendix) slowing would be observed in a few of the postcapillaries, and very soon what was termed "sludging" would appear, also called "stasis". The precise nature of this process was not determined, but it constantly occurred. The oedema described by Shumacker could not be observed microscopically, but as Shumacker says (as on page 11) the formed elements of the bloodwere seen progressively

to fill the small vessels, bringing to a halt the circulation through them. This stasis was reversible, at the beginning at least, since mechanical pressure or changes in the blood flow in adjacent vessels would break up the red cell masses. This spread of the stasis was also constantly observed, and the term "spread" is used in following descriptions in two ways, rather indiscrimately. In one instance the spread meant just what is implied here, a progression of the stasis along a continuous channel. More often the term was employed to signify the appearance of stasis in separate,not necessarily connected channels, somewhat as an infectious disease spreads through a neighbourhood, although this is not strictly a correct usage at all, even though convenient. For the purposes of the experiments, the exact meaning of the term does not really matter.

Recording of Observations

In all the records of the experimental observations there will be noted a boring and apparently useless repetition. The decision was made early to indicate variables in the technique within the text of the descriptions for each preparation, as opposed to describing the general method at the beginning, and merely the bare variations with each animal. It seemed when the records were written up that such variables as how much anaesth-

etic was used and exactly how it was administered, and exact time lapses, etc were important, and such a diversity of details could not be indicated without much repetition.

Criteria for a Drug Effect

At the end of each section of the data dealing with the cold point experiments appears a summary table. The columns are headed by such titles as onset of stasis, spread of stasis, generalized stasis, resumed flow. These need explaining, because of the necessity for defining just what was meant by an effect due to the influence of some medication or treatment.

The time for the onset of the stasis was one item considered important. If stasis is such a pronounced feature of a freezing injury, any medication that could delay stasis or prevent its appearance altogether would be a valuable agent. This applied too for the extent in the development of the vascular stasis. A drug might not necessarily delay or prevent vascular stasis, but it might limit the extent to which it affected the frozen tissue. The idea that a drug is effective in frostbite therapy stemmed partly from the fact that the stasis was a reversible process, but mostly from the facts that procaine flushed over a static field would dilate the small vessels and cause a restoration in the circulation. This was hence regarded as a likely attribute in drugs effective in frostbite therapy.

Although these criteria might appear to be fairly objective and scientific, such was in fact just an illusion. Vascular fields in the rat mesoappendix were unfortunately found to have as much resemblence to each other as human faces have to other human faces. One mesoappendix might have only one or two wide areas sparsely crossed by a few fine capillaries that could possibly pass as capillary beds. Other preparations would be just crowded with vessels going every possible direction. When trying to specify as to what the extent of the stasis was, or how rapid the spread was, it is obvious that there were difficulties. On the whole, the observation of the mesoappendix was a very subjective matter.

A necessary feature of the microscopic experiments lay in the decision to alternate when possible the test and the control preparations. It took generally four hours to perform a microscopic experiment of ten or twelve animals. Conditions could and did change greatly in the room used for a laboratory over this time. Hence the testing of all the control animals followed by testing of the test rats was discarded as impractical.

Care of Animals

Rats, either Wistar or Hooded, in the 150 to 250 gram weight range were obtained in batches of fifty or so

when available upon requisition from separate breeding stock barns elsewhere on the campus. They were housed in a well ventilated windowless inside room, in tiered animal cages holding from three to six animals each. Fresh drinking water was available to the animals at all times and Purina Fox Chow Checkers were kept suspended in each cage in a feeding trough. All animals stayed healthy and well in these surroundings for indefinite periods of time. They were separated according to sex.

Preliminary microscopic observations using Compound F (17 hydroxy-cortisone 21 acetate)

Preliminary capillary experiments

In order to gain some experience with the use of the microscope in connection with capillary observations, certain tests were performed using the capillary epinephrine response as described by Zweifach, B. W. (14). Warner, D.L., the senior medical student mentioned so frequently in this thesis as a part-time helper with the project, while performing experimental work in connection with his graduating thesis for the Faculty of Medicine at this university, tested capillary response to various drugs including compound F., and he found certain effects due to the drug which greatly modified the capillary response to epinephrine. He found (7) that whereas a 1:4 million (abbreviation for one part in four million parts) dilution was the threshold concentration of epinephrine needed to elicit a vasomotor response as determined by metarteriole and precapillary constriction, that after injecting compound F. for a certain number of days the threshold response had decreased to 1:18 or 1:20 million dilution of epinephrine.

Because of the supposed delicacy of the technique of examining vascular responses under the micro-

scope, it was felt that a repetition of Warner's work, in part, especially if it could be confirmed, would serve both as an initiation into the technique and also would serve in a small measure to increase confidence in the reliability of later observations with localized cold experiments.

Initial epinephrine response tests

The purpose of the following tests was to try to determine the threshold epinephrine response of the metarterioles and precapillaries in the mesoappendix of the rat. Several adult male Wistar rats were selected and set aside for use on several successive days. The apparatus was set up as described and a simple Ringer-Locke saline drip solution used. Epinephrine chloride solution 1:1000 was maintained in a small flask at the temperature of the water bath, and several test tubes of drip solution were also kept warm, with carefully measured quantities of drip solution to permit rapid preparation of the diluted epinephrine so as to minimize the tendency for the epinephrine to decompose. This decomposition of the epinephrine, especially when warm and mixed with the drip solution, was a real problem, and as will be explained later possibly accounted for some of the conflicing results obtained.

Animal 1

A normal adult male Wistar rat weighing 135 grams was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally at zero minutes. At twenty-four minutes the preparation was mounted and ready on the microscope stage. The temperature of the drip solution was 33.5°C., and this was allowed to drip over the mesoappendix except when the test solution was flushed on with a medicine dropper. Approximately a thirty seconds after the test solution was applied, the drip would be resumed until the next test. Long enough an interval was allowed for the preceding epinephrine to be washed away and for the vessel to recover from its effect before any more was used. An interval of about five minutes was considered suitable, and this was found to be adequate. At thirty-two minutes 1:2 million epinephrine was applied and this gave a very strong response, classed as ++++. At thirty-seven minutes 1:4 million gave a ++ response, at forty-two minutes 1:6 million gave no response. At forty-seven minutes a 1:4 million dilution gave a +++ response. At fifty-two minutes a 1:5 million dilution gave a +++ response. At fifty-seven minutes a 1:6 million dilution gave a very doubtful response, classed as plus or minus. On the basis of this, and with Warner's agreement (he worked

with me for the first day) the threshold response was set at 1:5 million dilution.

Animal 2

A normal adult male Wistar rat weighing 145 grams was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally at zero minutes. The animal was ready on the microscope by twenty-three minutes. At thirty minutes there was no response to 1:7 million epinephrine (all dilutions were freshly prepared each time they were needed); at thirty-seven minutes no response to 1:6 million dilution; nor any response at forty minutes to 1:5 million. At forty-two minutes 1:4 million gave a $\nearrow$ response two minutes later which lasted only a minute. At forty-seven minutes 1:5 million gave no response, but at fifty-two minutes 1:3 million gave a $\nearrow\nearrow$. The preparation was discontinued at fifty-five minutes. The temperature of the drip solution at the mesoappendix was 36°C. The threshold response was considered to be on the basis of this preparation 1:4 million dilution of epinephrine.

Animal 3

On the following day, the same set-up was made ready as before, and a normal adult male Wistar rat weighing 150 grams was anaesthetized with 0.13 cc of 0.6% nembutal solution, at zero minutes. The preparation

was ready on the microscope at twenty-three minutes with the drip going. At twenty-six minutes 1:6 million gave no response, but three minutes later 1:5 million gave a +++ response. At thirty-two minutes 1:6 million gave a very doubtful effect, and the same with 1:5 million four minutes later. At thirty-nine minutes 1:5 million gave no response, but at forty-two minutes it gave a doubtful effect. At forty-five minutes a 1:4 million solution gave a +++ effect. At forty-nine minutes 1:5 million gave no response, nor did 1:4 million two minutes later. Finally, at fifty-four minutes 1:5 million failed to give any effect. It was noted that flushing on the adrenaline too soon after the preceding application gave an unsatisfactory response. Possibly the preparation was kept too long. On the basis of these observations, the threshold for the epinephrine response was felt to be 1:5 million dilution.

Animal 4

A normal adult male Wistar rat weighing 160 grams was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally, at zero minutes. At twenty-five minutes the preparation was mounted on the microscope stage and ready with the drip going at 36°C. At thirty-seven minutes 0.07 cc of

0.6% nembutal had to be repeated because of lightness of the anaesthesia. At thirty-nine minutes a 1:6 million dilution gave no response. At forty-three minutes a 1:5 million dilution gave a ++ effect, which was repeated at fifty minutes. At fifty-five minues 1:5 million epinephrine had no effect, nor did 1:4 million at sixty minutes. One minute later a drop of plain ice water gave a vigourous ++++ response, and at sixty three minutes 1:4 million epinephrine gave a + response. The preparation was discontinued at sixty-five minutes and the threshold response was considered to be 1:5 million dilution.

Animal 5

A normal adult male Wistar rat weighing 175 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, at zero minutes. At twenty minutes the preparation was ready with the drip going. At twenty-four minutes a 1:6 million dilution gave no response. At thirty-two minutes 1:5 million epinephrine gave a + response. Five minutes later, on repeating the 1:5 million, a doubtful effect was obtained. At forty-two minutes 1:4 million gave no response, but three minutes later the 1:4 million produced a ++ effect. It was noted (a) the saline

packs kept over the exposed bowel were too cool, and (b) there was some degree of general stasis in the mesoappendix first noticed about the forty-two minute period. There was also some blood loss from the abdominal incision which might have affected the general circulation. The preparation was discontinued at fifty minutes, and the threshold level for the epinephrine response was felt to be again 1:5 million.

Animal 6

The following day the same experiments were continued. No attempt was made to control the temperature of the drip solution falling on the mesoappendix (the faster the drip ran the shorter time it was in the connecting tubing and the less heat it lost on its way from the water bath) and the average temperature was about 37°C. Generally, in the following five animals much more spontaneous vasomotion was noted than at the previous temperature levels of 33 to 36°C. This may explain the slightly higher threshold levels obtained with this and the following preparations.

A normal adult male Wistar rat, weight not recorded, was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally. The temperature of the drip over the mesoappendix were 36.6°C. The preparation was ready at six minutes. At sixteen

minutes 1:7 million epinephrine gave a +++ response, which was repeated five minutes later, and at twenty-five minutes 1:8 million gave a ++ response. A minute later plain drip solution gave no response, nor did 1:8 million dilution at twenty-eight minutes, nor did 1:7 million dilution at thirty-one minutes. 1:6 million failed to elicite any response at thirty-four minutes and 1:5 million did the smae at thirty-seven minutes. 1:4 million gave a + response at forty minutes, and at 43 minutes cold 1:100,000 dilution gave a ++++ response. For reasons not recorded, it was felt that 1:4 million was the threshold, and not 1:7 or 1:8 million was would appear to be. This opinion would have better support if the interval between the applications of the epinephrine had been longer than three minutes.

Animal 7

A normal adult male Wistar rat, weight not recorded was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally, and fifteen minutes later 0.05 cc. had to be repeated to obtain a satisfactory depth of anaesthesia. The preparation was ready at twenty minutes, on the microscope, with the warm Ringer-Locke drip running. At

twenty-two minutes 1:10 million epinephrine gave no response, 1:8 million did not at twenty-six minutes, 1:7 million did not at twenty-nine minutes, and 1:6 million did not at thirty-two minutes. At thirty-five minutes 1:5 million dilution gave a very doubtful response, but 1:4 million gave a definite ✓ response at forty minutes, but no response at forty-five minutes. At forty-eight minutes cold 1:100,000 epinephrine gave a very strong ✓✓✓✓ response, and at fifty-three minutes 1:4 million gave a ✓✓ response. At fifty-six minutes 1:5 million failed to give any effect, but three minutes later 1:4 million gave a ✓ response. Finally at sixty-two minutes, 1:100,000 cold epinephrine gave a very strong ✓✓✓✓ response. The preparation was discontinued at sixty-five minutes and the threshold was considered to be 1:4 million dilution of epinephrine.

Animal 8

A normal adult male Wistar rat of unrecorded weight, was anaesthetized at zero minutes with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready with warm drip going at fourteen minutes. There was much vascular stasis throughout the vascular field of the mesoappendix. At twenty-four minutes 1:6 million dil-

ution of epinephrine gave a +++ response, and five minutes later 1:7 million also gave a +++ response. The vascular stasis worsened gradually, and the preparation was discontinued with no results obtained.

Animal 9

A normal adult male Wistar rat weighing 155 grams was anaesthetized at zero minutes with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready ten minutes later. At ten minutes 1:6 million dilution gave no response, but at fourteen minutes 1:5 million gave a + response. This was a very beautiful preparation to observe. At sixteen minutes 1:4 million gave no response, but five minutes later it gave a doubtful response. At twenty-six minutes 1:3 million dilution gave a ++ response. The epinephrine was obviously getting brown, and was made up again from fresh 1:1000 stock epinephrine. At thirty-five minutes this fresh solution gave at 1:4 million dilution a ++ response. At forty minutes 1:5 million gave also a ++ response. At forty-four minutes 1:6 million gave no response, but a minute later 1:5 million gave a ++ response. The preparation was discontinued at fifty minutes, and it was felt that 1:5 million was the threshold dilution for the epineph-

rine response.

Animal 10

A normal adult male Wistar rat weighing 155 grams was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally, and it was ready on the microscope stage with the warm drip going at five minutes. At seven minutes 0.05 cc of nembutal had to be repeated. At seventeen minutes 1:6 million dilution of epinephrine gave a ///// response. 1:7 million gave a ///// response at twenty minutes, and so did a 1:8 million solution at twenty-three minutes. At twenty-six minutes leukocytes were observed sticking to the walls of the larger vessels, and the preparation was discontinued as unphysiological. The temperature of the drip solution was 37°C. No results were recorded.

Summary of Observations

No.	Threshold epinephrine response in millionths		
1.	1:5		
2.	1:4		
3.	1:5	7.	1:4
4.	1:5	8.	No results
5.	1:5	9.	1:5
6.	1:4 ?	10	No results

Discussion of Observations

These observations agree with Zweifach, B.W. (14) who stated a 1:6 million dilution of epinephrine is just below the level required for a vascular response according to his technique. Here, with the doubtful exception of Animal 6, no threshold was found lower that 1:5 million, and incidentally, none was found higher than 1:4 million, which is rather an excellent result.

It was learned that the requirements for a physiological preparation are fairly strict, and some of the signs that a preparation was beginning to spoil were observed first hand. These lessons were put to good advantage later on the local cold experiments. Note that a plain Ringer-Locke saline drip was employed. There were found later advantages to using a gelatin-glucose enriched and buffered drip solution.

Observations with Compound F

The purpose of the experiments here outlined was to test the effect compound F would have on the epinephrine threshold response in the circulation of the rat mesoappendix. 2.0 mg. of compound F in saline suspension in a concentration of 25 mg. per cc. (that

is 0.08 cc. per injection) administered intraperitoneally every day for approximately two weeks, was the contemplated dosage level, prior to the microscopic observations.

Eighteen normal adult male Wistar rats were selected, and after marking the ears characteristically the initial weights were taken as recorded on the Data Sheet. The animals were divided into three groups of six each so that the injections could be started in a staggered manner to finish the series on different days for convenience in testing. The medication was given to three test animals in each group, and the remaining three were for controls to be examined alternately with the test animals. At the end of the series of injections with compound F, the epinephrine threshold response test was performed on each rat as in the previous experiment. Details for each animal are as follows:

Animal 1 (Control)

The rat was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally, having received no previous medication, and the preparation was ready on the microscope stage with the warm drip running at thirteen minutes. At twenty-five minutes there was a very doubtful response to 1:6 million dilution of epinephrine which could not be repeated at twenty-eight minutes. At thirty-one minutes 1:5 million dilution

Data Sheet

Animals 1 to 6

Control	160	-	-	-	-	-	-	-	-	-	-	-	-	192	-	T
	154	-	-	-	-	-	-	-	-	-	-	-	-	190	-	T
	166	-	-	-	-	-	-	-	-	-	-	-	-	204	-	T
Test	166	F	F	F	F	F	F	F	F	F	F	F	F	164	F	T
	175	F	F	F	F	F	F	F	F	F	F	F	F	180	F	T
	147	F	F	F	F	F	F	F	F	F	F	F	F	150	F	T
		i/p						s/c								
Day	1	2	3	4	5	6	7	8	9	10	11	12	13			

Animals 7 to 12

Control	144	-	-	-	-	-	-	-	-	-	-	-	-	206	-	T
	155	-	-	-	-	-	-	-	-	-	-	-	-	214	-	T
	146	-	-	-	-	-	-	-	-	-	-	-	-	211	-	T
Test	162	F	F	F	F	F	F	F	F	F	F	F	F	163	F	T
	172	F	F	F	F	F	F	F	F	F	F	F	F	198	F	T
	153	F	F	F	F	F	F	F	F	F	F	F	F	135	F	T
		i/p					s/c									
Day	1	2	3	4	5	6	7	8	9	10	11	12	13			

Animals 13 to 18

Control	173	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	248	-	T
	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	231	-	?
	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	227	-	?
Test	190	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	201	F	?
	145	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	162	F	?
	151	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	163	F	?
		i/p				s/c															
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			

(-, means not administered, F, injection, T, tested, ?, wasted, i/p, intraperitoneally, s/c subcutaneously)

gave a ✓ response, and three minutes later the same dilution gave a ✓✓✓ response. The preparation was discontinued and the threshold was recorded as 1:5 million.

Animal 2 (Test)

The rat was anaesthetized with 0.13 cc of 0.6% nembutal solution administered intraperitoneally after thirteen daily intraperitoneal injections of compound F. At fifteen minutes 0.03 cc. of the nembutal had to be repeated, and the preparation was ready at twenty-five minutes with the warm drip running. At thirty-one minutes a 1:10 million dilution gave a ✓✓ response, and four minutes later 1:10 million dilution gave a ✓✓✓ response. At thirty-nine minutes 1:14 million gave no response, and 1:10 million failed to give any response at forty minutes but gave a ✓✓✓ three minutes later. At fifty-one minutes 1:14 dilution gave no response. At fifty-four minutes a 1:10 million gave a very doubtful response, and so did 1:8 million at fifty-seven minutes. There was too much vascular stasis to be sure. Probably solutions weaker than 1:10 million would have given a response if they had been used first. The results of this test had to be discounted in view of the fact the preparation was not entirely physiological.

Animal 3 (Control)

The rat was anaesthetized with 0.15 ccof 0.6% nembutal solution administered intraperitoneally at zero minutes, and at ten minutes 0.05 cc. of nembutal solution had to be repeated. The preparation was ready at twenty minutes with the warm drip running. At twenty-four minutes 1:6 million dilution of epinephrine gave a very doubtful vascular response. At twenty-seven minutes 1:5 million gave a ++ response which was repeated in three more minutes. At thirty-three minutes 1:6 million did not give any response, and at thirty-seven minutes 1:5 million did not either. At forty minutes 1:4 million gave a very doubtful effect. At forty-two minutes 1:100,000 dilution gave a strong ++++ response. At forty-five 1:4 million gave a ++++ response, and at fifty-two minutes 1:5 million gave a +++ response. 1:6 million gave a +++-response both at fifty-two and fifty four minutes. At fifty-seven minutes 1:7 million gave a ++++ response and at sixty-one minutes 1:10 million gave also a ++++ response. Finally, at sixty-five minutes plain drip solution gave a ++++, and the preparation was discontinued because vascular stasis was quite obvious. Probably no results after forty-two minutes were valid, and 1:5 million was accepted as the threshold for this test.

Animal 4 (Test)

The rat was anaesthetized with 0.15 cc. of 0.6% nembutal solution administered intraperitoneally after thirteen daily intraperitoneal injections of compound F. At eighteen minutes the preparation was ready with the warm drip running, and 1:100million epinephrine solution gave a ++ response. At twenty-three minutes a 1:14 million dilution gave a ++ response, and at twenty-eight minutes a 1:16 million dilution gave a +++ response. At thirty-three minutes plain drip was flushed over the mesoappendix with no apparent effect on the blood vessels. At thirty-four minutes 1:20 million dilution was tested with no response. At thirty-nine minutes 1:18 million gave a doubtful response. At forty-two minutes 1:16 million gave a +++ response, and then at forty-seven minutes 1:18 was tried again and it gave a definite ++ response. In two minutes 1:20 million gave no effect but when 1:18 million was tried at fifty-one minutes it gave a +++ response. This was a wonderful preparation to work with. The preparation was discontinued at fifty-five minutes, and 1:18 million was accepted as the threshold level.

Animal 5 (Control)

The animal was anaesthetized with 0.13 cc. of

0.6% nembutal solution administered intraperitoneally at zero minutes, and the preparation was ready at ten minutes. At twelve minutes 1:10 million dilution of epinephrine gave no response, nor did 1:8 million at fifteen minutes, nor did 1:6 million at nineteen minutes. However at twenty-three minutes 1:5 million dilution gave a +++ response. This was accepted as a very definite threshold and the preparation was discarded at twenty-five minutes.

Animal 6 (Test)

The rat was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered intraperitoneally at zero minutes after thirteen daily intraperitoneal injections of compound F. At eleven minutes 0.05 cc. of nembutal had to be repeated (subcutaneously) and the preparation was ready at nineteen minutes. At twenty-five minutes 1:20 million dilution of epinephrine gave no vascular response. At twenty-eight minutes 1:18 million gave no response, but at thirty-one minutes 1:16 million gave a +++ response. Then at thirty-four minutes 1:18 million gave a ++ response, but at thirty-eight minutes 1:20 million gave no response. On this basis 1:18 million dilution was accepted as the threshold , and the preparation was discarded.

Animal 7 (Control)

The rat was anaesthetized with 0.13 cc. of 0.6%

nembutal solution administered intraperitoneally at zero minutes, and at seven minutes the preparation was ready on the microscope stage with the warm drip running. At eight minutes 1:10 million epinephrine resulted in no vascular response. At eleven minutes 1:6 million dilution gave a ƒƒƒ response, and at fifteen minutes 1:7 million gave a ƒƒ response which was repeated in three minutes. At twenty-one minutes 1:10 million gave no response. At twenty-four minutes 1:8 million gave a slight response. In view of the fact the preparation was possibly sensitized to epinephrine at this stage, although it was otherwise an excellent preparation, 1:7 million was accepted as the threshold value. The preparation was discontinued at twenty-eight minutes.

Animal 8 (Test)

The rat was anaesthetized with 0.15 cc. of 0.6% nembutal solution administered intraperitoneally at zero minutes, and at twenty minutes 0.06 cc. was repeated, also by intraperitoneal injection, and the preparation was ready at thirty minutes. At thirty-three minutes 1:20 million dilution of epinephrine gave no response. At thirty-seven minutes 1:18 million gave a ƒ, definite but slight, response. At forty minutes 1:16 million gave a ƒƒ response, and at both three and six minutes later 1:16 million gave a ƒ response.

1:18 million was accepted as the threshold value, and the preparation was discontinued at fifty minutes.

Animal 9 (Control)

The animal was anaesthetized with 0.15 cc. of 0.6% nembutal solution administered intraperitoneally at zero minutes, and the preparation was ready at seven minutes with the warm drip running. At eight minutes 1:7 million epinephrine solution gave no vascular response, nor did 1:6 million at eleven minutes, nor did 1:5 million at fourteen minutes. However, at seventeen minutes, 1:4 million gave a definite / response. This was a good preparation with excellent active vasomotion. At twenty-two minutes 1:4 million gave a // response. 1:4 million was accepted as the threshold, and the preparation was discontinued at twenty-five minutes.

Animal 10 (Test)

The animal was anaesthetized with 0.14 cc. of 0.6% nembutal solution administered intraperitoneally at zero minutes, and at five minutes 0.05 cc. were repeated, with 0.03 cc. more at thirteen minutes. The preparation was ready at eighteen minutes. At nineteen minutes 1:20 million epinephrine gave no apparent vascular reaction, but at twenty-two minutes 1:18 million gave a /// response. 1:18 million was accepted as the threshold and the preparation was discontinued at twenty-five minutes.

Animal 11 (Control)

The animal was anaesthetized with 0.14 cc. of 0.6% nembutal solution administered intraperitoneally, at zero minutes, with 0.05 cc. being repeated at both fifteen minutes and twenty-one minutes. At twenty-eight minutes 1:7 million epinephrine gave a ++ response. At thirty-one minutes 1:10 million gave a ++ which was repeated three minutes later. At thirty-seven minutes plain warm drip gave no response, but immediately after 1:10 million again gave a ++ response. The physiological nature of this preparation was not recorded, and 1:10 million must be accepted as the threshold. The preparation was discontinued at forty minutes.

Animal 12 (Test)

The animal was anaesthetized with 0.13 cc. of 0.6% nembutal solution, and the preparation was ready thirteen minutes after the intraperitoneal injection. At fourteen minutes 1:20 million epinephrine dilution gave a ++ response but some stasis developed subsequently in the area being observed. At seventeen minutes 1:20 million gave a +++ response, which was repeated at twenty minutes. Vascular stasis continued to develop in the mesoappendix until the preparation was discontinued at twenty-five minutes. 1:20 million was accepted as

the threshold value.

Animal 13 (Control)

The animal was anaesthetized with 0.14 cc. of 0.6% nembutal solution administered intraperitoneally at zero minutes followed in two minutes by 0.05 cc. more. At nine minutes, after mounting the preparation, 1:6 million epinephrine dilution failed to give any vascular response. At nineteen minutes 1:5 million gave a ++ and this was accepted as the threshold value.

Note

With animals 13 to 18, attempts were made to photograph the record of the vascular responses, but difficulties in managing the camera, etc., caused the remaining five animals to be wasted.

Summary of Observations

No.	Kind	Threshold dilution
11	Control	1:5 million
2.	Test	No results
3.	Control	1:5 million
4.	Test	1:18 million
5.	Control	1:5 million
6.	Test	1:18 million
7.	Control	1:7 million
8.	Test	1:18 million

Summary (Cont'd)

No.	Kind	Threshold dilution
9.	Control	1:4 million
10.	Test	1:18 million
11.	Control	1:10 million
12.	Test	1:20 million
13.	Control	1:5 million

Discussion of Observations

These observations rather satisfactorily confirmed Warner's findings as described, that injecting compound F into rats for a certain number of days decreased the epinephrine threshold response from a normal of 1:4 million dilution down to 1:18 million or 1:20 million dilution.

They also form a base line of capillary performance upon which later capillary behaviour was judged.

Record of Microscopic Observations

Here follows a record of observations made microscopically on the mesoappendix of the rat as outlined previously. Details of time periods and injections are given individually for each animal tested. The results are grouped by the drugs being tested, according to the order of the table of contents.

Procaine

Experimental observations with procaine hydrochloride

(A summary of the following results is given in table form after Animal 10.)

Animal 1 (Test)

0.20 cc of a 0.6% nembutal solution were injected intraperitoneally into a 280 gram adult male Wistar rat. The preparation was ready on the microscope stage in nine minutes. A few drops of one in four million dilution of epinephrine failed to give any vasomotor response in the capillary circulation of the mesoappendix, but this failure was attributed to deterioration of the epinephrine. Three minutes later, two percent procaine was dropped on, and the rate of blood flow in the vessels generally was noted to be increased. One minute after this, the cold point

was applied to a selected area of the mesoappendix with freezing for forty-five seconds, which was followed after thawing by increased blood flow. Two percent procaine was dropped on the mesoappendix, but vascular stasis was observed in a few capillary beds within twenty-seconds of thawing, although the central venule was functioning well. By three and a half minutes after thawing, there was stasis in the main venule in the injured area, giving blockage and stasis generally throughout the whole area affected by the freezing. Therefore it would not seem as though the procaine delayed the onset of vasostasis much following the freezing injury. Ten minutes later one or two capillaries were observed to be functioning at the edge of the injured area, and three minutes after this the warm saline drip was stopped, and two percent procaine was instilled over the mesoappendix. This resulted in a more rapid flow in the capillaries around the periphery of the injured area, but there was no apparent effect on any of the stasis within the frozen area. Four minutes later more two percent procaine was applied. In two minutes one large vessel that had been static was functioning, and in another minute a couple of small capillaries at the edge of the injured area were flowing. Two minutes later more procaine was applied, which resulted in a slight trickle of blood corpuscles through the main static area. Three minutes later more procaine

resulted in almost immediate opening up of the main static area, although only temporarily, since the circulation closed down again on resuming the warm saline drip. Three minutes later more procaine again opened up the same channels, with increased vasomotion observed in the vessels tributary to this main venular channel. The preparation was discontinued thirty-five seconds after the initial freezing.

Animal 2 (Test)

A male Wistar rat in the 200 gram range was anaesthetized with 0.15 cc of 0.6% nembutal solution, and the preparation was ready seven minutes later. Two percent aqueous procaine was applied to the mesoappendix, and the speed of capillary blood flow was observed to be increased. One minute later, more procaine was flushed over the mesoappendix, and one minute still later the cold point was applied to a selected area with freezing for thirty seconds. This resulted in immediate vasoconstriction throughout the affected area, followed by marked vascular dilatation. Procaine was dropped on the field. Stasis in small venous channels appeared three and a half minutes after thawing, but the process was limited. Procaine was reapplied, and the stasis was noted to have spread to involve an adjacent channel, but within four minutes of thawing, the first channel opened up and resumed its blood flow. One minute after this the whole area was again in stasis, which was not

greatly relieved by further applications of the procaine. The preparation was not regarded at this time as being particularly physiological, and it was discarded thirteen minutes after the initial freezing injury.

Animal 3 (Control)

A normal male adult Wistar rat in the 200 gram range was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, but the preparation was discontinued because the whole mesoappendix was found to be in vascular stasis on mounting on the microscope stage. This stasis was not influenced by applications of two percent procaine.

Animal 4 (Control)

A normal adult male Wistar rat in the 200 gram range was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready in five minutes. The cold point was applied with freezing for forty-five seconds, and within sixty seconds of thawing stasis was observed in the venules that had been frozen. This stasis did not change in the following five minutes, and the preparation was discontinued.

Animal 5 (Test)

A normal adult male Wistar rat, in the 200 gram weight range was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready in six minutes. Two percent procaine was applied locally on the mesoappendix, and the

rate of capillary blood flow was observed to be generally increased. Two minutes later the cold point was applied with freezing for thirty seconds. Procaine was applied immediately after thawing, and again thirty seconds after this. Forty-five seconds after thawing, the blood flow in the injured area was greatly slowed, with vasodilatation especially of the venules, but only two and a half minutes after thawing was any stasis apparent, and then only in two small capillaries, with a third involved thirty seconds later. Three and a half minutes after thawing the warm saline drip was resumed, and ninety seconds later one third of the injured area was in vascular stasis with increased vasomotion observed in the remainder of the vessels in the frozen afea. Thirty seconds later the drip was stopped and procaine was flushed over the mesoappendix, with increased blood flow resulting, and only one small area remained static. Two minutes afterward the warm saline drip was resumed, with increased slowing and stasis, which, in all except one large connecting venule, was completely reopened by procaine (and also except in the two above described static areas). By twelve minutes after the freezing injury, the circulation in the rest of the mesoappendix was apparently quite normal, and the preparation was discontinued.

Animal 6 (Control)

A normal a_dult male Wistar rat in the 200 gram weight range was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, and the

preparation was ready within five minutes. The cold point was applied with freezing for thirty seconds, followed by immediate rapid thawing. For twenty seconds after thawing there was vasoconstriction and then marked vasodilatation. By three and a half minutes after thawing there was still no stasis except in one venous channel, but when the warm Ringer drip was started there was spreading stasis within thirty seconds. The preparation was discontinued.

Animal 7 (Test)

A normal male adult Wistar rat in the 200 gram weight range was used. It was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready in six minutes. Two percent procaine was flushed over the mesoappendix, and then the cold point was applied with freezing for thirty-five seconds. In two and a half minutes after thawing, stasis started to appear in small vessels. One minute later the warm saline drip was resumed, with spread of the stasis, although the main channels remained open. The uninjured areas of the mesoappendix had apparently normal circulation. One and a half minutes later the saline drip was discontinued and procaine was applied. In ninety seconds many of the channels opened up and resumed circulation, but increased vascular tone gave more vasomotion in these channels. By ten minutes after thawing it was noted that the general area of stasis was not really greatly affected, in comparison with what it had

been, and in comparison with the surrounding areas of the mesoappendix.

Animal 8 (Control)

A normal adult male Wistar rat in the 200 gram weight range was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready in five minutes, with warm saline solution dripping on the mesoappendix. The drip was stopped and the cold point was applied with freezing for thirty seconds. Within thirty seconds of thawing stasis had started and was spreading rapidly in the injured area. Two and a half minutes later the drip was resumed which completed the generalized vascular stasis within the injured area. The preparation was discontinued.

Animal 9 (Test)

A normal adult male Wistar rat in the 200 gram weight range was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready in seven minutes, mounted, and with the warm saline drip running. This was stopped, and two percent procaine was instilled locally over the mesoappendix. The cold point was next applied, with freezing for thirty seconds. The procaine was applied immediately afterward, and greatly increased blood flow was noted. By two and a half minutes after thawing, no stasis was observed, and good vasomotion was apparent

throughout the injured area. Procaine was again re-applied, and by five and a half minutes after thawing, there was still excellent blood flow with vasomotion, but no stasis had developed. Two minutes later slight capillary stasis generalized over the injured area was readily obliterated with half percent procaine. One minute later the warm saline drip was started with immediate general capillary stasis resulting in the injured area. The drip was stopped, with sub-total resumption of blood flow in the whole field. The preparation was discontinued ten minutes after the initial freezing injury.

Animal 10 (Control)

A normal adult male Wistar rat in the 200 gram weight range was used. It was anaesthetized with 0.15 cc of 0.6% nembutal solution administered intraperitoneally. Ten minutes later the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Quite extensive vaso-dilatation followed the thawing, and within ninety seconds of thawing, stasis started which rapidly became extensive. By four and a half minutes following thawing the whole injured area was in stasis except for one large channel. The preparation was discontinued five and a half minutes after thawing.

Summary of Results with Procaine

No.		Onset Stasis	Spread	Generalized	Flow Resum'd
1	Test	20"	Yes	3½'	+++
2	Test	3½'	No	Finally	+++
3	Control	No results, preparation discarded			
4	Control	45"	Rapid	Yes	None
5	Test	2½'	Slight	Drip caused	+++
6	Control	3½'	Total stasis when drip started after thawing		
7	Test	2½'	When saline drip resumed		+++
8	Control	30"	Rapid when drip resumed		None
9	Test	7½'		General when drip resumed	+++
10	Control	90"	Rapid	4½'	None

Discussion of Results

The unsuitability of plain Ringer-Locke saline solution as a perfusion drip is readily apparent. In spite of this uncontrolled factor, it is felt that two percent procaine applied locally to the mesoappendix both before and after a standard freezing injury with the cold point for thirty seconds could be relied on to both delay the onset of stasis and reopen clogged and static vessels onceethe stasis had occurred.

The average length of time required for the onset of stasis in the test animals was nearly three and a half minutes after thawing, whereas in the control runs the corresponding time was approximately ninety

seconds. Thus it would appear that two percent procaine delays the onset of vascular stasis in a frozen capillary bed after application of the cold point with freezing for thirty seconds by an average of two minutes.

Before freezing of the capillary bed, two percent procaine applied topically to the mesoappendix gave greatly increased flow of blood in all vessels observed, both in velocity and in apparent volume of flow. This was thought to be due to a vasodilatory effect, with the site of action not determined. The procaine could act directly on the small blood vessels or through inhibition of the vasoconstrictor impulses. It was important to observe that additional topical application of two percent procaine frequently aided or caused the opening up of channels in stasis, presumably through releasing vasoconstriction, and thereby allowing a greater volume of blood at a more effective hydrostatic and pulsile pressure to flush the vessel free of the sludge of cells.

It was noted on resuming the drip after freezing that vascular stasis in many channels developed almost immediately, but often such stasis could be overcome by local application of two percent procaine. It was thought the plain saline drip solution used aided the transfer of the liquid component of the blood through the damaged capillary walls, and hence increased the sludging of cells in the vessels.

Repeat Experiments with Procaine Hydrochloride

In this instance, the above trials were repeated

using a Ringer-Locke saline solution modified with gelatin and glucose. A summary of the following results is given in table form after Animal 17.

Animal 11 (Test)

A normal adult male Wistar rat in the 300 gram weight range was anaesthetized with 0.20 cc of 0.6% nembutal solution administered subcutaneously and the preparation was ready within twenty-eight minutes. Procaine two percent was flushed over the mesoappendix and then the cold point was applied with freezing for thirty seconds.

The preparation had to be discarded because it was not sufficiently anaesthetized. The rat clawed its intestines, and rolled off the microscope stage three or four times, with general vascular stasis throughout the mesoappendix, making evaluation impossible.

Animal 12 (Test)

A normal adult male Wistar rat in the 300 gram weight range was anaesthetized with 0.20 cc of 0.6% nembutal solution administered intramuscularly, supposedly. Twenty minutes later 0.10 cc more nembutal was given, and the animal was ready in four minutes. The Ringer-locke gelatine-glucose drip was discontinued and two percent procaine was flushed over the mesoappendix. The cold point was applied with freezing for thirty seconds. Procaine was flushed on immediately after, at which time the vessels were closed tightly and empty of cells. Within sixty seconds of thawing, a few blood

cells were flowing through. Procaine was reapplied and by three minutes of thawing ninety percent of the vessels in the injured area were open and functioning. Restarting the gelatine-glucose drip did not effect any further stasis.

Further tests were performed with this preparation. Thirty seconds after the drip was restarted the injured area was about ninety percent normal with a few small capillaries in stasis. Sixty seconds later two percent procaine was applied. This put the whole area into stasis, which recovered entirely with resumption of the warm drip, but shortly after, stasis redeveloped in the whole injured area. Further procaine gave only a little restoration of circulation which was aided by resuming flow of the drip. This was a very interesting preparation to watch, and it showed that the Ringer-Locke drip containing gelatine and glucose is much more physiological. Note that the procaine was an entirely aqueous solution, and not a saline one.

Animal 13 (Control)

A normal adult male Wistar rat in the 300 gram weight range was anaesthetized with 0.20 cc of 0.6% nembutal solution administered intramuscularly and the preparation was ready in twelve minutes, with the warm drip solution running over the mesoappendix. The cold point was applied to a selected area with freezing for thirty seconds. Within thirty seconds of thawing there was much vascular stasis, and within two and a half min-

utes of thawing the whole injured area was in complete and irreversible stasis except for two or three main large channels.

Animal 14 (Test)

A normal adult male Wistar rat in the 300 gram weight range was anaesthetized with 0.20 cc of 0.6% nembutal solution administered intramuscularly. The preparation was ready in thirty-one minutes, but the animal was discarded because the whole mesentery was in vascular stasis from the animal's kicking.

Animal 15 (Test)

A normal adult male Wistar rat in the 300 gram weight range was anaesthetized with 0.30 cc of 0.6% nembutal solution, and was not ready because the anaesthetic was not deep enough. The intramuscular route was regarded as impractical.

Animal 16 (Test)

A normal adult male Wistar rat in the 300 gram weight range was anaesthetized with 0.20 cc of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready seven minutes later. Procaine was flushed over the mesoappendix after stopping the drip and the cold point was applied to the mesoappendix with freezing for thirty seconds. Thirty seconds after thawing there was complete stoppage of circulation in the injured area. Procaine was applied twice with no effect. When the warm gelatine-glucose saline drip was restarted two minutes after thawing, one half of the static chann-

els opened, and in another minute sixty-five percent were functioning. In one and a half minutes procaine gave stoppage of flow. When the warm drip was restarted fifteen seconds later the whole injured area was flowing one hundred percent, although two small ecchymotic areas were noted. Two minutes later procaine stopped blood flow in the injured area, and resumption of the drip restarted it completely.

Animal 17 (Control)

A normal adult male Wistar rat in the 300 gram weight range was anaesthetized with 0.20 cc of 0.6% nembutal solution administered intraperitoneally, and in eight minutes the preparation was ready with the drip flowing. The cold point was applied with freezing for thirty seconds, and thirty seconds after thawing there was general small capillary stasis throughout the injured area. By four minutes after thawing the stasis was spreading to larger channels, and in another minute the stasis was general and nearly complete. Two and a half minutes later procaine was applied and a very marked recovery occurred, although it was incomplete. There was increased vasomotion, rather than any vasospasm. Three minutes later stasis returned and the preparation wasdiscontinued.

Summary of Results with Procaine

No.		Onset Stasis	Spread	Generalized	Flow Resumed
11	Test	No results because of general stasis			
12	Test	Minimal	Minimal	None	+++
13	Control	30"	Rapid	2½'	None
14	Test	No results because of general stasis			
15	Test	No results because of general stasis			
16	Test	Unusual			+++
17	Control	30"	Immediate	30"	None

Conclusions

These additional results with locally applied procaine are too variable and too few to be of any significance. The value of adding gelatine and glucose to the Ringer-Locke solution was apparent.

In the case of animal 16, some value in immediate warming (or thawing) of the frozen area with warm drip fluid was suggested.

Further Observations with Procaine (Parenteral)

In this run, procaine was used in the rather high dosage of 200 mg per kilogram body weight, administered parenterally. Note that the following tests were performed by a senior medical student who was helping with the project on a part time basis. His methods and observations could not be identical with those used elsewhere. His use of the term "general stasis" is here used to imply stasis throughout the

vessels of the whole mesoappendix, rather than merely in the injured area as is implied elsewhere.

Animal 1 (Test)

A normal adult male Hooded rat weighing 160 grams was injected subcutaneously with 1.6 cc of two percent procaine, and in ten minutes the animal was anaesthetized with 0.1 cc of 0.6% nembutal solution administered by subcutaneous injection. The preparation was mounted on the microscope stage and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds followed immediately by rapid thawing. Stasis began within one minute after thawing, and it spread to involve about one quarter of the capillaries in the injured area.

Animal 2 (Control)

A normal adult female nonpregnant Hooded rat weighing 180 grams was anaesthetized with 0.1 cc of 0.6% nembutal solution by subcutaneous injection. The animal expired before any observations could be made.

Animal 3 (Test)

A normal adult nonpregnant female Hooded rat weighing 170 grams was injected subcutaneously with 1.7 cc of two percent procaine. The animal expired before any observations could be made.

Animal 4 (Test)

A normal adult nonpregnant female Hooded rat weighing 160 grams was injected subcutaneously with 1.6 cc of a two pereent procaine solution. In five

minutes it was anaesthetized with 0.07 cc of 0.6% nembutal solution administered by subcutaneous injection, and when ready the preparation was mounted on the microscope stage. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing through resumption of the warm Ringer-Locke gelatine-glucose drip. Within thirty seconds of thawing stasis was observed, which spread to involve half of the vessels in the injured area in three minutes of thawing. Note that arterioles and metarterioles in the injured area did not develop stasis, but the capillaries and the collecting venules were widely affected. Resumption of flow was seen in one medium sized venule. There was no general (see definition above) stasis in the mesoappendix.

Animal 5 (Control)

A normal adult nonpregnant female Hooded rat weighing 170 grams was anaesthetized with 0.08 cc of 0.6% nembutal solution, and when ready the preparation was mounted on the microscope stage. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing through resumption of the warm drip solution. Stasis developed in forty-five seconds after thawing and it spread to involve nearly all the vessels in the injured area by four minutes of thawing. There was no general stasis noted or resumption of flow.

Animal 6 (Test)

Animal 6 (Test)

A normal adult nonpregnant Hooded female rat weighing 160 grams was injected subcutaneously with 1.6 cc of two percent procaine, and twenty-five minutes later the animal was anaesthetized with 0.10 cc of 0.6% nembutal solution also administered by subcutaneous injection. The preparation was mounted on the microscope and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis developed within three minutes of thawing, and spread to involve about one tenth of the vessels in the injured area. There was no resumption of flow noted or any general stasis.

Animal 7 (Control)

A normal adult nonpregnant Hooded female rat weighing 150 grams was anaesthetized with 0.09 cc of 0.6% nembutal solution administered subcutaneously, and the preparation was mounted on the microscope. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis developed in thirty seconds of thawing, with spread to involve one quarter of the vessels in the injured area in five minutes. There was no general stasis and no resumption of flow noted.

Animal 8 (Test)

A normal adult nonpregnant Hooded female rat weighing 160 grams was injected subcutaneously with 1.6 cc of two percent procaine, and in seventy-five minutes

it was anaesthetized with 0.1 cc of 0.6% nembutal solution administered by subcutaneous injection. The preparation was mounted and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis developed in one minute, spreading to involve seven-eighths of the vessels in the injured area. N o resumption of flow or general stasis was observed.

Animal 9 (Control)

A normal adult nonpregnant Hooded female rat weighing 170 grams was anaesthetized with 0.10 cc of 0.6% nembutal solution administered subcutaneously. The cold point was applied to a selected area of the mesoappendix with freezing, followed by immediate rapid thawing. Stasis began in forty-five seconds after thawing, and spread to involve two thirds of the vessels in the injured area. No general stasis or resumption of flow was observed.

Animal 10 (Test)

A normal adult nonpregnant Hooded female rat weighing 160 grams was injected subcutaneously with 1.6 cc of two percent procaine, and in twenty-five minutes the animal was anaesthetized with 0.09 cc of 0.6% nembutal solution. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Stasis commenced within forty seconds of thawing, spreading to involve four short segments of capillary

in the injured area (a very slight effect).

Animal 11 (Control)

A normal adult nonpregnant Hooded female rat weighing 150 grams was anaesthetized with 0.10 cc of 0.6% nembutal solution administered subcutaneously. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing through resumption of the warm drip. Stasis started in thirty seconds after thawing, and spread to involve nearly all the vessels in the frozen area by three minutes of thawing.

Animal 12 (Test)

A normal adult nonpregnant Hooded female rat weighing 170 grams was injected subcutaneously with 1.7 cc of two percent procaine, and fifty-five minutes later the animal was anaesthetized with 0.10 cc of 0.6% nembutal solution also administered by subcutaneous injection. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Stasis commenced in fifty seconds after thawing, and spread to involve almost half of the vessels in the injured area in approximately five minutes.

Animal 13 (Control)

A normal adult nonpregnant Hooded female rat weighing 160 grams was anaesthetized with 0.10 cc of 0.6% nembutal solution administered subcutaneously. The cold point was applied to a selected area of the

mesoappendix with freezing for thirty seconds followed by immediate rapid thawing through resumption of the warm drip. Stasis commenced in thirty seconds after thawing, spreading to involve three quarters of the vessels in the injured area in three minutes after thawing.

Animal 14 (Control)

A normal adult nonpregnant Hooded female rat weighing 180 grams was anaesthetized with 0.10 cc of 0.6% nembutal solution, and when ready the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing through resumption of the warm drip. Stasis commenced in thirty seconds after thawing, and spread to involve about half the vessels in the injured area in four minutes.

Summary of Results with Parenteral Procaine

No.		Onset Stasis	Spread	Final Extent	Flow Resumed
1	Test	60"	Slow	25%	None
2	Control	Died, no results			
3	Test	Died, no results			
4	Test	30"	Slow	50%	Some
5	Control	45"	Slow	95% in 4'	None
6	Test	180"		10%	None
7	Control	30"		25% in 5'	None
8	Test	60"		7/8 of vessels	None
9	Control	45"		2/3 of vessels	None

Summary Cont'd

No.		Onset Stasis	Spread	Final Extent	Flow Resumed
10	Test	40"		4 capillaries	None
11	Control	30"		Almost all in 3'	None
12	Test	50"		50% in 5'	None
13	Control	30"		75% in 3'	None
14	Control	30"		50% in 4'	None

Impression of Results

"The procaine treated animals seemed to be better protected against stasis produced by freezing injury as performed. However, differences from control observations were slight and it was difficult to say if they are significant." See discussion on page 311.

Priscoline

Experimental observations with Priscoline

This drug was tested parenterally in a dosage of 1.0 mg Priscoline per kilogram body weight. A summary of the following results is given in table form after Animal 9.

Animal 1 (Test)

A normal adult nonpregnant female Wistar rat weighing 170 grams was injected subcutaneously with 0.17 cc of a diluted Priscoline solution containing 0.17 mg., and twenty-four minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution administered by subcutaneous injection. The preparation

was ready in nine minutes and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing stasis started in several venules. A minute later a few venules reopened, and by another minute the reopening of the static venules was rather good. Stasis stopped extending to involve new vessels within three and a half minutes of thawing. A minute later vessels were observed to continue to shut down and restart blood flow, certainly to a far greater extent than any other normal controls so far tested during this project. The preparation was discontinued seven minutes later with no further change noted.

Animal 2 (Test)

A normal adult nonpregnant female Wistar rat weighing 170 grams was injected subcutaneously with 0.17 cc of a diluted Priscoline solution containing 0.17 mg, and sixteen minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution administered by subcutaneous injection, with 0.05 cc more being injected eleven minutes later. The preparation was ready in twelve minutes after the first dose of nembutal and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing with the warm drip solution. Within thirty seconds of thawing there had developed three ecchymotic areas. A minute later stasis was noted

in two capillary beds. In another minute several short venules were static, and within sixty seconds more, stasis was general throughout the whole of the injured area. The animal had gasping respirations. By another four minutes a few vessels had reopened. Two minutes later much of the stasis had been overcome except in the smaller channels. In four minutes the animal was again becomming apnoeic, with slowing of the blood flow and stasis. Four minutes later the animal was hyperpnoeic with restoration of the circulation. The preparation was discarded twenty minutes after thawing, in what was regarded as a Cheyne-Stokes type of respiration.

Animal 3 (Control)

A normal adult nonpregnant female Wistar rat weighing 165 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and the preparation was ready in twenty-six minutes. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds followed by immediate rapid thawing with the warm drip solution. Vascular stasis commenced forty-five seconds after thawing, and in fifteen seconds another capillary bed was involved. By sixty seconds more, several areas were static, and the process slowly spread. In three minutes only the arterio-venous shunts were open, and no resumption of any circulation was noted. The preparation was discontinued eight minutes after thawing.

Animal 4 (Test)

A normal adult nonpregnant female Wistar rat weighing 190 grams was injected subcutaneously with 0.19 cc of a diluted Priscoline solution containing 0.19 mg., and twenty-two minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution administered by subcutaneous injection. The preparation was ready in fifteen and a half minutes, and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis started within thirty seconds of thawing and spread rapidly. By ninety seconds after thawing the stasis became fairly generalized over the injured area. No resumption in blood flow was noted. Within five and a half minutes after thawing no further change was observed, and the preparation was discontinued.

Animal 5 (Control)

A normal adult nonpregnant female Wistar rat weighing 160 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously. Eleven minutes later 0.05 cc were repeated, and the preparation was ready on the microscope stage in four more minutes. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Within thirty seconds stasis started, which spread rapidly. By another two minutes the stasis had become generalized within the injured area. By seven minutes after thaw-

ing no further change was noted, and the preparation was discontinued. No resumption of any circulation was noted.

Animal 6 (Control)

A normal adult nonpregnant female Wistar rat weighing 220 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution and in seven minutes 0.05 cc were repeated. The preparation was ready on the microscope stage twelve minutes later, and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing, stasis developed in two capillary beds, and the process spread during the following sixty seconds. Thirty seconds later several areas throughout the injured area were in vascular stasis. No resumption of circulation was noted, and the preparation was discontinued seven minutes after thawing with no further changes being observed.

Animal 7 (Test)

A normal adult nonpregnant female Wistar rat weighing 170 grams was injected subcutaneously with 0.17 cc of a diluted Priscoline solution containing 0.17 mg., and in nineteen minutes the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution. The preparation was ready in nine minutes, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Capillary stasis

developed within thirty seconds of thawing in two capillary beds. By another ninety seconds several beds had become static. By another two minutes no resumption of any circulation had occurred, and the preparation was discontinued ten minutes after thawing, with no further changes being noted.

Animal 8 (Test)

A normal adult nonpregnant female Wistar rat weighing 160 grams was injected subcutaneously with 0.16 cc of a diluted Priscoline solution containing 0.16 mg., and twenty-eight minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution. The preparation was ready on the microscope stage in twelve minutes, and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds followed by immediate rapid thawing. Vascular stasis started sixty seconds after thawing, and by another sixty seconds three capillary beds and one large venous channel were affected. A minute later the venous channel flushed free. By six more minutes a few more small venules became static, and the preparation was discontinued ten minutes after thawing.

Animal 9 (Control)

A normal adult nonpregnant female Wistar rat weighing 170 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution, administered subcutaneously, and in ten minutes 0.05 cc were repeated. The preparation was mounted and ready on the microscope stage in

six minutes, and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Extensive vascular stasis developed throughout the injured area within thirty seconds of thawing. By another sixty seconds there was still fairly good blood flow, but no restoration of any of the circulation. The animal died while being observed four and a half minutes after thawing. It would seem that such a preparation could not be considered physiological, and the observations are not included in the summary table.

Summary of Results with Priscoline

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	30"	Slight for 3½'	None	+++
2	Test	90"	Rapidly	2½'	+
3	Control	45"	Slowly	3'	None
4	Test	30"	Rapidly	90"	None
5	Control	30"	Rapidly	2½'	None
6	Control	30"	Rapidly	2'	None
7	Test	30"	Slowly	2'	None
8	Test	60"	Slowly	None	+
9	Control	30"	Rapidly	30"	None (Physiological State Unlikely)

Comments

The average time with the test animals for stasis to occur after rapid thawing was 48", whereas the same value with the controls was 34". There was little difference between the test runs and the controls in

the length of time it took for generalized vascular stasis to develop in the injured area. Resumption of circulation after stasis had developed occurred only with the test animals, but not consistently so. It would seem that Priscoline was of no significant value in delaying the onset of vascular stasis, or in generally protecting the vascular bed.

Further Observations with Priscoline

In the following tests, Priscoline was tested parenterally in a dosage of 2.0 mg per kilogram body weight. A summary of the following results is given in table form after Animal 14. Note that these tests were performed by a senior medical student who was helping with the project on a part time basis. His methods and observations could not be identical with those used elsewhere. His use of the term "general stasis" is here used to imply stasis throughout the vessels of the whole mesoappendix, rather than merely within the injured area as is implied elsewhere.

Fourteen animals were employed, all being normal adult male Wistar rats in the 200 gram weight range. When ready after anaesthetizing with a stated amount of 0.6% nembutal solution, the animal was mounted on the microscope stage, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing effected by resumption of the warm Ringer-Locke gelatin-

glucose drip solution. The reactions of the circulatory channels were noted as outlined below:

Animal 1 (Test)

Weight of animal was 190 grams. It was injected subcutaneously with 0.2 cc of a diluted Priscoline solution containing 0.4 mg. Thirteen minutes later 0.2 cc of nembutal were injected subcutaneously, but the animal expired before any testing could be done.

Animal 2 (Test)

An animal weighing 200 grams was injected subcutaneously with 0.2 cc of a diluted Priscoline solution containing 0.4 mg., and eight minutes later it was anaesthetized with 0.2 cc of nembutal. Vascular stasis started sixty seconds after the thawing, which spread to involve five or six other capillary fields within four minutes. There was no general stasis or resumption of circulation noted.

Animal 3 (Control)

An animal weighing 200 grams was anaesthetized with 0.20 cc of nembutal injected subcutaneously. Stasis started in thirty seconds after thawing, spreading to involve three quarters of the capillaries in the injured field in five minutes. There was no general stasis or resumption of flow noted in any vessel.

Animal 4 (Test)

An animal weighing 190 grams was injected subcutaneously with 0.20 cc of a diluted Priscoline solution containing 0.4 mg., and ten minutes later it was anaesth-

etized with 0.20 cc of nembutal. Stasis began ninety seconds after thawing, with spread to involve half the capillaries in the injured area. There was no general stasis or resumption of circulation.

Animal 5 (Control)

An animal weighing 190 grams was anaesthetized with 0.20 cc of nembutal. Stasis developed within twenty seconds after thawing, spreading to involve three quarters of the capillaries in the frozen area in four minutes. There was no general stasis, and no resumption of flow noted.

Animal 6 (Test)

An animal weighing 200 grams was injected subcutaneously with 0.20 cc of a diluted solution of Priscoline containing 0.4 mg., and twenty minutes later it was anaesthetized with 0.20 cc of nembutal. No stasis was observed for two minutes following thawing, at which time two small capillaries became static. There was no spread of the stasis, no generalized stasis, and no resumption of circulation in the affected channels.

Animal 7 (Control)

An animal weighing approximately 200 grams was anaesthetized with 0.20 cc of nembutal, but it expired before it could be mounted on the microscope stage.

Animal 8 (Test)

An animal weighing 210 grams was injected subcutaneously with 0.20 cc of a diluted Priscoline solution

containing 0.4 mg., and twelve minutes later the animal was anaesthetized with 0.20 cc of nembutal also administered subcutaneously. Vascular stasis developed in ninety seconds after thawing, to involve about one quarter of the capillaries in the injured area. No general stasis and no resumption of circulation was noted.

Animal 9 (Control)

An animal weighing 210 grams was anaesthetized with 0.17 cc of nembutal administered by subcutaneous injection, and twenty minutes later 0.07 cc were repeated. Stasis started thirty seconds after the thawing and spread to involve half of the vessels in the injured area. No general stasis or resumption of any circulation was noted.

Animal 10 (Test)

An animal weighing 200 grams was injected subcutaneously with 0.20 cc of a diluted solution of Priscoline containing 0.4 mg., and twenty minutes later it was anaesthetized with 0.22 cc of nembutal. Stasis developed in thirty seconds, and spread to involve one third of the capillaries in the injured area. There was no general stasis. Some of the vessels began to go into stasis, the cells clumped together, and the circulation slowed, and then stopped for a few seconds, and then resumed flow.

Animal 11 (Control)

An animal weighing 200 grams was anaesthetized

with 0.20 cc of nembutal administered subcutaneously. Stasis developed in thirty seconds, spreading to involve almost all the capillaries in the frozen area.

Animal 12 (Test)

An animal weighing 200 grams was injected subcutaneously with 0.20 cc of a diluted solution containing 0.4 mg. of Priscoline, and fifteen minutes later it was anaesthetized with 0.20 cc of nembutal also administered by subcutaneous injection. Stasis started within thirty seconds of thawing, and spread to involve three quarters of the vessels in the injured area.

Animal 13 (Control)

An animal weighing 190 grams was anaesthetized with 0.20 cc of nembutal. Stasis started in forty-five seconds after thawing, spreading to involve nine tenths of the vessels in the injured area. Two petechial hemorrhages were produced also. No general stasis developed, or any resumption in circulation.

Animal 14 (Control)

An animal weighing 190 grams was anaesthetized with 0.20 cc of nembutal administered by subcutaneous injection. Stasis started after forty-five seconds after thawing, spreading to involve three quarters of the vessels in the injured area. No general stasis or any resumption in the circulation was noted.

Summary of Results with Priscoline

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	Died during observation - no results			
2	Test	60"	5-6 beds in 4'	None	None
3	Control	30"	75% in 5'	None	None
4	Test	90"	50% of caps	None	None
5	Control	30"	75% in 4'	None	None
6	Test	120"	2 small caps only	None	None
7	Control	Died during observation - no results			
8	Test	90"	25% of caps	None	None
9	Control	30"	50% of vessels	None	None
10	Test	30"	1/3 of caps	None	None
11	Control	30"	Rapid	General	None
12	Test	30"	75% of vessels	None	None
13	Control	45"	90% of vessels	None	None
14	Control	45"	75% of vessels	None	None

Comments on Observations

The onset of stasis after thawing in the control animals occurred on the average 35" later, whereas in the test runs, Priscoline appeared to delay the stasis to 70", just twice as long. Also the extent of the stasis was consistently less in the Priscoline treated animals than it was in the controls. To quote the observer of these tests, "Priscoline as tested resulted in some delaying in the onset of stasis, and in some lessening in its extent. No resumption of flow in the affected vessels was noted. However, results are not regarded as being definite enough or consistent enough to

be really significant."

Benadryl

Experimental observations with Benadryl

Benadryl was tested at a dosage level of 0.50 mg. per kilogram body weight. 10 cc Steri-vials of Benadryl (10 mg. per cc) were used, and a 1:100 dilution was prepared by placing 0.2 cc. into 19.8 cc of water. In this way, volume of dose per animal ranged about a 0.5 cc amount which would be of reasonable size for absorption from a subcutaneous injection. The pH of the Ringer-Locke gelatin-glucose drip solution was adjusted before starting to a value of 7.0.

Animal 1 (Control)

A normal adult nonpregnant female Wistar rat weighing 130 grams was anaesthetized with 0.1 cc of 0.6% nembutal solution administered intramuscularly, and in eight minutes a further 0.05 cc were injected. Seven minutes later the preparation was mounted on the microscope stage and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within forty seconds after thawing vascular stasis appeared and spread somewhat, but the preparation was not a very good one, and further observations were not recorded.

Animal 2 (Test)

A normal adult nonpregnant female Wistar rat weighing 110 grams was injected subcutaneously with 0.55 cc of a Benadryl solution containing 0.055 mg.,

and twenty-seven minutes later the animal was anaesthetized with 0.1 cc of 0.6% nembutal solution administered intramuscularly. In seven minutes the preparation was ready and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds after thawing stasis was noted in one capillary bed. By two minutes after thawing four capillary beds were involved. Slight ecchymosis was noted. In another minute and a half one large venous channel opened and resumed circulation. In another minute only four capillary beds were closed off, and circulation in the injured area was excellent. After another three minutes conditions remained unchanged, and the blood flow in the rest of the mesoappendix was excellent. The animal was rather small, and lightly anaesthetized, making observation difficult, but the field for testing was a good one. The preparation was discontinued nine and a half minutes after thawing.

Animal 3 (Test)

A normal adult nonpregnant female Wistar rat weighing 125 grams was injected subcutaneously with 0.65 cc of a solution containing 0.065 mg. of Benadryl. Thirty-eight minutes later the animal was anaesthetized with 0.1 cc of 0.6% nembutal solution administered subcutaneously, and in sixteen minutes the preparation was mounted and ready on the microscope stage. The cold tip was applied to a selected area of the mesoappendix

with freezing for thirty seconds. Within thirty seconds after rapid thawing, stasis was observed in a couple of the larger venules, but by another sixty seconds the flow in these was resumed with stasis in three capillary beds remaining. By another sixty seconds four capillary beds were in stasis. By four minutes after thawing the one large channel had been opening and closing intermittently for the previous two minutes. In two minutes more, the blood in the other large channel was free flowing. A minute later two of the capillary beds reopened and flowed normally. While admittedly there were a couple small capillary beds and a few short pieces of venous channels closed off, this area was enormously better than could be expected without prior treatment with Benadryl. One had the impression of the cohesive properties of the blood cells, and especially their adhesive tendencies to stick to the injured vein wall, being greatly reduced by Benadryl. By fourteen minutes after thawing the situation was essentially unchanged. Normal vasomotor tone was present, and the preparation was discontinued.

Animal 4 (Control)

A normal adult nonpregnant female Wistar rat weighing 120 grams was anaesthetized with 0.1 cc of 0.6% nembutal solution administered intramuscularly, and 0.05 cc administered subcutaneously. In nine

minutes the preparation was mounted and ready on the microscope stage. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. By thirty seconds after rapid thawing there was a very slight amount of ecchymosis at one site in the injured area. Stasis started sixty seconds after thawing and spread rapidly to involve three large capillary beds. After two more minutes no channels had reopened, stasis steadily progressing toward the venous side of the circulation to involve the large venules. The injury inflicted on this preparation was regarded as quite minimal in comparison with the other runs. By five minutes after thawing, vasomotion was observed in larger venous channels with intermittent stasis, and a minute later one large venous channel, that was in stasis quite continuously, reopened and flowed apparently normally. Three minutes later the preparation was discontinued with no further changes noted.

Animal 5 (Test)

A normal adult nonpregnant female Wistar rat weighing 140 grams was injected subcutaneously with 0.7 cc of a solution containing 0.07 mg. of Benadryl. Twnety-one minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and thirteen minutes later the preparation was ready on the microscope stage. The cold tip was applied to a selected area of the mesoappendix with

freezing for thirty seconds, and rapidly thawed with warm drip solution. Thirty seconds after thawing there was much capillary stasis present, and venous obstruction in a fairly large circumscribed area within the injured field. After another five and a half minutes the situation was unchanged. Actually these observations should be discounted because the whole mesoappendix was more or less in vascular stasis.

Animal 6 (Control)

A normal adult nonpregnant female Wistar rat weighing 130 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and the preparation was ready eleven minutes later. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds with immediate rapid thawing afterward. Within thirty seconds of thawing, capillary stasis was observed which spread rapidly and in another thirty seconds fifty percent of the vessels in the injured area were involved. By another minute no revascularization was observed in the static channels. Five minutes after this the preparation was discontinued. A few arterio-venous channels were flowing well, but generally the injured area was unchanged. Ninety percent of the vessels in the injured area were in stasis when the preparation was discontinued.

Animal 7 (Test)

A normal adult nonpregnant female Wistar rat weighing 120 grams was injected subcutaneously with 0.6 cc of a solution containing 0.06 mg. of Benadryl. Thirty-three minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and within nine minutes the preparation was ready for testing. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds and thawed rapidly immediately afterward. Within thirty seconds of thawing three capillary beds had closed off. In another thirty seconds one large venous channel became static, but opened up again five minutes later. Nine minutes after thawing one of the static capillary beds opened up. There was no further change in two more minutes observation and the preparation was discontinued.

Animal 8 (Control)

A normal adult nonpregnant female Wistar rat weighing 120 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and the preparation was ready for testing in twenty minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of radpid thawing, capillary and venous stasis was starting, and it spread rapidly to close off approximately one half of the circulation in

another thirty seconds. By another minute only two arterio-venous shunts were flowing in the injured area. In another two and a half minutes no further change in the circulation was noted, and the blood flow in the rest of the mesoappendix was normal. The preparation was discontinued six and a half minutes after thawing.

Animal 9 (Test)

A normal adult nonpregnant female Wistar rat weighing 115 grams was injected subcutaneously with 0.575 cc o f a solution containing 0.0575 mg. o f Benadryl, and forty-two minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously. The preparation was ready in eight minutes and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Hyperemia was noted thirty seconds after rapid thawing, and in another thirty seconds vascular stasis in the injured area spread to involve two or three capillary beds. After another six minutes no further change was noted. Active vasomotion was apparent in all channels that were not static. No channels appeared to reopen, the static ones being of the order of capillaries and prevenules, forming approximately forty percent of the vessels within the injured area. The circulation in the rest of the mesoappendix was normal. By nine minutes following thawing, one capillary bed reopened, and another bed did likewise a minute later. The preparation was

discontinued four minutes later.

Summary of Observations with Benadryl (0.5 mg/Kg)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Control	40"	Yes	Incomplete	None
2	Test	30"	Yes	None	++
3	Test	30"	Yes	None	+++
4	Control	30-60"	Rapid	Incomplete	?
5	Test	No results - Whole mesoappendix in stasis			
6	Control	30"	Rapid	Almost	None
7	Test	30"	3 Cap beds	None	+++
8	Control	30"	Rapid	Not A-V shunts	None
9	Test	60"	3 Cap beds	None	+++

Discussion of Observations

Benadryl did not appear to have any great effect on the time required for vascular stasis to develop as compared with control animals, but it appeared to limit the spread of the stasis. There was also evidence of increased resumption of flow in the static vessels. However, there are only four test and four control animals. These numbers are far too few for really significant conclusions to be drawn. Further testing with Benadryl is indicated, especially with a higher dosage level.

Active vasomotion was found to be a prominent feature in the re-opened vessels in the injured area, indicating that the freezing injury was unduly mild or

that the antihistaminic action on the endothelial cells must have been effectual to a certain degree.

Control animal number four requires some comment in that the reopening of channels, as marked "?", meaning doubtful, might be explained by the fact that without doubt the injury by freezing to this preparation was really minimal both as regards to duration and extent.

In spite of such explanation, and such apparent good results, it would not seem justifiable to credit the antihistamine used with such great effect unless the tests were repeated.

Further Experimental Observations with Benadryl

In the following tests with Benadryl, 1.0 mg. per kilogram body weight was the dosage level used. This did not appear to have any adverse effect on the animals.

Animal 1 (Test)

A normal adult nonpregnant female Wistar rat weighing 150 grams was injected subcutaneously with 0.75 cc of a solution containing 1.15 mg. of Benadryl, and thirty-five minutes later the animal was anaesthetized with 0.75 cc of 0.6% nembutal solution administered subcutaneously. Seven minutes later 0.05 cc and five minutes still later 0.025 cc of nembutal were injected also subcutaneously. The preparation was ready two and a half minutes after the last nembutal injection, and the cold point was applied to a selected area of the

mesoappendix with freezing for thirty seconds. Except for one static capillary bed not near the injured area, no vascular stasis developed within eight and a half minutes of thawing. By eleven and a half minutes of thawing, this capillary bed was open and flowing. It is again to be noted that this particular capillary bed was well outside the limits of the freezing injury. By fourteen and a half minutes of thawing there was no further change, and the preparation was discontinued. The effect of the Benadryl, if any, on the stasis in this isolated vessel cannot be explained.

Animal 2 (Control)

A normal adult nonpregnant female Wistar rat weighing 150 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution, and in twenty minutes a further 0.05 cc were injected, both being administered subcutaneously. The preparation was ready within another ten minutes, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. There was stasis within thirty seconds of thawing which spread rapidly. By another minute the stasis was still spreading, and within one and a half minutes of thawing, only the main arterio-venous shunts were flowing within the injured area, although the circulation in the rest of the mesoappendix was apparently normal. The preparation was discontinued three minutes later with no apparent change being noted.

There was no resumption of blood flow noted in any static channel.

Animal 3 (Test)

A normal adult nonpregnant female Wistar rat weighing 150 grams was injected subcutaneously with 0.75 cc of a solution containing 115 mg. of Benadryl, and within thirty-three minutes the animal was anaesthetized with 0.125 cc of 0.6% nembutal solution administered subcutaneously, a further 0.04 cc being injected within eight minutes. The preparation was ready in two more minutes, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. In one and a half minutes after rapid thawing there was noted the start of a quickly spreading stasis throughout the injured area. By three and a half minutes of thawing, one of the static vessels flushed clear, but shut down again. In three minutes more, another channel flushed clear, and seven minutes still later another channel flushed clear in a different part of the injured area. No further extension of any stasis was observed. By twenty-one and a half minutes after thawing little further change was noted and the preparation was discontinued.

Animal 4 (Control)

A normal adult nonpregnant female Wistar rat weighing 120 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution and in eight minutes another

0.025 cc were injected also subcutaneously. Within four minutes the preparation was ready, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing there was stasis which spread rapidly throughout the injured area. There was still further spread noted by another two minutes. In three minutes more, one of the main static venous channels opened and resumed circulation apparently normally. By another three minutes there was no further change noted, and the preparation was discontinued ten and a half minutes after thawing, at which time it was noted another small venous channel had reopened.

Animal 5 (Test)

A normal adult nonpregnant female Wistar rat weighing 150 grams was injected subcutaneously with 0.75 cc of a diluted solution containing 15 mg. of Benadryl, and thirty-one minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, with 0.04 cc more being injected ten minutes later. The preparation was ready two minutes after the second nembutal injection and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of rapid thawing, stasis in one venous channel was noted which backed up into the injured area. In another

minute a second venous channel acted similarly, and the same with a third within the next minute. By four and a half minutes after thawing the whole injured area was practically in stasis. No channels were observed to reopen. The preparation was discontinued.

Animal 6 (Control)

A normal adult nonpregnant female Wistar rat weighing 140 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and the preparation was ready within twelve minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, and then rapidly thawed. Stasis was observed to start within thirty seconds of thawing, and it spread slowly so that by six and a half minutes after thawing only three venules and capillaries were observed to be in stasis. In all probability the freezing injury was too slight. By another minute there was no further change noted and the preparation was discontinued.

Animal 7 (Test)

A normal adult nonpregnant female Wistar rat weighing 135 grams was injected subcutaneously with 0.65 cc of a diluted solution containing 0.13 mg. of Benadryl, and in twenty-four minutes the animal was anaesthetized with 0.10 cc of 0.6% nembutal solution injected subcutaneously, with 0.025 cc being repeated in seven minutes. In another five minutes the preparation was ready and the cold point was applied with

freezing for thirty seconds to a selected area of the mesoappendix. Within thirty seconds of thawing, beginning capillary stasis was observed which in another thirty seconds had become rather general except for large arteriovenous channels. By another five minutes many smaller channels reopened, but not much change took place in the next three minutes. Twenty-six minutes after thawing one large venous channel that appeared to be in irreversible stasis opened up and resumed apparently normal circulation. The preparation was discontinued in another eight minutes with little further change except for some general extension of the stasis within the injured area of the mesoappendix.

Animal 8 (Test)

A normal adult nonpregnant female Wistar rat weighing 140 grams was injected subcutaneously with 0.70 cc of a diluted solution containing 0.14 mg. of Benadryl and in forty-seven minutes the animal was anaesthetized with 0.125 cc of 0.6% nembutal solution administered subcutaneously. The preparation was ready in another twenty minutes and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Stasis first appeared three and a half minutes after thawing, and in only three small capillaries. In three minutes more, one of the capillaries

opened up with resumption of apparently normal circulation. By another four minutes the stasis spread to involve two capillaries connecting adjacent arteriovenous shunts. The preparation was discontinued one minute later with no further change being noted.

Animal 9 (Control)

A normal adult nonpregnant female Wistar rat weighing 140 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, with 0.025 cc. being repeated in eleven minutes. The preparation was ready in another three minutes and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing stasis had started, and it spread rapidly. By another six minutes a few large vessels were observed to be slowing, but really little further change was noted. The preparation was discontinued six and a half minutes after thawing.

Animal 10 (Control)

A normal adult nonpregnant female Wistar rat weighing 160 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and was ready on the microscope stage within twenty-three minutes. The field was somewhat obscured by fat in the mesoappendix, but enough of the circulation was visable to warrant continuing with the preparation. The cold tip was applied to a selected area of the

mesoappendix with freezing for thirty seconds, and rapidly developing stasis started within thirty seconds of thawing. No restoration of flow was observed in any of the vessels, and the preparation was discontinued five and a half minutes after thawing.

Animal 11 (Test)

A normal adult nonpregnant female Wistar rat weighing 135 grams was injected subcutaneously with 0.65 cc. of a diluted solution containing 0.13 mg. of Benadryl, and in thirty-five minutes the animal was anaesthetized with 0.125 cc of 0.6% nembutal solution administered subcutaneously. The preparation was ready in eleven minutes and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed immediately afterward with rapid thawing. In thirty seconds after thawing there was observed beginning stasis of the circulation in small vessels which spread rapidly to become more or less general in the injured area within four and a half minutes of thawing. No vessels re-opened to restore circulation, and the preparation was discontinued two minutes later.

Summary of Observations with Benadryl (1.0 mg./Kg)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	None	None	None	Yes ?
2	Control	30"	Rapidly	1½'	None

Summary (Cont'd)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
3	Test	1½'	Rapidly	None	++++
4	Control	30"	Rapidly	2½'	+
5	Test	30"	Rapidly	4½"	None
6	Control	30"	Slowly	None	None
7	Test	30"	Rapidly	60"	++
8	Test	3½'	None	None	++
9	Control	30"	Rapidly	None	None
10	Control	30"	Rapidly	60"	None
11	Test	30"	Rapidly	4½'	None

Discussion of Observations

Benadryl injected to the extent of 1.0 mg. per kilogram body weight had no apparent adverse effects on the rats tested.

From the somewhat arbitrary values in the above summary table, the beneficial effects of Benadryl in the test animals were inconsistent, and they conflicted with equally inconsistent values in the controls.

The particular vascular area chosen for injury by application of the freezing point, as well as the exact duration of the freezing are rather uncontrollable variables entering into consideration of the significance of these results. Possible repetitive tests on two or more areas of the same mesoappendix would be an improvement, although the time factor and the viability

of the preparation would be big obstacles.

It would seem that Benadryl in the dosage used had no significant influence beyond the errors inherent in the experiment. This conclusion agrees with that obtained above when the dosage of 0.5 mg. per Kilogram was tested.

Further Experimental Observations with Benadryl

The following tests were performed to determine the influence of Benadryl, in a dosage level of 1.5 mg. per kilogram of body weight, administered by subcutaneous injection one half hour before the observation of a freezing injury on the mesoappendix. The results are summarized in table form after animal 10.

Animal 1 (Test)

A normal adult nonpregnant female Wistar rat weighing 130 grams was injected subcutaneously with 0.39 cc of a diluted solution containing 0.195 mg. of Benadryl, and twenty-nine minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution. The preparation was ready in twelve minutes, and the cold point was applied to a selected area of the mesoappendix with freezing injury for thirty seconds. Within sixty seconds of thawing, a slight vascular stasis was starting, really just a slowing in the blood flow. By another minute it was spreading. One channel was observed to open and resume

circulation. By another two minutes there was no further spread. This is an excellent area of the mesoappendix with many small capillary channels and the fact that the stasis was so limited, together with a fairly strong freezing injury certainly makes the observed facts significant. After another two minutes one large connecting vein was in stasis, and by eight minutes after thawing some stasis was apparent in other parts of the mesoappendix, so that the preparation was discontinued.

Animal 2 (Control)

A normal adult nonpregnant female Wistar rat weighing 140 grams was anaesthetized with 0.15 cc of 0.6% nembutal solution administered subcutaneously, and the preparation was ready on the microscope stage in seventeen minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis started within thirty seconds of thawing, and spread rapidly. By three minutes after thawing the stasis was slowly extending but was by no means becoming generalized. Some ecchymoses were present. Two minutes later, except for the arterio-venous channels, stasis was generalized, and no reopening of the channels was noted. Four minutes later the preparation was discontinued.

Animal 3 (Test)

A normal adult nonpregnant female Wistar rat

weighing 130 grams was injected subcutaneously with 0.39 cc. of a diluted solution containing 0.195 mg. of Benadryl, and forty-five minutes later the animal was anaesthetized with 0.15 cc. of 0.6% nembutal solution administered subcutaneously. The preparation was ready fifteen minutes later and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. There was no stasis within ninety seconds of thawing, but in a minute more, stasis started in one small venous channel only. Two minutes later this static venous channel opened for half its length, and then was flushed free by a connecting arteriole-capillary tributary. After another two minutes there was no further change. The circulatory system in the whole injured area was functioning well. Five minutes later the preparation was discontinued with no further changes being noted.

Animal 4 (Control)

A normal adult nonpregnant female Wistar rat weighing 135 grams was anaesthetized with 0.15 cc. of 0.6% nembutal solution, administered by subcutaneous injection, and the animal was mounted and ready on the microscope stage in eleven minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. There was stasis

starting within thirty seconds which spread rapidly. Two and a half minutes later the stasis was still spreading, but not yet generalized. In two more minutes the stasis had become rather generalized through out the injured area, and no revascularization of any channels was noticed. The preparation was discontinued three minutes later with no further changes observed.

Animal 5 (Test)

A normal adult nonpregnant female Wistar rat weighing 135 grams was injected subcutaneously with 0.39 cc. of a diluted solution containing 0.195 mg. of Benadryl, and thirty-eight minutes later the animal was anaesthetized with 0.15 cc. of 0.6% nembutal solution administered subcutaneously. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis occurred in several venous channels in the injured area within sixty seconds after thawing. In another thirty seconds there was no further spread in the stasis, although a good sixty percent of the vessels in the injured area were affected. In three more minutes no resumption of circulation in any static vessel was observed, and the preparation was discontinued two minutes later.

Animal 6 (Control)

A normal adult nonpregnant female Wistar rat

weighing 170 grams was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered by subcutaneous injection, and the preparation was ready in thirteen minutes. The cold point was applied three minutes later to a selected area of the mesoappendix with freezing for thirty seconds. Stasis developed within thirty seconds of thawing and spread rapidly. By another thirty seconds, several ecchymotic areas had developed. By ninety seconds after thawing, stasis had become rather generalized except in the large shunt channels. By two minutes later, no resumption of circulation had been noted, and the stasis was still extending. The preparation was discontinued four minutes later.

Animal 7 (Test)

A normal adult nonpregnant female Wistar rat weighing 140 grams was injected subcutaneously with 0.42 cc. of a diluted solution containing 0.21 mg. of Benadryl, and twenty-eight minutes later the animal was anaesthetized with 0.15 cc of 0.6% nembutal solution, administered by subcutaneous injection. The preparation was ready in nine minutes, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of rapid thawing, one small capillary bed was in stasis. By another two minutes the stasis had spread slowly to involve several other capillary beds. In three more minutes there was no

further involvement. No resumption of circulation was noted, and although this area was very similar to the subsequent control prparation, there was much less stasis. By three more minutes, the preparation was discontinued, with no further change. There was no apparent difference from the following control run.

Animal 8 (Control)

A normal adult nonpregnant female Wistar rat weighing 120 grams was anaesthetized with 0.15 cc. of 0.6% nembutal solution, administered subcutaneously, and the preparation was ready in thirteen minutes. The cold point was applied to a selected area of the mesoappendix with freezing, followed by rapid thawing. Within thirty seconds of thawing stasis started which spread rapidly, with the development also of a few ecchymoses. Two and a half minutes later there were no further changes in the smaller static vessels. It was interesting to observe the events in this preparation. Some of the larger channels would fill up with concentrated cellular sludge, and then repeatedly flush free. By five more minutes no further changes had occurred. The area of stasis was quite complete within the injured field, being a patchy involvement of the smaller venules. Two minutes later the preparation was discontinued, and no reopening of the static vessels was noted.

Animal 9 (Test)

A normal adult nonpregnant female Wistar rat weighing 130 grams was injected subcutaneously with 0.39 cc of a diluted Benadryl solution containing 0.195 mg., and thirty-one minutes later the animal was anaesthetized with 0.15 cc. of 0.6% nembutal solution administered by subcutaneous injection. The preparation was ready in sixteen minutes, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. There was no stasis present by thirty seconds after rapid thawing, and after another minute, stasis formed in one venule only. A minute later a second capillary bed became static. Two minutes later there were only the two capillary beds static, and the rest of the circulation within the injured area appeared normal. Two and a half minutes later no further change developed, with no resumption of circulation being noted; and the preparation was discontinued.

Animal 10 (Control)

A normal adult nonpregnant female Wistar rat weighing 130 grams was anaesthetized with 0.15 cc. of 0.6% nembutal solution, administered subcutaneously, and the preparation was ready in eight minutes. The cold point was applied to a selected area of the mesoappendix with freezing, followed immediately by rapid thawing. Within thirty seconds of thawing

there was rapidly spreading stasis, which became generalized throughout the injured area within another minute. No recanalization was noted, and the preparation was discontinued three minutes later.

Summary of Observations with Benadryl (1.5 mg./Kg)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	60"	Slowly	None	Slight
2	Control	30"	Slowly	5'	None
3	Test	None	None	None	
4	Control	30"	Rapidly	3'	None
5	Test	60"	Slowly	60% in 90"	None
6	Control	30"	Rapidly	90"	None
7	Test	30"	Slowly	Incomplete	None
8	Control	30"	Rapidly	Incomplete	None
9	Test	90"	Slowly	2 Cap Beds	None
10	Control	30"	Rapidly	90"	None

Discussion of Observations

Not considering the test animal, number three, which did not develop stasis, the average time taken for the test preparations to develop stasis was sixty seconds, just twice as long as for the controls, which took thirty. Further, the stasis spread more slowly and was much less complete than in the case of the controls. There was no difference as regards any resumption of flow.

These considerations would not appear to shift

the decision more favourably towards Benadryl having a protective effect on the mesoappendix circulation against a standard freezing injury.

Etamon

Experimental observations with Etamon Chloride

The following trials were for the purpose of testing the effect of Etamon chloride (Tetra-ethyl ammonium chloride) in a dosage level of 25 mg. per kilogram body weight, on the capillary beds of the rat mesoappendix after a standard freezing injury. Normal adult male Wistar rats were used, four test animals, averaging 310 grams, and four controls, averaging 280 grams. The procedure was as before, using Ringer-Locke gelatine-glucose solution. The pH was adjusted to the first change to pink using sodium bicarbonate and methyl red indicator. The Etamon solution was administered to all test animals at one time, using 0.10 cc. (0.1 gm./cc), providing 10 mg. per animal, which is slightly larger than 25 mg. per kilogram. The drug was administered subcutaneously. A summary of the following observations is given in table form after the second Animal 8.

Animal 1 (Control)

0.20 cc. of 0.6% nembutal solution was administered intraperitoneally for anaesthesia, and in

seven minutes the preparation was ready, mounted on the microscope stage with the drip flowing. One and a half minutes later a selected area of the mesoappendix was frozen with the cold point for thirty seconds. Thirty seconds after thawing there was noted a very marked hyperemia, and then the flow of the drip solution was resumed. Within another thirty seconds, general vascular stasis had developed, which was somewhat incomplete. In ninety seconds more, some clearing, but only temporary, was noted in a few of the larger channels. One minute later no further change was observed. A main central arteriole was flowing uninterruptedly, and the vascular stasis seemed to be entirely on the venous side of the circulation. The preparation was discontinued two and a half minutes later.

Animal 2 (Test)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution one hour and fourteen minutes after the Etamon had been administered. The preparation was ready eight minutes after anaesthetizing. A state of hyperemia was noted in the vessels of the mesoappendix. Three minutes later the cold tip was applied with freezing for thirty seconds, with rapid thawing and resumption of flow of the warm drip solution. Within sixty seconds of thawing stasis was observed to be commencing. By a minute

and a half more, the condition of the circulation in the injured area was found to be much better than with the previous control animal, but considering the varying factors, the difference was not great enough to be truly significant unless present consistently in all the test and control animals. By four and a half minutes after thawing, the stasis had spread somewhat, but there was still very good circulation throughout the injured area. Two minutes later a main venous channel which had been static was noted to be open. Good vasomotion was also noted throughout the mesoappendix in spite of the Etamon (dose too small?). A minute later many smaller static channels resumed flow, and two minutes still later, eighty percent of the channels once static were open and functioning well. In three minutes (twelve and a half minutes after thawing), generalized vascular stasis was present in the injured area. Two minutes later no further change occurred, and the preparation was discontinued.

Animal 3 (Control)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered intraperitoneally, and in seventeen minutes the preparation was ready. The cold tip was applied with freezing for thirty seconds. By thirty seconds after rapid thawing not much hyperemia occurred, but after sixty seconds,

vascular stasis started. In sixty seconds more, this stasis was spreading rapidly, and by two minutes there was a slow insidious spread to all vessels with no more vasomotion observed. The preparation was discontinued one minute later.

Animal 4 (Test)

The animal was anaesthetized with 0.20 cc of 0.6% nembutal solution two hours and seven minutes after the Etamon had been administered. Within six minutes the preparation was ready, and moderate hyperemia was noted. The cold tip was applied with freezing for thirty seconds, and then immediate rapid thawing was achieved as before with resumption of the warm saline drip. A very beautiful capillary bed was available for observation. Stasis first appeared ninety seconds after thawing. By another minute stasis spread over a large venous area. By eight minutes further, no vessels reopened or resumed any circulation. Two minutes later a main vascular channel reopened, and within three more minutes it showed active vasomotion. No further change was noted for ten minutes. Part of the venous area did not resume flow. The preparation was discontinued.

Animal 5 (Control)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered intraperitoneally,

and the preparation was ready in ten minutes. The cold tip was applied with freezing for thirty seconds, and stasis was observed to commence within thirty seconds of thawing. By another sixty seconds the stasis was complete and irreversible, and the preparation was discarded three minutes later with no further change observed.

Animal 6 (Test)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered intraperitoneally three hours and seven minutes after the Etamon was injected. The preparation was ready in five minutes, and excellent vasomotion was noted. The cold point was applied with freezing for thirty seconds. By thirty seconds after freezing good blood flow through the injured area was still present. Slight and temporary vascular stasis was noted one minute later, and in another minute permanent stasis was noted in a few small capillaries. By another four minutes the situation was much the same, with some slowing of the flow in one venous channel. Three minutes later 2% procaine at room temperature was instilled over the mesoappendix with stoppage of the whole circulation. The preparation was discarded five minutes later (fourteen and a half minutes after thawing).

Animal 7 (Control)

The animal was anaesthetized with 0.20 cc. of

0.6% nembutal solution and the preparation was ready in nine minutes. The cold point was applied with freezing for thirty seconds. Within thirty seconds of thawing great hyperemia was noted, with vascular stasis developing and spreading rapidly. Within another minute, thirty percent of the injured area was in stasis, which did not increase by another three minutes. Three minutes later larger vessels were slowing, and by fourteen and a half minutes after thawing, over half the vessels in the injured area were in stasis. The preparation was discontinued four minutes later.

Animal 8 (Test)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered intraperitoneally four hours and five minutes after the Etamon had been given. The preparation was ready in nine minutes, and the cold point was applied with freezing for thirty seconds. Stasis started three minutes after thawing, and four minutes later was found to be complete in some parts of the injured area, but temporary in others. The preparation was discontinued one minute later.

(Summary table will be found after the following series of repeat tests)

Repeat Experiments with Etamon Chloride

The following is a repetition of the above run,

but using 25 mg. of Etamon (tetra-ethyl ammonium chloride) per test animal, administered subcutaneously. The test animals averaged 315 grams, the controls 290 grams, and all were normal adult male Wistar rats. The heaviest of the test animals, weighing 355 grams, died fifteen minutes after its injection of Etamon, and another similar male rat weighing 330 grams was substituted, being given 25 mg. of Etamon also subcutaneously.

Animal 1 (Control)

The animal was anaesthetized with 0.20 cc of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready in eight minutes. The cold point was applied with freezing for thirty seconds, and within thirty seconds of thawing vascular stasis developed in venous channels, rapidly becomming generalized over the injured area. There was no further change by another three minutes and the preparation was discontinued.

Animal 2 (Test)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution intraperitoneally thirty-two minutes after the Etamon was injected. The preparation was ready in four minutes and the cold tip was applied to the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing some small

capillary stasis was apparent and by another thirty seconds many large venous channels were in stasis. In two minutes slow blood flow was observed through one large venous channel that had been in stasis. During the following ten minutes it was noted that the infeeding capillaries could not keep up the flow and the circulation in the whole of the injured area ceased. These were adjacent venous systems, and a large part of the stasis was undoubtedly due to the fact that the arteriole between was injured by the freezing and its flow stopped. Two minutes later the preparation was discontinued. Little essential difference was noted here from the previous control preparation.

Animal 3 (Control)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered intraperitoneally and the preparation was ready in six minutes. The cold point was applied to a selected area of the meso-appendix with freezing for thirty seconds, and within thirty seconds of thawing, generalized stasis was observed over the entire injured area. There was no further change after three and a half minutes and the preparation was discarded.

Animal 4 (Test)

The animal was anaesthetized with 0.20 cc of 0.6% nembutal solution administered intraperitoneally

one hour and five minutes after the Etamon injection. The preparation was ready in six minutes and the cold point was applied to a selected area of the meso-appendix with freezing for thirty seconds. Within thirty seconds of thawing there was general slowing in the venous channels with many of the connecting channels pumping blood into the area. Vascular stasis started two and a half minutes after thawing. By another five minutes, although there was scant flow through one channel, generally the degree of stasis was unchanged. The preparation was discarded seven and a half minutes after thawing.

Animal 5 (Control)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution and the preparation was ready in twenty minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing there was generalized irreversable venous stasis starting. By two and a half minutes later, the field was unchanged and the preparation was discarded.

Animal 6 (Test)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered intraperitoneally one hour and fourty-four minutes after the Etamon was injected. The preparation was ready in eight minutes and the cold point was applied to a selected area of

the mesoappendix with freezing for thirty sedonds. By thirty seconds after thawing great hyperemia was noted generally, but no stasis appeared by two minutes after thawing. There was a good brisk blood flow throughout the frozen area. Two minutes later stasis was noted in one small venous channel, and increased vasomotion in others. In another minute the stream was noted to slow temporarily in a few of the larger venous channels. In thirty seconds stasis was noted to be spreading to a couple venous areas, although there was generally a good circulation. The preparation was discarded seven minutes after thawing with no further changes observed.

Animal 7 (Control)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution intraperitoneally, but when the preparation was mounted six minutes later, general stasis was present throughout the mesoappendix, and the animal was discarded.

Animal 8 (Test)

The animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered intraperitoneally two hours and eight minutes after the Etamon injection. The preparation was ready in five minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Small capillary stasis was noted within thirty seconds after thawing. By another sixty seconds there was slowing of

the flow and stasis in large venous channels. Three and a half minutes later one large static venous channel opened up, and in another thirty seconds another did likewise, and flowed normally. It would seem that the larger channels stay open provided there is any blood draining into them from the capillaries. The preparation was discarded eight minutes after thawing with no further change being noted.

Summary of Observations with Etamon

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Control	60"	Rapidly	60"	Temporary in larger channels
2	Test	60"	4½'	12½'	++++
3	Control	60"	Fast, then slow	2'	None
4	Test	90"	150"	None	+ at 10'
5	Control	30"	Rapidly	90"	None
6	Test	>90"	Incomplete by 6½'		None
7	Control	30"	Rapidly	90"	None
8	Test	180"	Steady	7'	None
1	Control	30"	Rapidly	60"	None
2	Test	30"	Slow	14'	++ at 3'
3	Control	30"	→	30"	None
4	Test	150"	None	None	None
5	Control	30"	→	30"	None
6	Test	240"	At 4'	None	None

Summary (Cont'd)

No.	Onset Stasis	Spread	Generalized	Flow Resumed
7	Control	Animal discarded because of general stasis		
8	Test	30"	Slight	None	at 5' & 5½'

Discussion of Observations

Variable effects from parenteral injections of Etamon would be expected in this experiment since there was with succeeding animals, an increased interval between the time of initial administration of the drug and the performance of the test. However, a superficial examination of the above results would suggest Etamon had a definite effect which was sustained for up to four hours following administration. On the average, with the test animals, vascular stasis did not start for 110 seconds after thawing, whereas with the controls the corresponging time was 40 seconds, almost three times as long. It would seem that the spread and extent of the stasis was much less with the Etamon treated animals.

However, because the drip solution seemed at the finish of the tests to be more deeply pinkish than would be expected for neutrality, its pH was tested on an electric pH meter. The determination was not done for a day or so following the tests, the fluid being stored in tightly stoppered flasks in an electric regrigerator. When determined, the pH measured 8.9 which is admittedly somewhat past neutrality. The exact effect of this more

alkaline drip solution was not studied, but the significance of the above results was considered invalid, and the tests were repeated, as follows, using a drip solution pretested on an electric pH meter, and carefully buffered to a pH of 7.10.

Repeat Experiments with Etamon

In the first few of the following tests, Etamon was used at a dosage level of 100 mg. per kilogram body weight. The observations are summarized in table form at the end of Animal 13.

Animal 1 (Test)

A normal adult nonpregnant female Wistar rat was used, weighing 225 grams. 22.5 mg. of Etamon chloride was injected subcutaneously, and fifteen minutes later 0.15 cc of 0.6% nembutal solution was injected intraperitoneally. Five minutes later 0.05 cc. more was injected also intraperitoneally. Within ten more minutes the animal was sufficiently anaesthetized to permit continuing. The mesoappendix was mounted on the microscope stage and the cold point was applied with freezing for forty seconds. Within thirty seconds of thawing a dispersed fine capillary vasostasis was observed, and a minute later some variable stasis was noted in the larger channels. By two and a half minutes after thawing the stasis was beginning to spread and in another two minutes the stasis

was quite generalized in the injured area, although the arterioles were pulsating to some degree. The preparation was discontinued with no further changes being noted.

Animal 2 (Test)

A normal adult nonpregnant female Wistar rat weighing 280 grams was used. 28 mg. of Etamon was injected subcutaneously and with twenty-three minutes 0.15 cc. of 0.6% nembutal solution was injected intraperitoneally. The preparation had to be discarded after mounting the mesoappendix because the circulation was totally invisible due to the amount of fat present.

Animal 3 (Control)

A normal adult nonpregnant female Wistar rat weighing 240 grams was used. 0.15 cc. of 0.6% nembutal solution was injected intraperitoneally, but after mounting, the mesoappendix five minutes later showed the animal had to be discarded because of generalized vascular stasis.

Animal 4 (Control)

A normal adult nonpregnant female Wistar rat weighing 205 grams was anaesthetized with 0.15 cc. of 0.6% nembutal solution, administered by intraperitoneal injection, and an additional 0.05 cc. was injected in ten minutes and again in seven more minutes. After mounting the mesoappendix, the cold point was

applied with freezing for thirty seconds. Within thirty seconds of thawing, rapidly spreading vascular stasis was noted throughout the injured area. The smaller venules became involved in ninety seconds more, but the arterioles were unaffected and the arterio-venous shunts to the larger veins appeared to be functioning normally. Circulation was observed to be apparently normal in the reat of the mesoappendix. By three minutes after thawing the circulation was quite occluded in the injured area, except for the larger vessels. Two minutes later the preparation was discontinued with no further change noted.

Animal 5 (Control)

A normal adult nonpregnant female Wistar rat weighing an unrecorded amount. 0.15 cc. of 0.6% nembutal solution was injected intraperitoneally. After mounting the mesoappendix, the preparation had to be discarded because the amount of fat present totally obscured the circulation.

Animal 6 (Test)

A normal adult nonpregnant female Wistar rat weighing 165 grams was used. 16.5 mg. of Etamon chloride was injected subcutaneously. The animal was found dead in its cage ten minutes later.

Animal 7 (Test)

A normal adult nonpregnant female Wistar rat

weighing 155 grams. 15.5 mg. of Etamon chloride was injected subcutaneously into the dorsum of the animal, and fifteen minutes later 0.10 cc. of 0.6% nembutal solution was injected intraperitoneally. Five minutes later the preparation was mounted on the microscope stage with excellent vasomotion observed, but the animal died one minute later.

Animal 8 (Test)

A normal adult nonpregnant female Wistar rat weighing 185 grams was used. 18.5 mg. of Etamon chloride was injected subcutaneously and twelve minutes later 0.085 cc. of 0.6% nembutal solution was injected intraperitoneally. Four minutes later the preparation was ready, with the vascular bed dilated and sluggish, but some vasomotion was noted, although no stasis was observed. The cold point was applied with freezing for thirty seconds, and within thirty seconds of thawing, capillary and venous stasis was observed to have occurred. In another minute the circulation closed down completely within the injured area. By seven and a half minutes after thawing, general stasis was present throughout the mesoappendix, and it was felt this animal should not be admitted to the data because of obvious toxic effects from the start due to the Etamon.

Note:

From the above and previous observations, it

would appear that Etamon chloride at a dosage level of 100 mg. per kilogram was too toxic, killing at least 50% of the animals. Further trials will be limited to a smaller dosage.

Animal 9 (Test)

A normal adult nonpregnant female Wistar rat weighing 160 grams was used. 8.0 mg. of Etamon chloride was injected subcutaneously and thirty seconds later 0.11 cc. of 0.6% nembutal solution was injected intraperitoneally. Within twelve minutes the preparation was ready and the cold point was applied with freezing for thirty seconds. Within thirty seconds after thawing, spreading stasis was observed in all except the large vessels and the arterio-venous shunts, within the injured area, which was a relatively small field. Two minutes later it was noted that the adjacent areas were quite hyperemic, although no spread of the vascular stasis occurred within another two minutes. Generally this was an excellent preparation as far as obtaining a good vascular bed for observation was concerned. At the edges of the static area pulsations could be seen entering the closed channels from arterioles. No reopening of any vessels was observed. The preparation was discontinued eight and a half minutes after thawing.

Animal 10 (Test)

A normal adult nonpregnant female Wistar rat weighing 165 grams was injected subcutaneously with 8.6 mg. of Etamon chloride, and in fourteen minutes it was anaesthetized with 0.115 cc. of 0.6% nembutal solution administered intraperitoneally, with 0.05 cc. more being given in five minutes. The preparation was ready within thirteen minutes after the second nembutal injection, but the animal had to be discarded because of too much vascular stasis generally in the mesoappendix.

Animal 11 (Control)

A normal adult nonpregnant female Wistar rat weighing 165 grams was anaesthetized with 0.125 cc. of 0.6% nembutal solution intraperitoneally, and the preparation was ready in six minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds and within thirty seconds of thawing, stasis had started and some ecchymosis was present. In another minute it was observed that the stasis was limited to only one capillary bed. By three and a half minutes after thawing the larger channels in the area were slowing, but open. This field and the degree of cold injury were very comparable to the situation in Animal 9.

Animal 12 (Test)

A normal adult nonpregnant female Wistar rat

weighing 170 grams was injected with 8.5 mg. of Etamon chloride subcutaneously and within four minutes 4.0 mg. was given additionally, making the total dosage about 75 mg. per kilogram body weight. Fourteen minutes later 0.10 cc. of 0.6% nembutal solution was injected intraperitoneally and in another twelve minutes the preparation was ready. The blood vessels in the mesoappendix were observed to be very much dilated, and hyperemic. However, the cold point was applied to a selected area with freezing for thirty seconds. Within thirty seconds of thawing all circulation in the mesoappendix stopped. The animal was considered moribund, and was discarded without further observation.

Animal 13 (Test)

A normal adult nonpregnant female Wistar rat weighing 160 grams was injected subcutaneously with 8.0 mg. of Etamon chloride, and fourteen minutes later it was anaesthetized with 0.075 cc. of 0.6% nembutal solution administered intraperitoneally. The preparation was ready in six minutes and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing there was beginning venous stasis which spread rapidly, and by two and a half minutes after thawing the whole area was static. Adjoining areas were observed to be normal, with trickling of

blood into the injured area from the arterioles at the periphery. The stasis did not spread further. No channels were observed to open up and resume flow. The preparation was discontinued five and a half minutes after thawing.

Summary of Observations with Etamon

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	30"	Rapidly	5½'	None
2	Test	No result, fat obscured circulation			
3	Control	No result, general vascular stasis			
4	Control	30"	Rapidly	5'	None
5	Control	No result, fat obscured circulation			
6	Test	No result, animalfound dead in cage			
7	Test	No result, animal died on microscope stage			
8	Test	No result, preparation not physiological			
9	Test	30"	Rapidly	30", limited	None
10	Test	No result, general vascular stasis			
11	Control	30"	None	None	None
12	Test	No result, animal moribund on microscope			
13	Test	30"	Rapidly	2½'	None

Discussion of Observations

Etamon chloride in excess of 50 mg. per kilogram body weight is too toxic for practical use.

The number of animals with valid data in this series is too limited for any conclusions to be made. There were only three test animals and two controls.

Observations with the control preparations

agree fairly well with controls in other experiments. In view of this, it would seem that no value above the error intrinsic with the method could be claimed for Etamon. There was no apparent delay in the onset of stasis, no apparent decrease in the spread or extent of the stasis, and no evidence that Etamon enabled any of the static channels to open up and resume circulation.

Hydergine

Experimental Observations with Hydergine

A summary of the following data appears in table form after Animal 15.

Animal 1 (Test)

A female non pregnant adult Wistar rat weighing 185 grams was used. It was injected subcutaneously with 0.33 cc. containing 0.1 mg. of Hydergine. This seemed to have some effect in making the animal more docile, and twenty-six minutes later, 0.10 cc. of 0.6% nembutal solution was injected intraperitoneally for anaesthesia. Within seven minutes the preparation was ready, but the animal had to be discarded because a slowly spreading vascular stasis was observed throughout the mesoappendix.

Animal 2 (Test)

An adult nonpregnant female Wistar rat weighing 185 grams was injected subcutaneously with 0.33 cc.

containing 0.1 mg. of Hydergine, and twenty-three minutes later, the animal was anaesthetized with 0.075 cc. of 0.6% nembutal solution administered intraperitoneally. In seven minutes the preparation was ready, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing a rapidly spreading general small vessel stasis was observed, but noteworthy was the fact that two and a half minutes after thawing, a few small vessels that were in vascular stasis appeared to reopen, and by ten minutes after thawing quite a marked resumption of the circulation was noted in many vessels that otherwise would certainly not have been expected to open up. It is possible that vasomotion was observed, but it is more likely that the resumption of flow was merely a matter of a passive flushing of the sludged cells by the more effective hydrostatic and pulsating pressure permitted by the action of the Hydergine.

Animal 3 (Control)

An adult nonpregnant female Wistar rat weighing 185 grams was anaesthetized with 0.10 cc. of 0.6% nembutal solution administered intraperitoneally, and the preparation was ready on the microscope stage within seven minutes. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, and immediately following thawing,

stasis in capillary beds and small venous channels was noted. In another thirty seconds the large veins were slowing, and by two minutes after thawing the small venous stasis was slowly spreading over the injured area. A few larger venous channels opened up following a temporary arrest in flow, not exactly the same as the venous stasis otherwise described. After five minutes following thawing, the arterioles and arterio-venous shunts in the injured area were all regarded as free flowing, and the rest of the circulation in the mesoappendix was regarded as normal. The preparation was discarded.

Animal 4 (Test)

An adult nonpregnant female Wistar rat weighing 170 grams was injected with 0.33 cc. of Hydergine, containing 0.1 mg. of active principle, administered subcutaneously, and twenty-eight minutes later, 0.075 cc. of 0.6% nembutal solution was injected intraperitoneally. Within eight minutes the preparation was ready, and the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Vascular stasis in five capillary beds was starting within forty-five seconds after thawing, although this apparent delay in onset of fifteen seconds was not considered significant. It was noted that several normally functioning capillary beds were

interspersed through the static ones. By two and a half minutes following freezing, several of the static venous and capillary systems "flushed" open with active vasomotion observed in the precapillaries. It was noted fifteen minutes after thawing that one large venous system failed to resume complete flow, although a faint trickle of blood corpuscles did resume, possibly because of the twisting and moving of the animal under the light anaesthetic. One minute later the preparation was discontinued because of general vascular slowing generally throughout the mesoappendix.

Animal 5 (Control)

An adult nonpregnant female Wistar rat weighing 145 grams was anaesthetized with 0.075 cc. of 0.6% nembutal solution administered intraperitoneally. The preparation was ready in ten minutes, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within forty-five seconds after thawing there was venous slowing and stasis with rapidly spreading stasis in the venules in the injured area. Two and a half minutes after thawing the stasis was spreading but many normal capillary beds were functioning normally in between, and also in the very centre of the injured area. By seven and a half minutes following the thawing there was no further change to be noted. The stasis did not become

generalized nor complete in the affected area. The rest of the vasculature in the mesoappendix appeared relatively normal when the preparation was discontinued.

Animal 6 (Test)

An adult nonpregnant female Wistar rat weighing 145 grams was injected subcutaneously with 0.1 mg. of Hydergine contained in 0.33 cc., administered subcutaneously, and in twenty-five minutes 0.075 cc. of 0.6% nembutal solution was injected intraperitoneally. The preparation was ready ten minutes later, but the animal had to be discarded because of generalized vascular stasis and ecchymosis in the mesoappendix.

Animal 7 (Test)

An adult nonpregnant female Wistar rat weighing 140 grams was injected subcutaneously with 0.1 mg. of Hydergine contained in 0.33 cc., and in twenty-five minutes 0.05 cc. of 0.6% nembutal solution was administered intraperitoneally, with 0.025 cc. being repeated four minutes later. The preparation was ready four minutes later, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds after thawing, small capillary stasis spreading to other beds was noted, and in another minute a fairly extensive area was in stasis with only one large artery flowing.

Actually the injured area in this preparation was about four times as large as in animals 1 to 6, and it was felt that if Hydergine had any real value in aiding the flushing of cells through the static vessels, the area in stasis was too extensive for the cells to flush through such a long channel by means of arteriole pulsations. By twelve and a half minutes following thawing, no further change was noted. The rest of the circulation in the mesoappendix was regarded as good. At the periphery of the static areas a few arterioles were observed to be pulsating into the static vessels. The preparation was discontinued three minutes later.

Animal 8 (Control)

An adult nonpregnant female Wistar rat weighing 115 grams was anaesthetized with 0.05 cc. of 0.6% nembutal solution administered intraperitoneally and the preparation was ready within six minutes. The cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within forty-five seconds of thawing a rapidly spreading vascular stasis occurred. By two minutes after thawing, only the main channels and the arterio-venous shunts were observed to be flowing, and many of these were observed to be slowing. After a further three minutes, no change was noted, with no further spread

in the stasis, and no reopening of any vascular channels. The warm saline drip was not resumed with this preparation until eight minutes after thawing, and it was not observed to effect any immediate additional stasis, thus supporting the contention that the Ringer-Locke solution with gelatine and glucose added was fairly physiological. The preparation was discarded one minute later, with good blood flow in the rest of the mesoappendix.

Note:

The pH of the Ringer-Locke drip containing gelatine and glucose was adjusted before starting, using a Beckman pH meter and adjusting to a pH of 7.10 with sodium bicarbonate powder.

Animal 9 (Test)

An adult nonpregnant female Wistar rat weighing 175 grams was injected subcutaneously with 0.1 mg. of Hydergine, contained in 0.33 cc., and twenty-eight minutes later the animal was anaesthetized with 0.075 cc. of 0.6% nembutal solution administered intraperitoneally. Within eight minutes the preparation was ready, but the animal was discarded since generalized vascular stasis, and much ecchymosis were present on mounting the mesoappendix.

Animal 10 (Control)

An adult nonpregnant female Wistar rat weigh-

ing 185 grams was anaesthetized with 0.10 cc. of 0.6% nembutal solution administered intraperitoneally and seven minutes later the preparation was ready. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing generalized venous stasis was noted to be starting which spread rapidly. In one minute more the whole injured area was in vascular stasis. The preparation was discontinued with no further change, the remainder of the mesoappendix apparently having normal circulation.

Animal 11 (Test)

An adult nonpregnant female Wistar rat weighing 165 grams was injected subcutaneously with 0.1 mg. of Hydergine (in 0.33 cc.) and twenty-three minutes later it was anaesthetized with 0.05 cc. of 0.6% nembutal solution administered intraperitoneally, with 0.025 cc. more being given in four minutes. Eleven minutes after the first nembutal injection, the preparation was mounted on the microscope stage, ready for observation. The cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Within thirty seconds of thawing, vascular stasis was observed to be starting. In one minute more, many of the channels that appeared closed were again functioning, and this restor-

ation of circulation was not considered to be due to passive vasomotion. In another minute, there were only a few of the capillary beds in stasis, and five and a half minutes after thawing there were only two capillary beds in stasis. One minute later, one of the capillary beds reopened widely, and appeared to be freely flowing. By nine and a half minutes after thawing there was only one capillary bed showing vascular stasis, and the preparation was discontinued with the circulation in the remainder of the mesoappendix apparently normal.

Animal 12 (Test)

An adult nonpregnant female Wistar rat weighing 170 grams was injected with 0.1 mg. of Hydergine contained in 0.33 cc. subcutaneously, and twenty-five minutes later 0.075 cc. of 0.6% nembutal solution was injected intraperitoneally for anaesthesia. In another seven minutes the preparation was ready, but the animal was discarded because of extensive vascular stasis and ecchymosis throughout the mesoappendix circulation.

Animal 13 (Test)

An adult nonpregnant female Wistar rat weighing 150 grams was injected subcutaneously with 0.1 mg. of Hydergine contained in 0.33 cc. Thirty-three minutes later the animal was anaesthetized with 0.15

cc. of 0.6% nembutal solution administered intramuscularly. In nine minutes the preparation was ready and no vascular stasis was noted in the circulation of the mesoappendix. The cold point was applied with freezing for thirty seconds. Stasis first appeared sixty seconds after thawing, and in only three capillary beds, with, however, some slowing in the larger veins. In another two minutes a few of the closed arterio-venous shunts reopened a channel through. At nine and a half minutes after thawing, a very short venous channel reopened. By six minutes later there had occurred no further changes. The same four static capillary beds were still shut down with excellent arteriole flow through them in the arterio-venous channels. The remainder of the circulation appeared normal, with moderate hyperemia present throughout. The preparation was discontinued nineteen and a half minutes after thawing.

Animal 14 (Control)

An adult nonpregnant female Wistar rat weighing 150 grams was anaesthetized with 0.10 cc. of 0.6% nembutal solution administered intramuscularly, and seven minutes later the dosage was repeated with immediate death of the animal, presumably through intravenous injection.

Animal 15 (Control)

An adult nonpregnant female Wistar rat weighing 170 grams was anaesthetized with 0.10 cc. of 0.6% nembutal solution, administered intramuscularly in three divided sites, and eleven minutes later the preparation was ready on the microscope stage. The cold point was applied with freezing to a selected site in the mesoappendix for thirty seconds. Stasis was observed within thirty seconds of thawing, and was slowly spreading to involve in the next two minutes several capillary beds within the injured area. Four minutes later the preparation was discontinued, with no further change being noted.

Note:

The injection of the nembutal other than intraperitoneally appears to lessen the incidence of the vascular stasis which has been so wasteful of animals even before any testing could be done.

Summary of Observations with Hydergine

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	No results, mesoappendix in stasis			
2	Test	30"	Rapidly	Fairly gen'l	2½', max by 10'
3	Control	Immediate	2'	Almost total	None
4	Test	45"	Not even	None	2½' in many areas

Summary (Cont'd)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
5	Control	45"	Rapidly	None	None
6	Test	No results, mesoappendix in stasis			
7	Test	30"	Rapid within 1'	Almost total	None
8	Control	45"	Rapidly	Except A-V shunts	None
9	Test	No results, mesoappendix in stasis			
10	Control	30"	Rapidly	90"	None
11	Test	30"	Finally, only 1 Cap. bed	None	/// at 90"
12	Test	No results, mesoappendix in stasis			
13	Test	60"	To four Cap beds	None	// at 3'
14	Control	No results, intravenous nembutal injection			
15	Control	30"	Slowly	Yes	None

Discussion of Observations with Hydergine

It would seem warranted to refrain from intraperitoneal injections in case the material injected induces vascular stasis in the mesoappendix.

Control preparations behaved much as in other experiments. There was in the control animals customary failure of any restoration of circulation in blood vessels once they went into stasis following thawing. The Hydergine pretreated rats did not show a definite tendency that was consistent toward resumption of circulation after the vasostatic effect of the freezing injury. Other differences were not considered significant

following thawing.

Apresoline

Experimental Observations with Apresoline

A summary of the following observations will be found below in table form after Animal 11.

Preliminary test for toxicity

10 mg. of Apresoline was administered subcutaneously to a 200 gram nonpregnant adult female Hooded rat. In ninety minutes there were very obvious signs of a serious reaction. At the same time, 2 mg. of Apresoline was similarly administered to another animal of the same size, and in ninety minutes it was apparently normal.

Animals used

Adult male Hooded rats, five in all, in weight range of 190 to 220 grams were chosen for testing, and to these at one time was administered 2 mg. of Apresoline subcutaneously. By fifteen minutes, all five of the injected animals were obviously influenced by the drug, though not adversely, and were all lying quietly in their cages.

Five adult male Hooded rats in the weight range of 150 to 250 grams were selected to serve as controls, and these were not injected with Apresoline.

Animal 1 (Control)

The animal was anaesthetized with 0.1 cc. of nembutal solution 0.6% subcutaneously, and in five minutes 0.125 cc. more were administered. Stasis started within ninety seconds of thawing. There was minimal spread after three minutes of thawing, and the greatest part of the injured area was normal. Only twenty percent of the injured vessels were in stasis. There was no further extension by six minutes after thawing. No resumption of flow was observed.

Note:

The Ringer-Locke saline drip solution with gelatine and glucose had been buffered without use of the Beckman pH meter, and it was possible the solution when used was slightly too alkaline, but it appeared that the material was fairly physiological with no significant adverse effects on the vessels being noted.

Animal 2 (Test)

The animal was anaesthetized with 0.075 cc. of 0.6% nembutal solution which was augmented in fifteen minutes by 0.05 cc. more. No stasis was observed within thirty seconds of thawing, the first occurring three minutes after thawing. Only one capillary bed was involved by four and a

half minutes of thawing. This is an excellently preserved field to observe. No revascularization was noted, nor any further change by seven and a half minutes following thawing.

Animal 3 (Control)

The animal was anaesthetized with 0.125 cc. of 0.6% nembutal solution with no further nembutal being required. Freezing occurred forty-five minutes after the nembutal injection. After freezing, a small arteriolar haemorrhage was noted, which was not severe enough to affect the animal generally or the mesoappendix vasculature in particular. At nine and a half minutes after the thawing there was generalized vascular stasis throughout the injured area. No intermediate observations were made. These observations were not included in the summary table.

Animal 4 (Test)

The animal was anaesthetized with 0.125 cc. of 0.6% nembutal solution. Freezing occurred one hour and forty minutes after the Apresoline administration. The freezing took forty seconds before thawing could commence, and except for one strongly flowing arterio-venous anastamosis, there was generalized vascular stasis in the injured area to the extent of eighty percent within thirty seconds

of thawing. There was no further change noted by four and a half minutes after thawing, and no restoration of circulation apparent.

Animal 5 (Control)

There was no stasis apparent by five and a half minutes following thawing.

Animal 6 (Test)

The animal was anaesthetized with 0.125 cc. of 0.6% nembutal solution. Stasis commenced within thirty seconds of thawing. Many channels reopened and the blood flowed freely through them. By two and a half minutes after thawing all channels were restored. This was not the usual type of vascular stasis. The whole injured area became freely flowing, and remained so by seven and a half minutes after thawing when the preparation was discontinued.

Note:

Into another three male adult Hooded rats was injected 2.0 mg. of Apresoline subcutaneously.

Animal 7 (Test)

The animal was anaesthetized with 0.15 cc. of 0.6% nembutal solution, and freezing injury was inflicted seventy-five minutes after the Apresoline was administered. Stasis commenced within thirty seconds after thawing. By ninety seconds

there was rapid spread to the whole of the injured area. No further change occurred by five minutes after thawing, and no restoration of the circulation was noted.

Animal 8 (Control)

The animal was anaesthetized with 0.15 cc. of 0.6% nembutal solution. Stasis commenced within thirty seconds of thawing. Fifty percent of the injured area was in stasis within three minutes after thawing. No restoration of the circulation was observed.

Animal 9 (Test)

The animal was anaesthetized with 0.15 cc. of 0.6% nembutal solution. Freezing occurred approximately ninety minutes after administration of the Apresoline. Stasis occurred in only one capillary bed, and in that by sixty seconds after thawing. There was also one small site of ecchymosis. There were no further changes by five minutes after thawing, and no restoration of the circulation.

Animal 10 (Test)

The animal was anaesthetized with 0.15 cc. ofnembutal 0.6% solution. Freezing occurred approximately two hours after administration of the Apresoline. Stasis occurred within thirty seconds of thaw-

ing. Within sixty seconds of thawing, fifty percent of the injured area developed vascular stasis. Only one arterio-venous bridge was functioning by three minutes after thawing. No restoration of the static circulation was observed.

Animal 11 (Control)

The animal was anaesthetized with 0.10 cc. of 0.6% nembutal solution. Stasis developed within fifteen seconds of thawing, and rapidly spread to involve thirty percent of the injured area within sixty seconds after thawing. Much ecchymosis was present in the static area. The stasis slowly spread to involve fifty percent of the injured area within three minutes after thawing. No restoration of the circulation was noted.

Addendum

In the case of Animal 2, freezing occurred one hour after the Apresoline injection. With Animal 6, it was two hours and ten minutes.

Summary of Observations with Apresoline

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Control	90"	Minimal	20% in 3'	None
2	Test		Slowly	1 Cap. bed in $4\frac{1}{2}$'	None
3	Control	Results not included			
4	Test	30"	80% in 30"	80% in $4\frac{1}{2}$'	None

Summary (Cont'd)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
5	Control	None by 5½'			
6	Test	30", but complete restoration by 2½'			
7	Test	30"	Rapidly	Complete by 90"	None
8	Control	30"	Steady	50% in 3'	None
9	Test	60", in only one capillary bed			None
10	Test	30"	50% in 60"	90% in 3'	None
11	Control	15"	30% in 60"	50% in 3'	None

Discussion of Observations

No difference can be claimed between the behavior of the test and the control group in respect to time before onset of stasis, extent of the stasis, or resumption of flow in static channels.

Chlor-Tripolon

Experimental Observations with Chlor-Tripolon

Into each of six adult nonpregnant female Wistar rats in the 150-200 gram weight range was injected subcutaneously 5 mg. of Chlor-tripolon maleate dissolved in water to a strength of 50 mg. per cc. (0.1 cc. for each animal). The injection was made one hour before preparaing the animals for microscopic observation. Six similar animals were selected as controls. Each animal was anaesthetized before preparation with a subcutaneous injection of 0.15 cc.

of 0.6% nembutal solution. A summary of the following observations in table form will be found after Animal 12. The usual drip solution was used.

Animal 1 (Control)

Stasis started within thirty seconds of thawing. Twenty percent of the injured area was in stasis within sixty seconds after thawing, and sixty percent within three minutes. One venule opened and closed intermittently. There were no further changes observed within five minutes after thawing. No restoration of the circulation was noted.

Animal 2 (Test)

Stasis started within fifteen seconds after thawing. Within sixty seconds after thawing, stasis was present in the whole of the injured area except for one large arterio-venous bridge. Three small ecchymoses were present within the frozed area. No further change was noted within five minutes after thawing, and no resumption of flow was observed.

Animal 3 (Control)

Stasis started within thirty seconds after thawing. Sixty percent of the injured area was involved within forty-five seconds after thawing. After two minutes only one large venous channel was flowing within the injured area. There was no further change noted after four minutes, and no restoration of circulation in any static channel.

Animal 4 (Test)

Stasis commenced sixty seconds after thawing, in one capillary bed only. A little distance away one venule was observed to be opening and closing, but this vessel became static two and a half minutes after thawing. Stasis spread to only one other capillary bed by three and a half minutes after thawing. This amount of reaction was a rather less effect than would be expected from the amount of vascularity in the injured area. By five minutes after thawing twenty percent of the injured area was in stasis. No restoration of the circulation was noted. The Ringer-Locke gelatin-glucose drip was not observed to have any appreciable harmful effect on the behaviour of the vessels in the preparation.

Animal 5 (Control)

Stasis started thirty seconds after thawing. There was rapid spread to involve twenty-five percent of the injured area in stasis within sixty seconds. Sixty percent of the area was static by three minutes after thawing. Affew venules were observed to start and stop their flow of blood, with no real stasis in these channels. There was no further change by five minutes, and no resumption of flow observed.

Animal 6 (Test)

Stasis was noted throughout the mesoappendix

on mounting and the preparation was discarded.

Animal 7 (Control)

Stasis occurred within fifteen seconds after thawing. It became generalized in the injured area within forty-five seconds after thawing. One venous channel was observed to be flushing open every little while. The whole injured area was static by three and a half minutes after thawing, and no further change was noted by five minutes, with no resumption of flow apparent.

Animal 8 (Test)

Stasis started within fifteen seconds of thawing, and became generalized throughout the injured area by sixty seconds. No resumption of flow was noted except in a few of the larger venules that were not totally static. No further change was noted by five minutes after thawing.

Animal 9 (Control)

Stasis started within forty-five seconds after thawing. Twenty-percent of the injured area was involved within two minutes after thawing, and seventy-five percent in four minutes. No resumption of flow was noted.

Animal 10 (Test)

Stasis started within fifteen minutes after thawing, and was total in the injured area within

sixty seconds except for one central venous channel that stopped and started for another sixty seconds only. There was no further change noted by three minutes after thawing.

Animal 11 (Control)

Stasis started forty-five seconds after thawing. Only one capillary bed became involved by stasis by three minutes after thawing. No further changes were noted by five minutes after thawing.

Animal 12 (Test)

Stasis started sixty seconds after thawing, spreading by two minutes after thawing. By four minutes after thawing eighty percent of the injured area was in stasis, and by five minutes after thawing the area was one hundred percent static. No resumption of flow was noted.

Summary of Observations with Chlor-Tripolon

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Control	30"	20% in 60"	60% in 3'	None
2	Test	15"	Rapid	90% in 60"	None
3	Control	30"	60% in 45"	90% in 2'	None
4	Test	60"	Slight	Two beds in 3½'	None
5	Control	30"	25% in 60"	60% in 3'	None
6	Test	Preparation discarded			
7	Control	15"	General in 45"	100% in 3½'	None

Summary (Cont'd)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
8	Test	15"	Rapid	General in 60"	None
9	Control	45"	20% in 2'	75% in 4'	None
10	Test	15"	90% in 60"	100% in 2'	None
11	Control	45"	None	1 Cap bed in 3'	None
12	Test	60"	80% in 4'	100% in 5'	None

Discussion of Observations

The animals pretreated with Chlor-Tripolon could be claimed to differ in no respect from those control animals not pretreated with Chlor-Tripolon.

Rutin

Experimental Observations with Rutin

All the observations regarding rutin microscopically were performed by a senior medical student helping with the project part time. His method of timing is slightly varied from that used elsewhere, and he uses the term "general stasis" to apply to the whole vascular bed of the mesoappendix mount, whereas the term is used elsewhere to apply only to the vessels within the field actually frozen by the cold point, the so-called injured area. When stasis develops throughout thewhole preparation, such a mounting is regarded as unphysiological, and the animal is discarded, and the results are not included.

The test animals were prepared with rutin prior to the experimental observation by selecting six normal adult male Wistar rats, placing them in one cage, and putting into their drinking water the ground up rutin tablets. 600 mg. of rutin was placed in about 250 cc. of water, and the animals were observed to be drinking the water satisfactorily. This was kept before them for five full days, besides their usual feed, and then they were tested in the usual manner as outlined below. Eight similar animals were selected as controls. These were managed in a like manner, but did not receive any rutin. The following observations are summarized in table form after Animal 14.

Animal 1 (Control)

A male Wistar rat weighing 220 grams that had received no prior medication with rutin was anaesthetized with 0.15 cc. of 0.6% nembutal solution injected subcutaneously. The preparation was ready twelve minutes later, but was discarded because it fell off of the microscope stage.

Animal 2 (Control)

A male Wistar rat weighing 180 grams that had received no prior treatment with rutin was anaesthetized with 0.15 cc. of 0.6% nembutal solution, injected subcutaneously, but the preparation had to be

discarded (reason not recorded).

Animal 3 (Control)

An untreated male Wistar rat of 190 grams was anaesthetized with 0.15 cc. of 0.6% nembutal solution. A selected area of the mesoappendix was frozen for thirty seconds with the cold point apparatus, followed by immediate rapid thawing with the warm drip solution which had been discontinued during the freezing procedure. Vascular stasis began within thirty seconds after thawing and spread to involve about six capillary beds by five minutes after thawing. There was no generalized stasis or any apparent restoration of blood flow in the static vessels. Numerous capillaries and one medium sized venule were involved.

Animal 4 (Control)

A normal adult male Wistar rat of 190 grams that had received no prior medication was anaesthetized with 0.15 cc. of 0.6% nembutal solution. Seventeen minutes later a further 0.05 cc. of nembutal had to be administered, and the animal was satisfactorily anaesthetized fifteen minutes later. A selected area of the mesoappendix was frozen for thirty seconds followed by immediate rapid thawing. Stasis began within thirty seconds of thawing, and spread

to involve five or six capillary beds in two minutes. There was no general stasis and no resumption of flow in those vessels in which stasis occurred. Capillaries and small collecting venules were involved.

Animal 5 (Control)

A 170 gram normal adult male Wistar rat which had received no prior treatment was anaesthetized with 0.15 cc. of 0.6% nembutal solution requiring an additional 0.05 cc. twice within the following fifteen minutes. Vascular stasis began within thirty seconds after thawing and involved about ten capillary beds within four minutes. There was no general stasis or resumtpion of flow. Capillaries and small venules were affected.

Animal 6 (Test)

A 170 gram normal adult Wistar male rat treated with rutin was anaesthetized with 0.15 cc. of 0.6% nembutal solution followed in fifteen minutes by another 0.05 cc. Following rapid thawing, vascular stasis began within thirty seconds and spread to involve three or four capillary beds by four minutes after thawing. There was no general spread. Restoration of circulation was noted in a collecting venule two minutes after thawing. Capillaries and small collecting venules were involved in the stasis.

Animal 7 (Control)

A normal adult male Wistar rat of 170 grams

previously untreated with rutin was anaesthetized with 0.15 cc. of 0.6% nembutal solution, without any additional nembutal being required, which resulted in a lightly anaesthetized animal. No stasis occurred in thirty seconds. Reversal of direction of circulation was noted in some of the medium sized venules within one minute of thawing, but no stasis was observed anywhere in the field.

Animal 8 (Test)

A normal adult male Wistar rat of 170 grams, previously treated with rutin, was anaesthetized with 0.17 cc. of 0.6% nembutal solution, followed in fifteen minutes with 0.07 cc. more, both injections being given subcutaneously. Stasis began within forty-five seconds of rapid thawing, and spread slightly to involve about three capillary beds within five minutes after thawing. Stasis was slight and involved only about one third of the vessels in the injured area. No general stasis or restoration of circulation in the static vessels was noted. Capillaries and small venules were involved in the stasis.

Animal 9 (Test)

A normal adult male Wistar rat of 160 grams was anaesthetized with 0.15 cc. of 0.6% nembutal solution after full pretreatment with rutin. Stasis commenced seventy-five seconds after thawing, and spread to involve two or three capillary beds in four

minutes. About one third of the vessels in the injured area were involved. No general stasis or resumption of flow was observed to occur. Capillaries and small collecting venules were affected.

Animal 10 (Test)

A normal adult male Wistar rat of 210 grams previously treated with rutin was anaesthetized with 0.21 cc. of 0.6% nembutal solution administered subcutaneously. Vascular stasis began with one minute after rapid thawing, and spread to involve by four minutes after thawing only four or five capillary beds. About two thirds of the vessels in the injured area were involved. No general stasis or resumption of flow was observed. Capillaries and small collecting venules were involved in the stasis.

Animal 11 (Control)

A normal adult male Wistar rat of 220 grams not previously treated with rutin was anaesthetized with 0.18 cc. of 0.6% nembutal solution followed in twenty minutes with 0.08 cc. more. The animal expired during observation, and no results are recorded.

Animal 12 (Test)

A normal adult male Wistar rat of 180 grams, having received prior treatment with rutin orally, was anaesthetized with 0.20 cc. of 0.6% nembutal solution injected subcutaneously. Stasis commenced

within thirty seconds after rapid thawing, and it spread to involve about six capillary beds within three minutes after thawing. Two thirds of the vessels in the injured area were in stasis, but there was no generalized stasis throughout the preparation. Circulation was observed to be resumed in two vessels. Capillaries and small venules were involved in the static process.

Animal 13 (Control)

A normal adult male Wistar rat of 190 grams not previously treated with rutin was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered subcutaneously. Stasis commenced within sixty-five seconds after thawing, spreading so as to involve three capillary beds within three minutes. About one quarter of the capillaries in the injured area were involved. There was no stasis or resumption of circulation in the static channels. Capillaries only were involved in the process.

Animal 14 (Test)

A normal adult male Wistar rat of 190 grams previously treated with rutin, was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered subcutaneously. There was generalized vascular stasis present on mounting the mesoappendix, and the preparation was discarded.

Summary of Observations with Rutin

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Control	No results, fell off microscope stage			
2	Control	No results, discarded			
3	Control	30"	6 beds in 5'	None	None
4	Control	30"	5-6 beds in 2'	None	None
5	Control	30"	10 beds in 4'	None	None
6	Test	30"	3-4 beds in 4'	None	✓
7	Control	None			
8	Test	45"	3 beds in 5'	None	None
9	Test	90"	2-3 beds in 4'	None	None
10	Test	60"	4-5 beds in 4'	None	None
11	Control	No results, expired during observation			
12	Test	30"	6 beds in 3'	None	✓
13	Control	65"	3 beds in 3'	None	None
14	Test	No results, general stasis on mounting			

Discussion of Observations

The six test animals, for the five days before the experiments took place, were kept in one cage and allowed to drink from the same drinking bottles. This together with the fact the rutin tended to settle somewhat to the bottom of the container probably caused an uneven distribution of the medication among the rats.

The average time after thawing for stasis to commence in five test animals was 50". Substantially the same time, 60", held for four control animals (not

considering Animal 7 in which no stasis occurred). In animals 6 and 12, definite resumption of flow in static channels occurred. To quote the opinion of the observer of these experiments, "Rutin shows some delaying in the onset of stasis, but slight if any decrease in the area involved. In two animals the channels resumed flow. These results cannot be considered definite or consistent enough to warrant positive conclusions, but the drug appears promising, and the tests should be repeated with care to ensure an accurate dosage per animal."

Further observations with Rutin

Six rats as noted below were fed Rutin carefully so that each received twenty-five mg. per day for four days, followed by one hundred mg. per day for four more days. They showed no adverse effect from the medication. Seven other animals were selected as controls. As mentioned these observations were made by a senior medical student. A summary in table form appears after Animal 13.

Animal 1 (Test)

A normal adult nonpregnant female Wistar rat weighing 190 grams, previously treated with rutin orally was anaesthetized with 0.1 cc. of 0.6% nembutal solution administered subcutaneously. Vascular stasis

was observed to occur immediately after rapid thawing, which spread to involve seven eighths of the injured area within two and a half minutes after thawing. No generalized stasis occurred, and no resumption of circulation in the static areas was observed.

Animal 2 (Control)

A normal adult nonpregnant female Wistar rat weighing 200 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution. Vascular stasis occurred immediately after thawing which rapidly spread to involve all vessels in the injured area.

Animal 3 (Test)

A normal adult nonpregnant female Wistar rat of 195 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution. Stasis started within forty seconds after thawing, and spread to involve half the vessels in the injured area within three minutes after thawing.

Animal 4 (Control)

A normal adult nonpregnant female Wistar rat weighing 200 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution. The whole mesoappendix went into vascular stasis from the freezing, and the preparation was discarded.

Animal 5 (Test)

A normal adult nonpregnant female Wistar rat

weighing 160 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution administered subcutaneously. Stasis began within forty seconds after thawing, and involved only four short segments of capillaries.

Animal 6 (Control)

A normal adult nonpregnant female Wistar rat weighing 180 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution. Stasis began in fifty-five seconds after thawing and spread to involve one third of the vessels in the frozen area by four minutes after thawing.

Animal 7 (Test)

A normal adult nonpregnant female Wistar rat weighing 200 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution administered subcutaneously. Stasis developed within fifty seconds after thawing, spreading to involve one quarter of the vessels in the injured area.

Animal 8 (Control)

A normal adult nonpregnant female Wistar rat weighing 180 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution. Stasis began in sixty seconds after thawing and involved only two or three capillaries. There was resumption of flow in several small capillaries.

Animal 9 (Test)

A normal adult nonpregnant female Wistar rat weighing 180 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution. Stasis started forty-five seconds after rapid thawing, involving three or four short segments of capillaries.

Animal 10 (Control)

A normal adult male Wistar rat weighing 200 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution administered subcutaneously. Stasis started within one minute after thawing to involve half the vessels in the injured area.

Animal 11 (Test)

A normal adult nonpregnant female Wistar rat weighing 190 grams was anaesthetized with 0.13 cc. of 0.6% nembutal solution. Stasis started sixty seconds after rapid thawing and spread to involve one third of the vessels in the injured area in three minutes.

Animal 12 (Control)

A normal adult male Wistar rat weighing 200 grams was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered subcutaneously. Stasis began forty-five seconds after thawing and spread to involve two thirds of the vessels in the injured area.

Animal 13 (Control)

A normal adult male Wistar rat weighing 200 grams was anaesthetized with 0.13 cc. of 0.6% nembutal solution administered subcutaneously. Stasis began forty-five seconds after thawing, and spread to involve two thirds of the vessels in the injured area.

Summary of Observations with Rutin

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	Immediate	Slow	7/8	None
2	Control	Immediate	Rapid	Complete	None
3	Test	40"	Slow	1/2	None
4	Control	No results, preparation discarded			
5	Test	40"	Slow	Minimal	None
6	Control	55"	Slow	1/3	None
7	Test	50"	Slow	1/4	None
8	Control	60"	Slow	Minimal	〃
9	Test	60"	Slow	1/2	None
10	Control	60"	Slow	1/2	None
11	Test	60"	Slow	1/3	None
12	Control	30"	Slow	3/4	None
13	Control	45"	Slow	2/3	None

Discussion of Observations

As mentioned above, these observations using

rutin were performed by another worker. From reading the data, it would appear that the freezing injury might be a little more severe than was customary with the other tests. Even though this would prevent comparison with the controls of other experiments, the test and control animals of this run should be comparable.

The time required for the onset of stasis after the rapid thawing was identical for both series (average of six animals each), namely forty seconds. The speed and extent of the spread of the stasis was the same for both the test and the control series. It is also interesting to note that the only instance of resumption of flow in the static vessels occurred in a control animal.

To quote the impressions of the original observer, "The results of this experiment indicate that rutin does not alter the reaction of capillaries in the rat mesoappendix after freezing and rapid thawing."

Ascorbic Acid

This drug was not tested microscopically because the observed effects on a gross standard freezing injury did not warrant further investigation.

Histamine

Experimental Observations with Histamine

Histamine in the dosage level of 100 mg. per kilogram body weight was tested by parenteral injection. Summary table of the following observations appears after Animal 9.

Animal 1 (Test)

A normal adult male Wistar rat weighing 272 grams was injected subcutaneously with 0.27 cc. of a diluted histamine solution containing 100 mg. per cc., and fifteen minutes later the animal was anaesthetized with 0.075 cc. of 0.6% nembutal solution administered subcutaneously. 0.05 cc. of nembutal were repeated in ten minutes and another 0.025 cc. three minutes afterwards. The preparation was ready on the microscope stage seventeen minutes after the final injection, and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Generalized vascular stasis developed in the injured area within sixty seconds of thawing.

Animal 2 (Test)

A normal adult male Wistar rat weighing 241 grams was injected subcutaneously with 0.24 cc. of a

solution containing 100 mg. of histamine per cc., and sixty-three minutes later the animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution administered subcutaneously. When the preparation was ready, the cold tip was applied to a selected portion of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Stasis developed in thirty seconds after thawing, progressing rapidly to involve sixty percent of the injured area within three minutes after thawing.

Animal 3 (Control)

A normal male adult Wistar rat weighing 235 grams was anaesthetized with 0.1 cc. of 0.6% nembutal solution administered by subcutaneous injection. In twenty minutes a further 0.05 cc. was injected and in five minutes after that 0.05 cc. more. The preparation was ready in another eight minutes, and the cold tip was applied to a selected portion of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. General stasis developed in the injured area within thirty seconds after thawing.

Animal 4 (Test)

A normal adult male Wistar rat weighing 244 grams was injected subcutaneously with 0.24 cc. of a

solution containing histamine in the concentration of 100 mg. per cc. The animal was anaesthetized in twenty minutes with 0.20 cc. of 0.6% nembutal solution administered by subcutaneous injection, and when the preparation was ready on the microscope stage, the cold tip was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis did not develop in any vessel within seven minutes after thawing, at which time the preparation was discontinued. Good vasomotion was maintained throughout the observation period.

Animal 5 (Test)

A normal adult male Wistar rat weighing 247 grams was injected subcutaneously with 0.24 cc. of a solution containing 100 mg. of histamine per cc., and in twenty-eight minutes the animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution also administered by subcutaneous injection. Stasis developed within thirty seconds of thawing, and spread to involve thirty percent of the injured area within four minutes of thawing.

Animal 6 (Control)

A normal adult male Wistar rat weighing 208 grams was anaesthetized with 0.15 cc. of 0.6% nembutal solution administered by subcutaneous injection.

The preparation was mounted on the microscope stage and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Stasis developed within thirty seconds after thawing, rapidly spreading to become generalized within the injured area within ninety seconds after thawing.

Animal 7 (Test)

A normal adult male Wistar rat weighing 270 grams was injected subcutaneously with 0.27 cc. of a histamine solution containing 100 mg. per cc., and in thirty-five minutes the animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution also administered by subcutaneous injection. The preparation was mounted on the microscope stage and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Stasis developed within forty-five seconds after thawing, becomming quite generalized within the injured area.

Animal 8 (Control)

A normal adult male Wistar rat weighing 286 grams was anaesthetized with 0.25 cc. of a 0.6% nembutal solution, and when ready the preparation was mounted on the microscope stage. The cold point was

applied to a selected area of the mesoappendix with freezing for thirty seconds, followed by immediate rapid thawing. Stasis developed within sixty seconds after thawing, rapidly becomming generalized within the injured area by two and a half minutes after thawing.

Animal 9 (Test)

A normal adult male Wistar rat weighing 244 grams was injected with 0.24 cc. of a histamine solution containing 100 mg. per cc., and the animal was anaesthetized with 0.20 cc. of 0.6% nembutal solution. The preparation was mounted and the cold point was applied to a selected area of the mesoappendix with freezing for thirty seconds. Stasis started within thirty seconds after thawing, but at no time during the period of observation did it spread to involve more than twenty-five percent of the injured area.

Summary of Observations with Histamine Parenteral

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Test	30"?	Rapid	by 60"	None
2	Test	30"	Rapid	60% in 3'	None
3	Control	30"	Rapid	by 30"	None

Summary (Cont'd)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
4	Test	None			
5	Test	30"	Slow	30% in 4'	None
6	Control	30"	Rapid	by 90"	None
7	Test	45"	Rapid	Yes	None
8	Control	60"	Rapid	by 2½'	None
9	Test	30"	Slowly	ohly 25%	None

Discussion of Observations

These tests with parenteral histamine were somewhat of a trial to determine an effective dosage of histamine, and the recording was incomplete in a few respects. There were insufficient control animals used, for one thing. Some of the details regarding time intervals are lacking. None the less, it would seem that histamine, as here used, had some definite effects on the behavior of blood vessels after a localized freezing injury. Although no difference was apparent in the time after thawing for stasis to start, the extent and rate of spread of the stasis seemed to be much less in the histamine treated animals. It is doubtful if these findings are significant in view of the objections here raised.

The severity of the freezing injury would seem to be somewhat greater here than in other experiments.

Further Observations with Histamine

The following tests were performed to study the effect of histamine applied locally over the rat mesoappendix in 1:1000 dilution before freezing with the cold point (the warm Ringer-Locke gelatin-glucose drip being temporarily discontinued for this, but of course being resumed following freezing so as to effect an immediate rapid thawing, and to maintain a physiological preparation during the observation period). The usual technique was employed, and 150 gram normal adult nonpregnant female Wistar rats were employed, being anaesthetized fifteen minutes before experimentation with 0.15 cc. of 0.6% nembutal solution. A summary of the following observations appears in table form after animal 10.

Animal 1 (Control)

Stasis occurred within twenty seconds after thawing, which rapidly spread to involve half of the vessels within the injured area by sixty seconds after thawing. No resumption of flow was observed in the static vessels. It was noted that the large arterio-venous bridges were flowing normally even through the injured area.

Animal 2 (Test)

Histamine in 1:1000 concnetraion was flushed

over the mesoappendix prior to freezing. This was observed to give generalized increase in blood flow. After rapid thawing, stasis was observed in a larger venule within sixty seconds after thawing. By ninety seconds after thawing, small capillary stasis was found present, but in only one capillary bed within the injured area. There was no further change noted up to two and a half minutes after thawing when the preparation was discontinued.

Animal 3 (Control)

Stasis was noted within sixty seconds after thawing. This spread slowly to involve other capillary beds. One large venous channel clearly showed hemoconcentration. Half the injured area was found to be in stasis by two minutes after thawing. At this time, histamine, 1:1000, was instilled over the observation field, and this was noted to cause some dilatation of a venule with resumed blood flow in the large channel that had shown the hemoconcentration. There was no further change by five minutes after thawing. The histamine had no effect on the small channels that were definitely static. Ninety seconds after the histamine was applied, one of the small venules resumed flow, and the whole capillary bed it drained resumed circulation. This process

was not duplicated elsewhere within the injured area.

Animal 4 (Test)

Histamine 1:1000 was flushed over the mesoappendix prior to freezing. Generalized vascular stasis occurred within fifteen seconds after thawing. One or two channels opened up and resumed blood flow within sixty seconds after thawing, but this phenomenon involved only a small portion of the whole injured circulatory bed. No further changes were observed by five minutes after thawing.

Animal 5 (Control)

Small capillary bed stasis was present thirty seconds after thawing. A larger venous channel showed a temporary slowing and sludging of blood. No further development was observed except that this large channel later became static. Histamine 1:1000 was flushed over the area, and while it had no visible effect on this large static venule, it did cause the circulation to reflow in the capillary bed that first showed stasis.

Animal 6 (Test)

There was generalized vascular stasis within fifteen seconds after rapid thawing. No effect attributable to the histamine could be observed. There was no resumption of circulation in any vessel within the injured area.

Animal 7 (Control)

Stasis started within fifteen seconds after thawing, and became generalized throughout the injured area within forty-five seconds. One venous channel showed intermittency of flow. Histamine 1:1000 when flushed over the area had no apparent effect.

Animal 8 (Test)

Vascular stasis first appeared ninety seconds after rapid thawing. Some intermittency of stasis in a couple of the larger channels was attributed to the histamine instillation prior to freezing, but otherwise the stasis rapidly became general; throughout the injured area.

Animal 9 (Control)

Stasis occurred within thirty seconds of thawing and slowly became generalized within ninety seconds to involve approximately sixty percent of the injured area. No restoration of the circulation in any static vessel was noted. On flushing the field later with histamine 1:1000, circulation resumed in a large venous channel but only temporarily and intermittently.

Animal 10 (Test)

The mesoappendix was flushed with the warm

histamine 1:1000 solution, and then frozen in the usual manner. Stasis started within thirty seconds after rapid thawing, but in one capillary bed only, with no spread of the stasis within ninety seconds after thawing. Some sludging in larger channels was corrected completely by instilling more histamine 1:1000 over the area, and this effect was repeated after the vessel sludged when the saline drip washed the histamine away. No further change was noted by five minutes after thawing, and the preparation was discontinued.

Summary of Observations with Histamine (Topical)

No.		Onset Stasis	Spread	Generalized	Flow Resumed
1	Control	20"	Rapid	50% in 60"	None
2	Test	60"	None	None	None
3	Control	60"	Slow	50% in 2'	None
	Histamine gave slight resumption of flow				
4	Test			in 15"	✓
5	Control	30"	Slow	larger venules	None
	Histamine gave slight resumption in flow				
6	Test			in 15"	None
7	Control	15"	Rapid	in 15"	None
	Histamine gave no apparent effect				
8	Test	90"	Rapid	Yes	✓ ?
9	Control	30"	Slow	60% in 90"	None
	Histamine gave doubtful effect				
10	Test	30"	None		
	Histamine corrected sludging				

Discussion of Observations with Histamine

On the average, the test animals took forty seconds to develop stasis, while the controls required only thirty following rapid thawing. This was however not consistent enough to be significant.

Histamine applied topically over the control preparations after stasis had occurred frequently permitted the restoration of circulation in static channels. In comparing this action with a similar one obtained by flushing procaine over the mesoappendix, it might be suggested that the effect could be obtained through mere physical action of the weight of the fluid being applied from a height.

Histamine could not be definitely credited with any significant beneficial effect on the blood channels following a standard freezing injury.

Gross Frostbite Experiments

Reason for Gross Experiments

As mentioned previously, on page 18, there was no reason to believe that the capillaries of the rat mesoappendix functioned in the same way as the capillaries of the rat's hind leg, and since it was not the peritoneal contents that would be affected by frostbite, it was therefore necessary to test the same ten medications in order to find out whether they would influence the course of the lesions produced in the hind leg of the rat by a standard cold injury. Shumacker (4) very completely summarizes the standard injuries of a great many gross freezing experiments performed by several different investigators. In almost all of these the tendency was to compare the resulting conditions of a set of test extremities at a certain period after a specified cold injury to the resulting conditions of a similar set of control extremities the same length of time after the same specified cold injury. As an example of what is meant, an experiment of Shumacker, Radigan, Ziperman, and Hughs (4) is summarized. The tails of sixty-four mice were frozen for five seconds at -15°C., the animals having received no treatment. Four

animals (6.3%) showed what the authors termed an excellent result (grade 1 reaction), nine (14.1%) showed a good result, and fifty-five (85.9%) showed a poor result (grade 3 reaction). Then thirty-two mice were given a drug (Benadryl, but the details are not important) and put to the same freezing injury. Two animals (6.3%) showed an excellent result; four animals (12.5%) showed a good result, and twenty-eight animals (87.5) showed a poor result. There need be no discussion of these figures except to mention that they are obviously significant because of the numbers of animals used. However, in assessing the frostbite injury, and in comparing different animals, it seemed as though something more was needed. Frostbite, like any other pathological process, is a dynamic process. We cannot say to-day whether one individual is in a better condition than another, as far as the disease in question is concerned, unless we know how both were yesterday and how they will be tomorrow. Accordingly, in the following pages there will appear some attempt to record the gross frostbite results in a dynamic manner, and the concept, an arbitrary concept, granted, will be explained.

Standard Freezing Injury

Although it is somewhat trite to mention it, freezing is freezing, and the temperature at which the freezing injury is inflicted matters little provided the degree of cold is sufficient to freeze the extremity within a reasonable period of time. In the data summarized by Shumacker (4) ambient temperatures varying from -10°C. to -55°C. were used, provided usually by some mixture with carbon dioxide snow and usually ether in a beaker into which the extremity was dipped for a stated length of time. By passing carbon dioxide gas into a small beaker of ether it was found that a temperature of from -15°C. to -25°C. was readily obtainable, and hence this was the temperature range used for the bulk of the experiments reviewed by Shumacker. Therefore -20°C. plus or minus 5C.° was accepted as the temperature to be used for the standard injury. It was not always possible to obtain on any one day a temperature exactly -20°C. but it was usually possible to maintain throughout each day's work the same temperature, whether -15°C. or -25°C. In all experiments the value of -20°C. will be recorded, and where necessary this will be qualified by some such statement as "the animals suffered a slightly more severe freezing injury than

was usually the case." This might mean a freezing temperature of -23°C. was used. The difficulty in obtaining the exact temperature each day lay in several factors. The discharge nozzle for the carbon dioxide would freeze up to a varying extent from day to day. The amount of ether in the beaker would vary greatly, the warmth of the room would be somewhat of a factor, etc. For this reason, the observations of different experiments are hardly comparable.

The duration of the standard injury was also a problem, but this was more easily settled. The first gross experiment was a time experiment in which the length of the freezing varied from five seconds through twenty seconds to sixty seconds. As will be seen later, the five second injury produced too slight a reaction for standard use, and the sixty second injury produced far too severe a reaction. Actually the twenty second injury (possibly due to minor variations in degree of coldness, or variations in the amount of the extremity immersed) was not always ideal, but there seemed little virtue in reducing the time to fifteen seconds.

So, with these considerations, a twenty second freezing injury at -20°C. was accepted as

the standard freezing injury for the purposes of the experiments here performed.

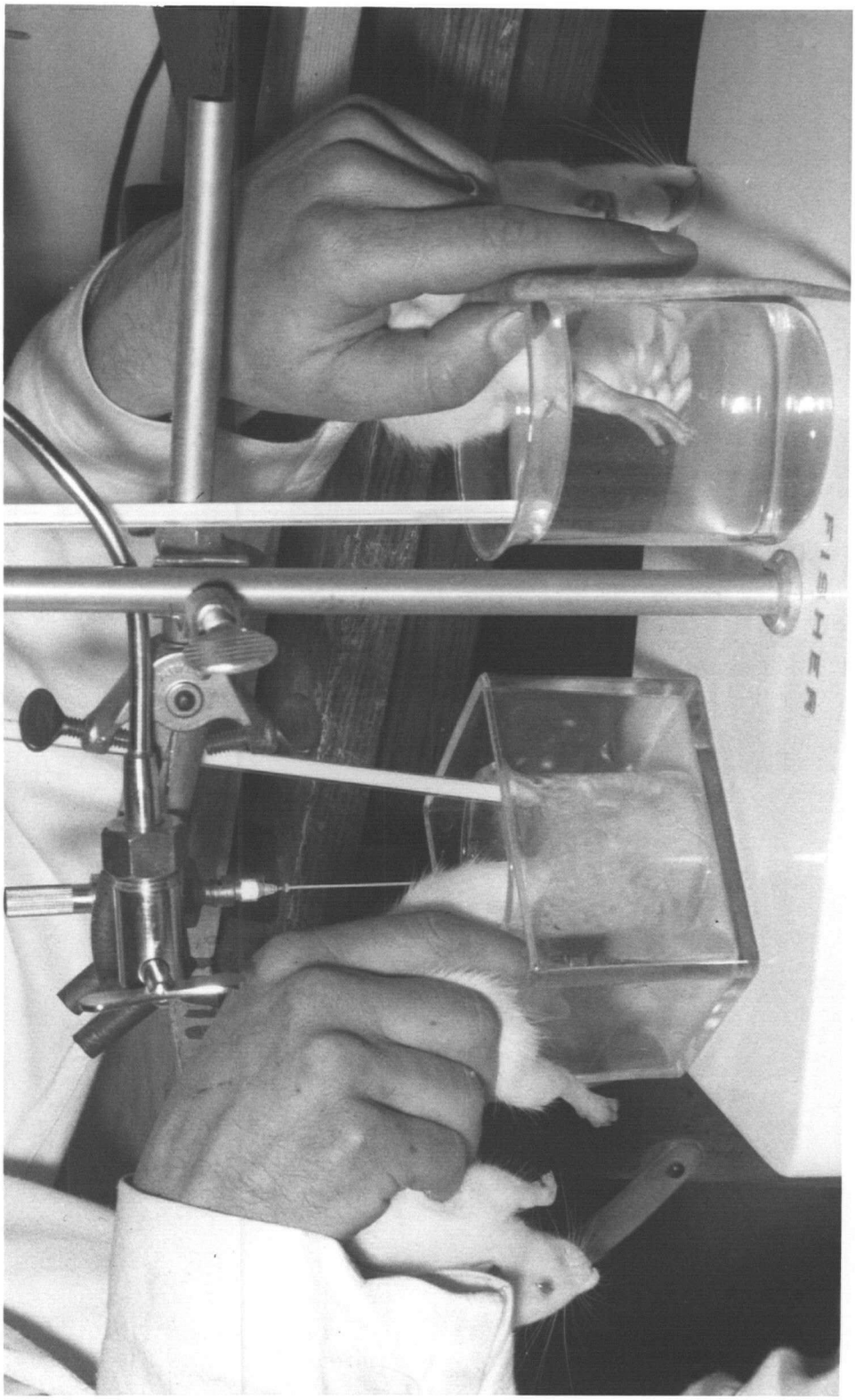
Manner of Thawing

There always has been a great controversy over whether a frozen extremity should be thawed slowly or rapidly. The time honoured method for frozen ears or noses (which more often than not were not "frozen" in the true sense of the word, but just cooled to the extent that the subcutaneous fat solidified thus blanching the skin) has been to rub the part actively with a handful of snow. This was one of the "certain widely accepted concepts based almost entirely upon armchair philosophy of the past" that Shumacker criticises so actively, and he perhaps more than any other person has gathered enough evidence together to show that this is erroneous and can be discarded, at least on an experimental level. Most of the experiments reviewed by Shumacker give immersion of the frozen extremity in warm water at 42°C. for a specified period ot time, usually two minutes, as the accepted method for rapid thawing. These standards were employed in the present experiments, and where possible (providing enough animals were available) both the rapid thawing at 42°C. and ordinary thawing at room temperature was used. This provided

additional control series for almost every experiment.

Apparatus

The accompanying photograph illustrates an actual freezing injury and rapid thawing in progress. The carbon dioxide gas was run in from an ordinary gas cylinder through maleable metal tubine to the control needle valve (this was the adapted Spencer Carbon Dioxide Microtome apparatus as adapted for the microscopic experiments, but with the expansion cold point needles disconnected and a simple number nineteen spinal anaesthetic needle attached) and then through a long needle into a small 125 cc. beaker half filled with ether. The gas bubbling in overflowed the ether, and a larger moat was provided to catch the spillage. A thermometer registering to -30°C. was kept suspended in the freezing mixture and read frequently throughout the performance. The animal was anaesthetized, and the hind leg was extended and dipped into the mixture almost to the fur line. Generally about ten to twenty seconds were required for the limb to cool down, and freezing was indicated by a sudden blanching of the skin and squirming of the animal. Such a limb when frozen was very



brittle, and could be snapped across much as a piece of glass of similar thickness. A stop watch, because of its large second hand that could be started with pressing a button, was generally used for timing. The timing was counted from the moment of this blanching, and the limb was removed from the freezing mixture exactly at the expiry of the time interval (twenty seconds). On the right hand side of the photograph is depicted the rapid thawing. The frozen hind leg of the animal just finished the freezing treatment is shown dipped in a beaker of plain tap water at exactly 42°C. as indicated on the thermometer suspended in the beaker. The leg was kept in the water for a minute or two until thoroughly limp and warm. In actual practice the rapid thawing part of the treatment was performed by a helper, and a larger preserving kettle was used instead of the beaker because the larger volume of water maintained its temperature much more constantly.

Recording the Observations

The course of the injured limbs was not recorded until the lapse of twenty-four hours after the freezing injury. The animals that were thawed at room temperature were merely placed back in their cages in quietness, except for any injection they

would be receiving. The feet of such animals would begin to thaw immediately, and any chance contact with the metal of the cage would aid the warming so that the limb would be limp (thawed) within five minutes usually. It would take another ten or fifteen minutes for the foot to warm up, and within three or four hours the leg would begin to show oedema. Usually the skin would be still bluish, but it would soon become engorged, pink, and warm. The feet of the animals thawed rapidly in the warm water were pink and warm as soon as they were returned to their cages, and within the following couple of hours they would be swollen, but still pink and warm.

On the following day, usually at the same time as the freezing had been done the animals were weighed, and the feet examined. Examination was made under four categories: oedema, colour change, moisture exudate or slough, and gangrene of toes or other parts of the foot. A constant comparison evaluation was attempted, and of course the same person did the examining, but when possible some other person present would be asked to corroborate the impressions. Then under each category, an attempt was made to assign a number to each obser-

vation. The number scale used for each category is outlined as follows:

Oedema	-	None apparent
	1	Minimal
	2	Moderate
	3	Incomplete
	4	As fully rounded as possible
Colour	-	Normal
	1	Pinker than normal
	2	Red
	3	Purple
	4	Blue, either cyanosis or beginning eschar
	5	Black Scab not graded.
Exudate	0	Dry
	1	Barely moist
	2	Quite moist or wet
	3	Infected ulcer or slough. Blood clot not graded
Gangrene	0	None apparent
	1	One toe gangrenous or missing
	5	Five toes gangrenous or missing
	6	Front half of paw gangrenous
	7	Whole paw gangrenous or missing
	8	Whole injured area gangrenous or missing

This classification was not designed completely at the very start of the experiments. One or two preliminary tests were performed in order to determine just exactly what changes to expect, and to construct a table that would best describe the lesions. The one adopted as outlined was certainly not the only one possible, and in a few respects it is not the best. For instance, the degree of swelling could have perhaps been more accurately measured by a water displacement method. But the method of assessing the changes had to be reasonably accurate only, and the biological experiments as performed did not require the exactness of an experiment in physics. Such a grading system as outlined on the preceding page was at all times convenient, rapid, and accurate within the limits of the error of the experiment.

On the following page is an example of how the injury changes were graded in practice. The colour print does not give the colour changes as accurately as the original transparency did, but it gives a rough illustration of what is meant by the accompanying description. The word "much" under the oedema column is synonymous with the word "incomplete" used before for a third degree oedema change.

Injury Index

As will be noted on the following Example sheet, the term Injury Index is introduced. This word, Injury

Example of Injury Calibration

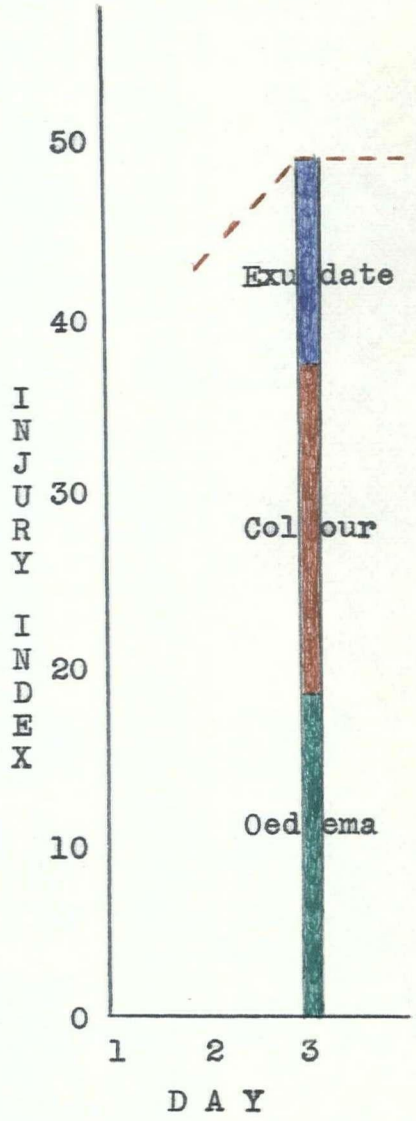
This is a photograph of the feet of group 5, which were frozen for twenty seconds, then thawed slowly at room temperature. The picture was taken on day three, forty-eight hours after the injury occurred.

Animal	Oedema	Colour	Exudate	Gangrene
1	Much	Blue	Wet	None
2	Much	Red	Wet	None
3	Much	Blue	Wet	None
4	Much	Purple	Wet	None
5	Much	Purple	Wet	None
6	Much	Purple	Wet	None

Converting these observations to the arbitrary number code, we have:

Animal	O	C	E	G
1	3	4	2	-
2	3	2	2	-
3	3	4	2	-
4	3	3	2	-
5	3	3	2	-
6	3	3	2	-
Totals	18	19	12	0

The sum of these four totals, here 49, is plotted on all subsequent graphs as the "INJURY INDEX". On the sample graph drawn on this page, the components of the ordinate are blocked in for illustration, but the other graphs would be too crowded with these additional details which can be read from the data tables when desired.



Index is merely an arbitrary number invented in order that the frostbite changes, on a time basis, could be represented graphically without too much crowding and hence confusion on the graphs. A sample graph is indicated with the standard scale used throughout all graphs, and the meaning of the ordinate points on the Injury Index line graph explained.

Selection of Gross Experiments

Gross experiments were performed as outlined in following pages. The details and purpose of each experiment is explained under the write-up for the experiment. Nine different runs were performed as outlined in the Table of Contents: (1) Duration of injury and rate of thaw, (2) procaine, (3) repeat procaine, together with Priscoline, (4) Benadryl, (5) Etamon and Hydergine, (6) Apresoline and Chlor-Tripolon, (7) rutin, (8) ascorbic acid, and (9) histamine. The reason for the particular grouping of the two drugs into one experiment was made only for convenience. The reasons for the dosages selected has already been explained.

Record of Gross Observations

Here follows a record of observations made grossly on the effects of a standard freezing injury on the hind leg of the rat as outlined previously. The results are grouped by the drugs being tested, according to the order of the table of contents.

Duration of Injury and Rate of Thaw

Experimental Observations with Time and Rate of Thaw

The purpose of the experiment described here was to test the effects of a standard cold injury as influenced by the duration of the frozen state, and also as influenced by the rate of thawing. Thirty-six normal adult nonpregnant female Wistar rats were selected, and they were divided into six groups of six animals each, with each group designed as follows:

Group: 1. A group to receive a freezing injury of five seconds duration, with rapid thawing in water at 42°C.

2. A group to receive a freezing injury of twenty seconds duration, with rapid thawing in water at 42°C.

3. A group to receive a freezing injury

of sixty seconds duration, with rapid thawing in water at 42°C.

4. A group to receive a freezing injury of five seconds duration, with slow thawing at room temperature.
5. A group to receive a freezing injury of twenty seconds duration, with slow thawing at room temperature.
6. A group to receive a freezing injury of sixty seconds duration, with slow thawing at room temperature.

Although all groups are numbered the same throughout all other experiments, in this particular instance, the numbering is independent. On Day 1, the day of freezing, each animal in a group was marked characteristically on the ear, and initial weights were recorded as on the Data Sheet. All animals were given by subcutaneous injection from 0.1 cc. to 0.125 cc. of 0.6% nembutal solution. Then, when anaesthetized, one hind leg was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen (at this moment the rat invariably squirmed, in

spite of adequate anaesthetic), the time was counted so that the leg was immersed and frozen for exactly the required length of time, depending on the group to which the animal belonged. The animals of Groups 4, 5, and 6 were placed in their cages and the feet were allowed to thaw at room temperature, taking approximately fifteen or twenty minutes to become limp and warm. The animals of groups 1, 2, and 3 were treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the appropriate time interval in the ether , into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then these animals were also returned to their cages and all thirty six were left quietly alone until they wakened from the effects of the nembutal. Observations were made as recorded in the tables, by the method described above for estimating the degree of frostbite injury under the categories of oedema, colour change, exudate and slough, and gangrene.

Data Sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of the freezing. They are necessarily as compact as possible. Symbols are:

Grp	Gm	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
1	250	260	1	1	-	-	260	½	1	-	-	267	½	½	-	-
	174	169	2	1	-	-	167	1	1	-	-	170	1	1	-	-
	168	171	2	½	-	-	172	1	1	-	-	172	½	½	-	-
	232	236	2	1	-	-	234	1	1	-	-	233	1	1	-	-
	204	201	1	1	-	-	202	½	1	-	-	198	1	1	-	-
	224	220	1	1	-	-	222	½	1	-	-	219	½	1	-	-
2	204	228	3	2	-	-	225	3	2	1	-	234	3	2	-	-
	234	237	3	2	1	-	229	3	1	1	-	228	2	1	-	-
	175	175	3	1	1	-	176	3	1	1	-	177	3	1	-	-
	231	225	3	1	1	-	227	3	2	1	-	226	3	2	-	-
	180	182	3	1	1	-	180	3	2	1	-	182	2	2	-	-
	210	207	3	1	1	-	201	3	2	1	-	202	3	2	-	-
3	194	200	4	2	1	-	191	3	2	2	-	192	3	3	2	-
	248	251	3	2	1	-	242	3	3	1	-	238	3	4	2	-
	230	241	3	2	1	1	233	3	2	2	1	230	3	3	2	1
	183	186	3	3	1	-	180	3	4	2	-	176	3	4	2	-
	238	232	3	2	1	-	229	4	2	2	-	224	3	3	2	-
	175	173	3	2	1	-	170	3	3	2	-	170	3	3	2	-
4	220	215	3	2	1	-	216	2	2	2	-	210	1	1	1	-
	240	235	3	2	1	-	232	3	3	2	-	226	3	3	2	-
	164	168	3	1	1	-	162	2	1	-	-	165	2	3	-	-
	195	196	3	2	1	-	191	2	2	2	-	186	1	2	1	-
	205	203	3	3	2	-	199	3	2	2	-	192	2	2	1	-
	215	210	3	2	1	-	210	3	2	2	-	206	3	3	1	-
5	234	226	3	3	2	-	225	3	4	2	-	214	4	4	2	-
	174	170	3	3	2	-	164	3	4	2	-	155	3	3	2	-
	173	168	3	3	2	-	163	3	3	2	-	157	3	3	2	-
	210	210	3	2	1	-	210	3	2	2	-	205	3	3	2	-
	216	215	3	2	1	-	212	3	3	2	-	204	3	3	2	-
	178	178	3	3	1	-	175	3	3	2	-	176	3	3	1	-
6	235	232	3	4	2	-	226	3	4	2	1	222	3	4	2	1
	215	214	3	4	2	-	207	3	4	2	-	205	3	5	2	-
	240	242	3	4	2	-	234	3	3	2	-	224	3	4	2	-
	199	199	3	4	1	-	189	3	4	2	-	184	2	5	2	1
	238	228	3	4	1	-	224	3	4	2	-	214	1	5	2	1
	197	197	1	4	-	-	188	3	5	2	8	187	3	5	2	8
Day	1	2					3					4				

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
1	260	½	½	-	-	267	½	½	-	-	265	½	1	-	-
	163	1	1	-	-	160	½	½	-	-	163	½	½	-	-
	175	½	1	-	-	181	-	-	-	-	178	-	-	-	-
	225	½	½	-	-	226	½	½	-	-	228	½	½	-	-
	190	1	1	-	-	194	1	1	-	-	196	½	½	-	-
	214	½	1	-	-	216	½	½	-	-	220	½	½	-	-
2	225	2	2	-	-	215	1	1	-	-	224	1	1	-	-
	224	1	1	-	-	226	1	1	-	-	230	½	1	-	-
	172	1	1	1	-	178	1	1	-	-	179	1	1	-	-
	211	2	1	-	-	214	1	1	-	-	223	1	1	-	-
	179	1	1	-	-	175	1	1	-	-	176	1	1	-	-
	194	2	2	-	-	198	1	2	1	-	203	1	2	-	-
3	186	3	3	2	-	178	3	3	3	-	184	3	3	3	-
	236	3	3	2	-	224	3	3	1	-	226	3	2	1	-
	226	3	3	2	-	231	3	3	3	-	232	2	3	3	-
	Dead														
	221	3	3	2	-	226	3	3	3	-	228	3	3	1	-
	171	3	3	2	-	172	3	3	3	-	170	3	3	1	-
4	204	2	2	1	-	205	1	2	-	-	210	3	3	-	4
	218	2	3	1	-	220	1	3	-	-	230	1	3	-	-
	168	1	2	-	-	174	1	1	-	-	180	1	-	-	-
	187	½	1	-	-	186	1	1	-	-	188	1	1	-	-
	188	½	1	-	-	191	1	1	1	-	195	1	1	-	-
	204	2	1	-	-	203	2	2	-	-	203	2	1	-	-
5	210	3	4	3	3	211	3	3	-	4	210	3	3	-	4
	157	3	3	2	4	158	3	3	3	6	159	3	3	2	6
	160	3	3	1	-	163	3	3	3	5	168	3	3	1	5
	202	3	2	-	-	200	2	3	1	-	204	1	2	1	-
	200	3	3	1	-	200	3	3	1	-	204	2	3	-	1
	157	3	3	3	4	186	3	4	1	1	189	3	5	-	4
6	214	3	3	2	8	210	3	4	3	8	212	2	4	1	8
	195	2	5	2	6	197	3	4	3	8	203	2	4	2	8
	220	1	5	2	7	216	3	4	3	8	225	2	4	-	8
	185	1	4	2	8	184	-	3	-	8	182	-	-	-	8
	215	2	4	2	8	210	-	3	-	8	208	-	-	-	8
	190	1	2	-	8	190	-	3	-	8	185	-	-	-	8
Day	5					6					7				

Grp, group, Gm, grams, O, oedema, C, colour change, E, exudate, G, gangrene, -, means not present. All animals were disposed of with chloroform fumes on Day 7.

Summary Sheet

Following the Data Sheets is a Summary Sheet which gives the average weight for each group each day, and under I, or Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes together for each group each day. Note that the values for Group 3 on days 5, 6, and 7 are underlined to indicate an extrapolation to make up for the animal that died.

Graphs

The values on the Summary Sheet are plotted on two simple line graphs, the first for the Injury Index changes from day to day, and the second for the weight changes. These are done in colours for easier reading, but each line is otherwise clearly labelled by the nature of the injury, rather than by group numbers.

Condition of the Animals

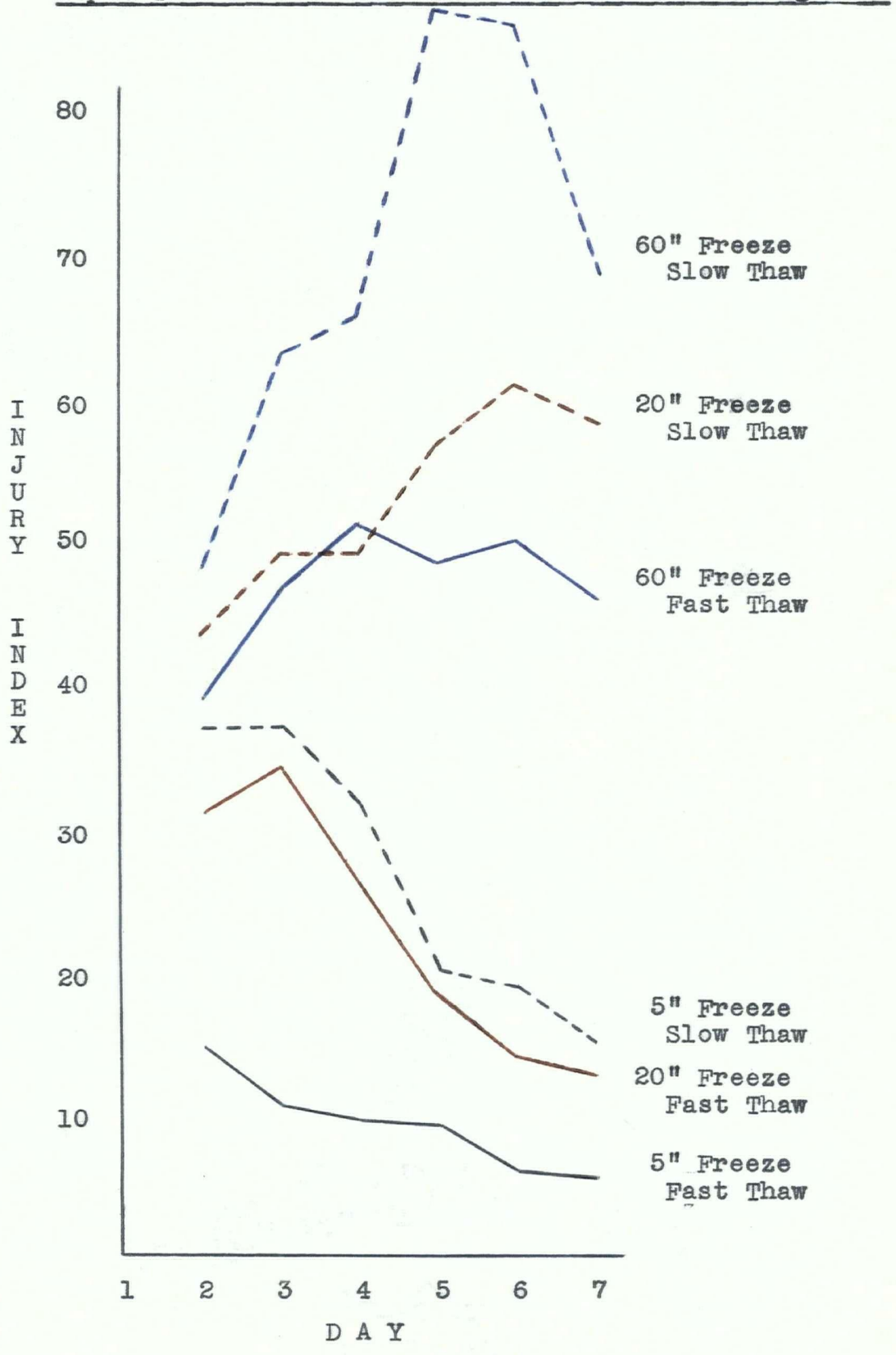
There is little need to include details of the rats themselves. Within a couple of hours of the freezing, all feet were grossly oedematous, bright

Summary Sheet

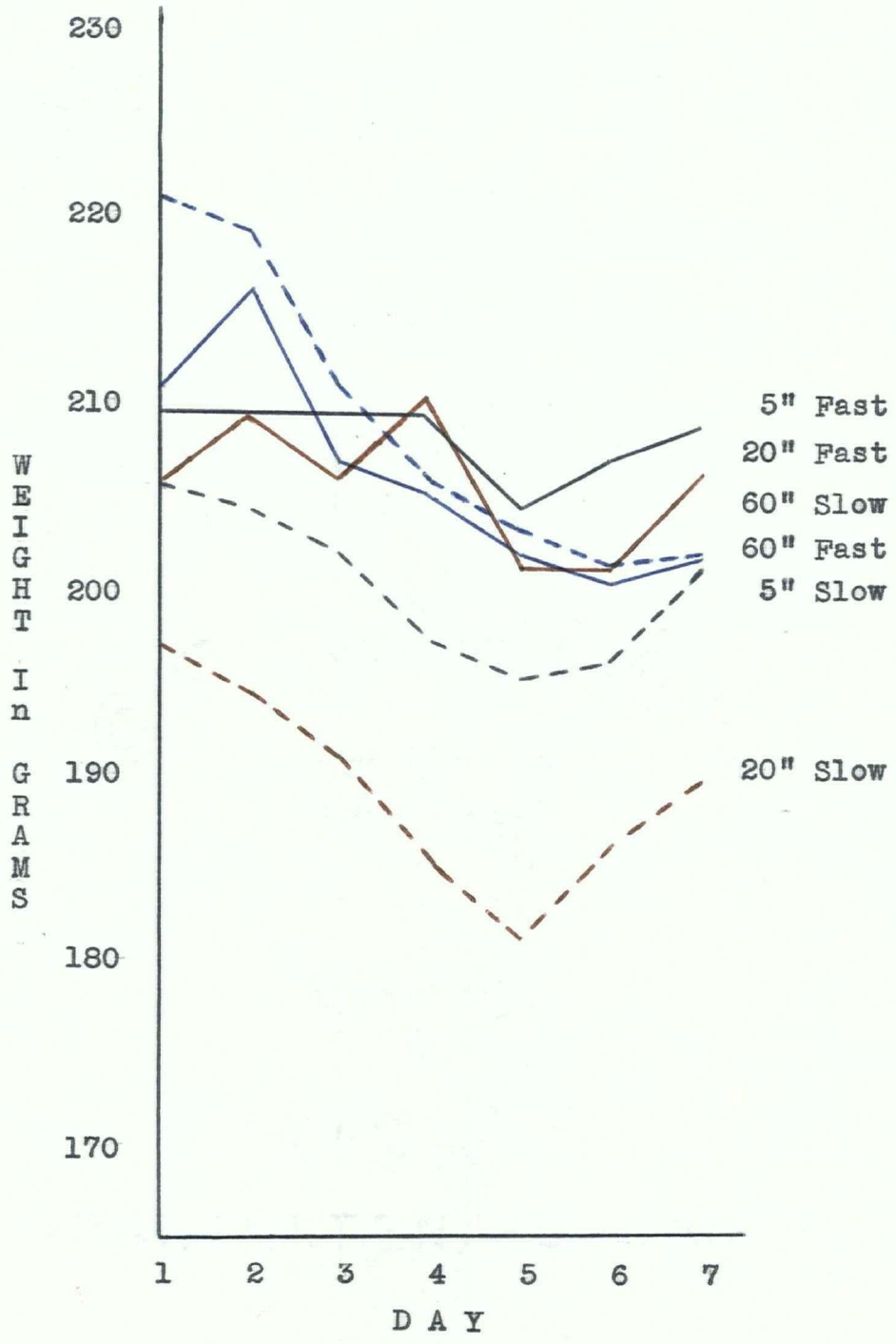
Grp	Gm	Gm	I	Gm	I	Gm	I
1	209	209	14½	209	10½	209	9½
2	206	209	31	206	34	210	26
3	211	216	39	207	47	205	51
4	206	204	37	202	37	197	32
5	197	194	43	191	49	185	49
6	221	219	48	211	63	206	66
Day	1	2		3		4	

Grp	Gm	I	Gm	I	Gm	I
1	204	9	207	6	208	5½
2	201	18	201	14	206	12½
3	202	48	200	50	202	46
4	195	20	196	19	201	15
5	181	57	186	61	189	58
6	203	88	201	87	202	69
Day	5		6		7	

Rapid Thaw Versus Slow Thaw - Effect of Freezing Time



Rapid Thaw Versus Slow Thaw - Effect of Freezing Time



pink, warm, and essentially nontender, although apparently numb for the animals hobbled around their cages apparently unaware of the positions of their injured feet. The day to day changes in the appearance of the of the feet may be readily imagined from the values on the Data Sheets. Generally the rats did well, and the graph showing weight changes inicates a fairly similar response through all groups. Individual rat weight changes mean little.

Discussion of Observations

The Injury Index graph gives a very clear separation of the various groups. An Index reading of 80 indicated a very severe reaction, and in this case the feet of Group 6's animals all sloughed off to the limits of the freezing, whereas, at the other extreme, by Day 7 it was almost impossible to detect any change in the feet of the animals of Group 1, except for a slight thickening of the skin over the dorsum of the paw.

There is first of all, as would be expected, a worse injury produced the longer the limb is frozen. The effect of the sixty second injury is worse than for the twenty, and the twenty worse than for the five. This response was consistent throughout the

graph.

Then there is secondly, well demonstrated, the great benefit to be derived from rapid thawing of the frozen limb in warm water at 42°C. This effect was also consistent throughout the graph.

Photographs

On Day 5, after a repeat dosage of the nembutal anaesthetic, all feet were photographed in colour by groups. The prints obtained were of too poor a quality to merit inclusion here. However, the picture of Group 5, labelled "Time 5" has been used earlier to illustrate how an Injury Index is calculated. It would be worthwhile to examine this page again in reference to this experiment.

Procaine

Experimental Observations with Procaine Hydrochloride

The purpose of the experiment here described was to test the effects of a standard cold injury as influenced by parenteral injections of procaine at a dosage level of 200 mg. per kilogram body weight administered hourly prior to the freezing injury and for forty-eight hours afterwards. Thirty normal adult male Wistar rats were selected, and they were divided into five groups of six animals each, with

each group designed as follows:

Group: 1. A group to receive a freezing injury of twenty seconds duration with slow thawing at room temperature.

2. A group to receive only the procaine injections as outlined.

3. A group to receive both the procaine injections and a freezing injury of twenty seconds duration with slow thawing at room temperature.

4. A group to receive a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A group to receive both the procaine injections and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

These groups are numbered identically throughout all subsequent gross experiments. Note that Group 1 here corresponds to Group 5 of the last experiment, and Group 4 here with Group 2 there. On day 1 of the experiment, the day of freezing, each animal was marked characteristically on the ear, and initial weights were recorded as on the Data Sheet. The animals of Groups 2, 3, and 5 received their initial

subcutaneous injection of procaine solution prepared at 20 mg. per cc. for ease in computing doses. Then, approximately twenty minutes later, the animals of Groups 1, 3, 4, and 5 received by subcutaneous injection an anaesthetizing dose of 0.6% nembutal solution ranging from 0.1 cc. to 0.125 cc. Then, when anaesthetized, one hind leg of each animal in these groups was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen (at this moment the rat invariably squirmed, in spite of adequate anaesthetic), the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals of Groups 1 and 3 were placed in their cages and the feet were allowed to thaw at room temperature, taking approximately fifteen or twenty minutes to become limp and warm. The animals of groups 4 and 5 were treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one

minute, until thoroughly thawed and warm. Then these animals were also returned to their cages and all thirty were left quietly alone (except for the hourly injections of procaine) until they wakened from the effects of the nembutal. Observations were made as recorded in the tables, by the method described above for estimating the degree of frostbite injury under the categories of oedema, colour change, exudate and slough, and gangrene.

Data sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. Days 1 and 2 include a record of the hourly injections. The symbols used are: Grp, group, Gm, grams, qlh, hourly, O, oedema, C, colour change, E, exudate and slough, G, gangrene, F, freezing, -, means not present or not administered, N, full dose of procaine, $\frac{1}{2}$, one half the dose of procaine, $\frac{1}{4}$, one quarter the dose of procaine (note that $\frac{1}{2}$ in the O C E G columns means half value). All animals not already dead were disposed of with chloroform fumes on Day 4.

Summary Sheet

Following the Data Sheets is a Summary Sheet

Data Sheet

Grp	Gm	qlh	Gm	O	C	E	G	qlh
1	264	-F----------	260	3	3	1	-	------
	310	-F----------	304	3	2	1	-	------
	180	-F----------	166	3	3	1	-	------
	287	-F----------	290	3	2	2	-	------
	254	-F----------	246	3	2	2	-	------
	318	-F----------	320	3	2	2	-	------
2	410	N-NNNNN---(Died)						
	295	N-NNNNN------	285					
	348	N-NNNNN----	335					
	241	N-NNNNN---	248					
	267	N-NNNNN---	254					
	268	N-NNNNN----	230					
3	279	NFNNNNN------ -(Died)						
	317	NFNNNNN (Died)						
	202	NFNNNNN------	192	2½	2	1	-	--(Died)
	306	NFNNNN--- (Died)						
	320	NFNNNN------	305	3	3	2	-	
	318	NFNNNN----	308	2½	3	1	-	
4	261	-F----------	262	3	1	1	-	------
	317	-F----------	312	3	1	1	-	------
	292	-F----------	286	3	1	1	-	------
	255	-F----------	250	3	1	1	-	------
	238	-F----------	232	3	1	1	-	------
	244	-F----------	240	3	1	1	-	------
5	283	NFNNNNN------	272	2½	1	1	-	
	242	NFNNNNN-N---	232	2½	1	-	-	
	210	NFNNNNN-----	204	2	1	-	-	
	282	NFNNNNN----	262	3	1	1	-	
	341	NFNNNNN-N---	335	2½	1	-	-	
	285	NFNNNNN----(Died)						

Day 1 2

Data Sheet

Grp	qlh	Gm	O	C	E	G	Gm	O	C	E	G
1	----------	270	3	3	2	-	260	3	4	2	-
	----------	310	3	2	2	1	303	3	3	3	1
	----------	165	3	3	2	-	164	3	4	2	-
	----------	295	3	3	1	-	292	3	3	2	-
	----------	252	3	3	1	-	249	3	4	2	-
	----------	323	3	3	2	-	311	3	4	3	-
2	(Dead)										
	----------	272					259				
	----------	323					303				
	----------	216					221				
	----------	241					245				
	----------	246					212	(Dying)			
3	(Dead)										
	(Dead)										
	(Dead)										
	(Dead)										
	----------	296	2	3	2	-	281	(Dead wt)			
	----------	297	2	4	-	-	274	2	3	1	-
4	----------	266	3	1	-	-	267	2	1	1	-
	----------	306	2	1	-	-	283	1	-	-	-
	----------	289	3	1	-	-	289	3	1	1	-
	----------	249	3	1	1	-	239	2	1	1	-
	----------	238	3	1	1	-	229	2	1	-	-
	----------	240	3	1	1	-	244	2	2	1	-
5	----------	270	1	1	-	-	262	2	1	-	-(Dead)
	----------	230	2	1	-	-	218	2	1	-	-(Dead)
	----------	199	2	-	-	-	196	2	1	-	-(Dead)
	----------	258	3	1	-	-	237	3	1	-	-
	----------(Dead)										
	(Dead)										

Day	2 (Cont)	3	4

Summary Sheet

Grp	Gm	Gm	I	Gm	I	Gm	I
1	269	264	41	269	46	263	55
2	284	270		259		246	
3	319	306	40	296	38	277	36
4	268	264	30	265	26	258	22
5	254	242	24	239	16½	226	19½
Day	1	2		3		4	

which gives the average weight for each group each day, and under I, or Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes apparent together for each group each day. Except for Groups 1 and 3 where no animals expired, all I values had to be extrapolated up to correspond to six animals per group. As will be explained below, this seriously reduces the signif icance of the data.

Graphs

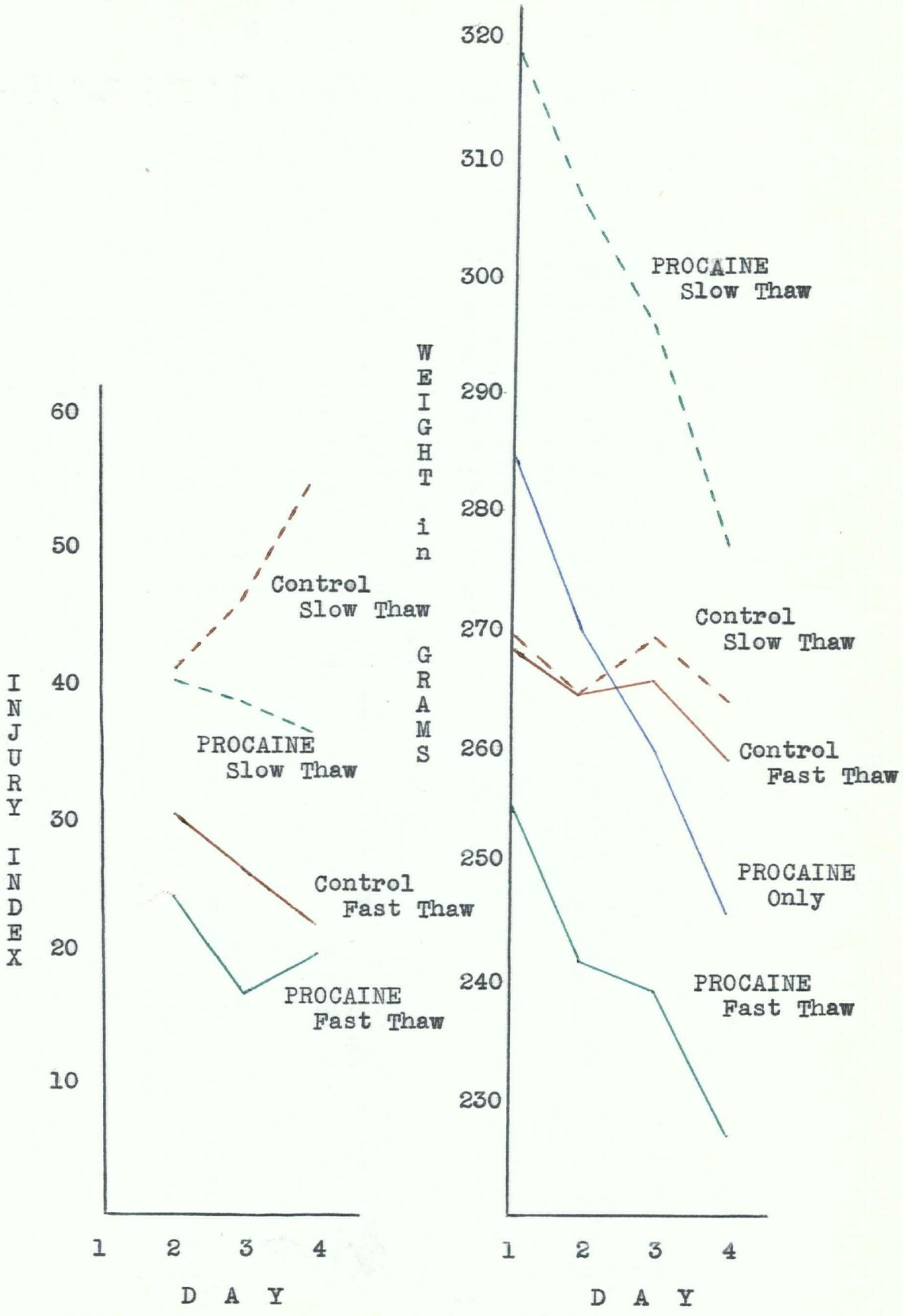
The values on the Summary Sheet are plotted on two simple line graphs, the first for the Injury Index changes from day to day, and the second for the weight changes. These are done in colours for easier reading (except for the Time graphs of the last experiment these and all succeeding gross graphs have identical colours), but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers.

Condition of the Animals

It will be obvious from the Data Sheets that the procaine in the dosage given took a terrible toll of the animals. Many, after the sixth dose, were comatose and in convulsions. This explains why such drastic reductions in the medication were necessary. Many died, and of those surviving, many sat limply huddled in their cages, weakly protesting every disturbance. The day to day changes in the appearance of the feet may be imagined from the values on the Data Sheets. These do not reflect the pitiful condition of the animals.

Discussion of Observations

Procaine in the dosage used is too toxic to be practical.


INJURY INDEX
60
50
40
30
20
10
Control
Slow Thaw
PROCAINE
Slow Thaw
Control
Fast Thaw
PROCAINE
Fast Thaw
1
2
3
4
D A Y
WEIGHT in GRAMS
320
310
300
290
280
270
260
250
240
230
PROCAINE
Slow Thaw
Control
Slow Thaw
Control
Fast Thaw
PROCAINE
Only
PROCAINE
Fast Thaw
1
2
3
4
D A Y

The Injury Index graph confirms the impression that rapid thawing at 42°C reduces the severity of the injury reaction. This is consistent both with the procaine treated groups and with the control groups.

Procaine treatment administered as outlined apparently resulted in less injury than was shown by the controls. The fact that the graphs for the procaine treated animals lie under the corresponding control graphs supports this claim. However, two animals never make a group, and the lack of any significance in these graphs is realized. They are included merely for interest.

The weight graphs attest to the toxic effects of the procaine. The procaine treated animals show a steady and rapid weight loss, while the controls tend to maintain their weight more steadily.

Procaine and Priscoline

Experiments with Procaine (Repeat) and Priscoline

The purpose of the experiment here described was to test the effects of a standard cold injury on the hind legs of rats as influenced by two different drugs. Because of the toxicity and mortality of the

procaine at the dosage level used in the last experiment (200 mg. per kilogram body weight) it was decided necessary to repeat the experiment using the procaine at half the dosage, viz. 100 mg. per kilogram, but administered by subcutaneous injection three hourly. The effects of priscoline were also tested at a dosage level of 50 mg. per kilogram body weight also administered subcutaneously at three hourly intervals. Thirty normal adult nonpregnant female Wistar rats were selected, and they were clearly divided into two groupings: one of eighteen animals for testing with procaine, and the other of twelve animals for the Priscoline tests. Each division was divided into groups of six animals each, with each group designed as follows:

Group: 2. A group to receive only the procaine injections as outlined.

4. A group to receive a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A group to receive both the procaine injections and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

Group: 4. A group to receive a freezing injury

of twenty seconds duration with rapid thawing in water at 42°C.

(Group) 5. A group to receive both Priscoline injections as outlined and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

These group numbers are identical throughout all gross experiments (except the first). On the day the freezing was done, Day 1, the animals of each group were marked characteristically on the ear, and initial weights were recorded as on the Data Sheet. The animals of Group 2 and both Groups 5 received two initial injections of their appropriate drug. The procaine solution was in a concentration of 10 mg. per cc. and the Priscoline was 2 mg. per cc. Then one hour approximately after the second dose of the drugs the animals of both Groups 4 and both Groups 5 were anaesthetized with 0.1 to 0.125 cc. of 0.6% nembutal solution administered subcutaneously. When anaesthetized, one hind leg of each animal in these groups was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched,

indicating the foot was completely frozen (at this moment the rat invariably squirmed, in spite of adequate anaesthetic), the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. All animals were treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then these animals were returned to their cages and left quietly alone (except for the three hourly injections) until they wakened from the effects of the nembutal. Observations were made as recorded in the tables, but the method described above for estimating the degree of frostbite injury under the categories of oedema, colour change, exudate and slough, and gangrene.

Data sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. Days 1, 2, and 3 include a record of the three hourly injections. The symbols used are: Grp, group, Gm, grams,

Data Sheet

Procaine

Grp	Gm	q3h	O	C	E	G	q3h	Gm	O	C	E	G	q3h
2	197	NN-NNNNNNNNN					NNNNNN	182					NNNN
	184	NN-NNNNNNNNN					NNNNNN	175					NNNN
	194	NN-NNNNNNNNN					NNNNNN	175					NNNN
	178	NN-NNNNNNNNN					NNNNNN	168					NNNN
	178	NN-NNNNNNNNN					NNNNNN	165					NNNN
	181	NN-NNNNNNNNN					NNNNNN	168					NNNN
4	188	--F---------	3	2	1	-	------	177	3	1	1	-	----
	195	--F---------	3	3	2	-	------	187	3	4	2	-	----
	170	--F (Died)											
	186	--F---------	3	2	1	-	------	175	3	2	1	-	----
	173	--F---------	3	3	-	-	------	161	3	3	1	-	----
	191	--F---------	3	2	-	-	------	181	2	1	-	-	----
5	201	NNFNNNNNNNNN	3	1	-	-	NNNNNN	187	3	1	-	-	NNNN
	189	NNFNNNNNNNNN	3	1	1	-	NNNNNN	174	2	1	-	-	NNNN
	170	NNF (Died)	3	2	-	-							
	207	NNFNNNNNNNNN	3	2	-	-	NNNNNN	190	3	3	-	-	NNNN
	201	NNFNNNNNNNNN	3	2	1	-	NNNNNN	187	3	2	-	-	NNNN
	194	NNFNNNNNNNNN	3	1	1	-	NNNNNN	182	3	1	-	-	NNNN
Day	1		2					3					

Priscoline

Grp	Gm	q3h	O	C	E	G	q3h	Gm	O	C	E	G	q3h
4	192	--F---------	3	3	1	-	------	187	3	2	1	-	----
	146	--F---------	3	3	1	-	------	150	3	3	1	-	----
	182	--F---------	3	3	2	-	------	175	3	3	1	-	----
	195	--F---------	3	2	2	-	------	192	3	2	1	-	----
	198	--F---------	3	1	2	-	------	194	3	1	-	-	----
	195	--F---------	3	2	2	-	------	180	3	2	-	-	----
5	185	PPFPPPPPPPPP	3	3	-	-	PPPPPP	181	3	3	1	-	PPPP
	204	PPFPPPPPPPPP	3	1	-	-	PPPPPP	198	3	2	1	-	PPPP
	197	PPFPPPPPPPPP	3	2	1	-	PPPPPP	190	3	1	-	-	PPPP
	184	PPFPPPPPPPPP	3	3	1	-	PPPPPP	179	3	3	-	-	PPPP
	205	PPFPPPPPPPPP	3	2	2	-	PPPPPP	198	3	2	1	-	PPPP
	206	PPFPPPPPPPPP	3	2	1	-	PPPPPP	200	3	1	1	-	PPPP
Day	1		2					3					

Data Sheet

Procaine

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
2	175					188					176					217				
	168					182					170					216				
	165					178					170					212				
	163					178					177					217				
	155					174					174					208				
	159					174					175					211				
4	168	2	2	1	-	174	2	2	2	-	173	2	2	2	-	175	1	2	3	-
	178	3	3	2	-	176	2	4	3	6	184	2	3	-	6	186	1	2	-	6
	(Dead)																			
	168	2	2	1	-	176	1	2	1	-	162	1	1	-	-	187	1	2	2	-
	155	2	3	1	-	164	1	3	-	-	168	1	2	-	-	184	1	4	1	-
	176	1	1	-	-	180	1	1	-	-	186	1	1	-	-	202	1	1	-	-
5	179	2	1	-	-	190	2	1	-	-	198	1	1	-	-	228	2	3	-	-
	167	2	1	-	-	174	2	2	-	-	178	1	2	-	-	198	2	2	1	-
	(Dead)																			
	182	3	3	1	-	190	2	1	-	-	194	2	3	-	-	208	2	3	-	-
	181	2	1	1	-	190	3	3	2	-	192	1	1	-	-	212	2	2	1	-
	176	2	2	1	-	190	2	1	-	-	197	1	1	-	-	214	2	2	3	-
Day	4					6					8					10				

Priscoline

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
4	184	2	2	-	-	196	1	3	1	-	205	1	2	-	-	228	1	2	-	-
	146	3	3	-	-	131	2	3	-	-	132	1	2	1	-	140	1	1	-	1
	171	3	4	2	-	172	3	4	2	-	176	3	3	1	-	193	2	3	3	-
	188	1	2	-	-	192	-	1	-	-	185	1	1	-	-	214	1	1	-	-
	184	2	2	-	-	202	-	2	-	-	204	1	2	1	-	235	1	2	-	-
	178	2	2	-	-	180	2	2	-	-	181	1	1	-	-	200	1	2	-	-
5	175	3	3	1	-	178	3	3	1	-	185	2	3	-	-	202	2	2	-	-
	188	2	1	-	-	190	1	1	-	-	197	1	1	-	-	207	1	2	-	-
	184	1	1	1	-	181	2	3	2	-	188	1	3	1	-	210	1	2	3	-
	172	3	2	1	-	172	2	2	-	-	170	1	2	-	-	194	1	2	2	-
	194	3	2	1	-	195	2	2	2	-	196	2	2	-	-	210	2	3	3	1
	197	3	2	1	-	187	2	2	1	-	194	1	2	1	-	205	1	3	2	-
Day	4					6					8					10				

Data Sheet

Procaine

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
2	201					207					220				
	203					205					210				
	198					201					213				
	207					199					205				
	194					201					200				
	193					194					200				
4	172	1	2	3	-	180	1	2	3	-	192	-	-	-	-
	181	1	1	-	6	186	1	1	-	6	189	1	1	-	6
	(Dead)														
	169	1	-	3	-	173	1	1	2	-	190	1	-	-	-
	167	1	3	3	-	175	-	2	-	3	183	-	-	-	1
	189	-	1	-	-	197	-	-	-	-	200	-	-	-	-
5	210	-	1	-	-	219	1	1	-	-	219	-	1	-	-
	180	1	3	-	-	184	1	2	1	-	197	1	1	2	-
	(Dead)														
	190	2	3	-	-	192	1	3	1	-	207	2	2	3	5
	202	2	2	1	-	204	2	2	1	-	220	1	2	1	-
	199	2	2	2	-	205	3	2	3	-	206	2	2	1	5
Day	11					12					13				

Priscoline

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
4	212	1	2	-	-	214	1	2	-	-	210	1	1	-	-
	133	1	2	1	-	134	1	2	-	-	149	1	2	1	1
	178	1	3	1	-	182	2	3	2	-	186	2	2	3	2
	193	-	1	-	-	193	-	1	-	-	210	-	-	-	-
	221	-	2	-	-	230	-	1	-	-	214	-	-	-	-
	184	-	2	-	-	191	1	1	-	-	210	-	-	-	-
5	180	1	2	1	-	180	1	2	2	-	200	-	-	-	1
	194	-	1	-	-	194	-	-	-	-	206	1	1	-	-
	197	1	2	-	-	197	1	2	1	-	214	-	1	-	-
	184	-	2	2	-	189	1	2	1	-	180	1	1	1	-
	195	2	2	3	-	202	2	2	3	-	210	1	2	3	2
	194	1	2	2	-	198	1	2	1	-	200	-	1	-	-
Day	11					12					13				

q3h, every three hours, O, oedema, C, colour change, E, exudate and slough, G, gangrene, N, injection of procaine, F, freezing, -, means not present or not administered, P, injection of Priscoline. All surviving animals were disposed of on Day 13 with chloroform fumes.

Summary Sheet

Following the Data Sheets is a Summary Sheet which gives the average weight for each group each day, and under I, or Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes apparent together for each group each day. Where an animal died, the Injury Index was extrapolated to raise the value to what could be expected for a complete group.

Graphs

The values on the Summary Sheet are plotted on two simple line graphs, the first for procaine and the second for Priscoline. The weight curves and the Injury curves are put onto the same corresponding graph, and plotted against time. The X marks on the graphs indicate missed days to permit the graph to

Summary Sheet

Procaine

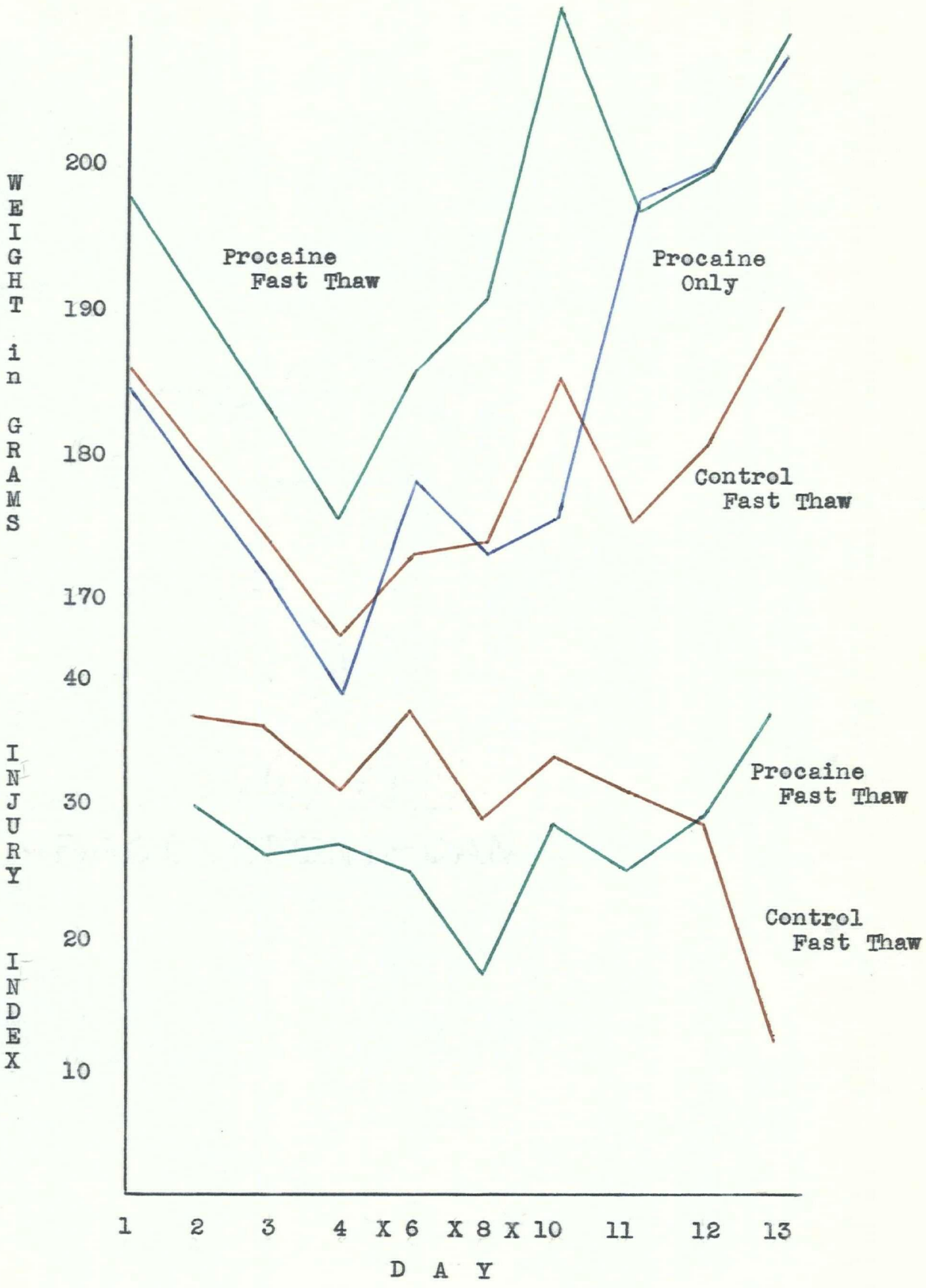
Grp	Gm	I	Gm	I	Gm	I	Gm	I	Gm	I
2	185		172		164		179		174	
4	187	37	175	36	168	31	174	37	175	29
5	198	30	184	26	177	27	187	25	192	17
Day	1		2		3		4		6	
	8									

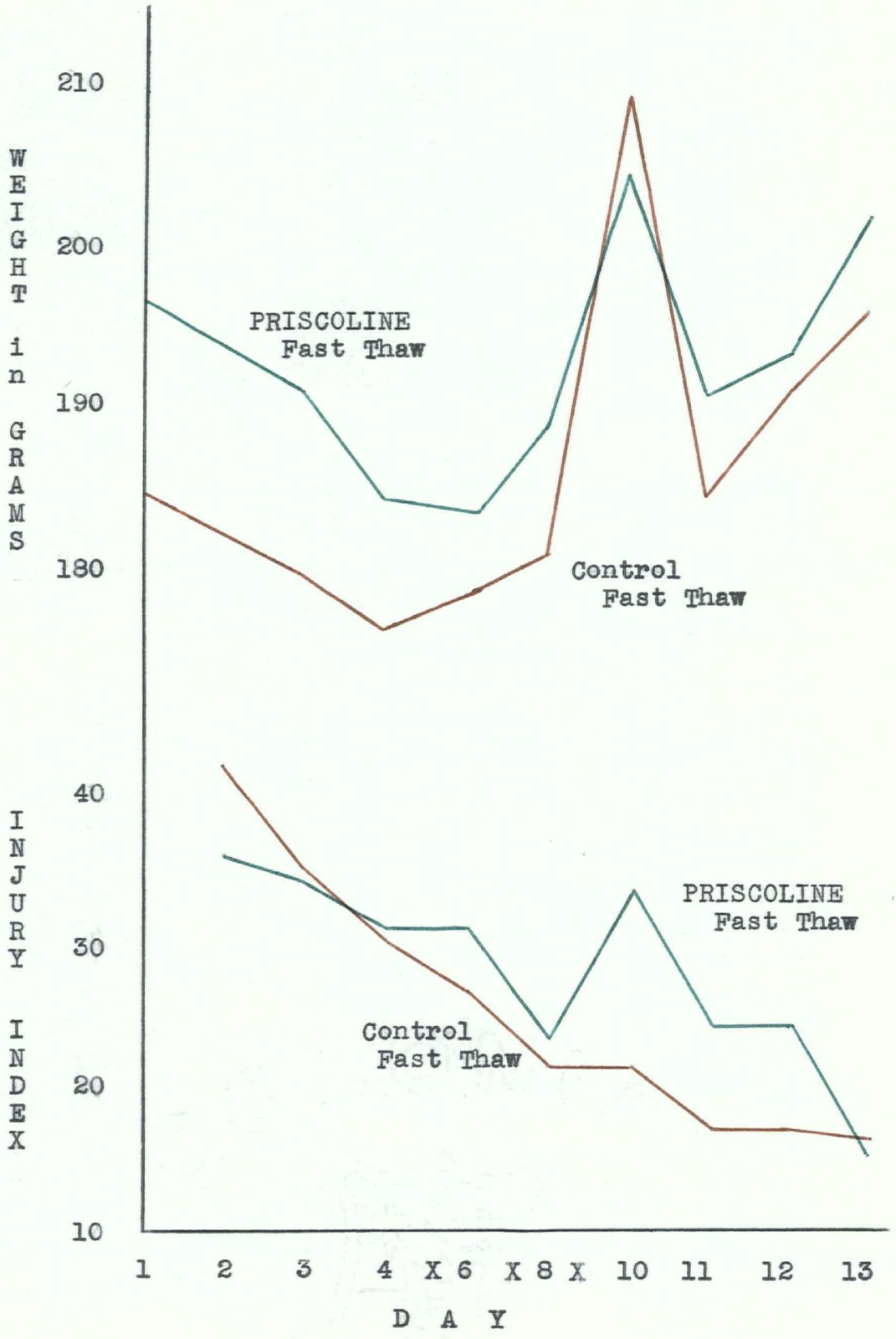
Grp	Gm	I	Gm	I	Gm	I	Gm	I
2	177		199		201		208	
4	187	34	176	31	182	28	191	12
5	212	28	198	25	201	29	210	37
Day	10		11		12		13	

Priscoline

Grp	Gm	I	Gm	I	Gm	I	Gm	I	Gm	I
4	185	42	180	35	177	30	179	26	181	22
5	197	36	191	34	185	31	184	31	188	23
Day	1		2		3		4		6	
	8									

Grp	Gm	I	Gm	I	Gm	I	Gm	I
4	210	22	185	17	191	17	196	16
5	205	33	191	24	193	24	202	15
Day	10		11		12		13	


WEIGHT in GRAMS
200
190
180
170
Procaine
Fast Thaw
Procaine
Only
Control
Fast Thaw
40
30
20
10
INJURY
INDEX
Procaine
Fast Thaw
Control
Fast Thaw
1
2
3
4
X 6
X 8 X 10
11
12
13
D A Y


WEIGHT in GRAMS
210
200
190
180
PRISCOLINE
Fast Thaw
Control
Fast Thaw
INJURY INDEX
40
30
20
10
PRISCOLINE
Fast Thaw
Control
Fast Thaw
1
2
3
4
X 6
X 8 X
10
11
12
13
D A Y

be contained within one page. These graphs are done in colours for easier reading (all graphs have identical colours except for the Time graphs of the first experiment), but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers.

Condition of the Animals

The animals receiving procaine were in much better condition on the reduced dosage received less often. However, they were somewhat affected, appearing rather out or sorts, and less active than healthy rats should be. The Pirscoline had no observable ill effect on the group of animals receiving it.

Discussion of Observations

A casual inspection of the Injury Index graphs for procaine and Priscoline would give the impression that procaine protected the rat legs and that the Priscoline permitted a worse injury. The key to the question lies in the fact that Injury Index lines for the control groups, Groups 4, on both the procaine and the Priscoline graphs should be more similar than they are, considering the fact they are for identical groups receiving the standard freezing injury, follow-

ed by rapid thawing, without any medication. Actually there was little significant difference in the appearance of the various groups of animals.

Weight changes were comparable between the various groups, indicating that none were adversely affected by the drugs.

Benadryl

Experimental Observations with Benadryl

The purpose of the experiment here described was to test the effects of a standard cold injury as influenced by parenteral injections of Benadryl at a dosage level of 2 mg. per kilogram body weight administered subcutaneously at three hour intervals prior to the freezing injury and for forty-eight hours afterwards. Thirty-six normal adult nonpregnant female Wistar rats were selected, and they were divided into five groups with each group designed as follows:

Group: 1. A group of eight animals to serve as a control group and to receive a freezing injury of twenty seconds duration with slow thawing at room temperature.

2. A group of five animals to serve as a

control also, and to receive only the Benadryl injections as outlined.

3. A group of eight animals to receive both the Benadryl injections and a freezing injury of twenty seconds with slow thawing at room temperature.
4. A group of eight animals to serve as a control, to receive a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.
5. A group of seven animals (one of the eight originally set aside died when being anaesthetized with nembutal, and was eliminated completely from the records) to receive both the Benadryl injections and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

The larger number of animals being used was decided on because several other animals in the same room had chest conditions and it was feared a few of the animals in this experiment might later develop infection and have to be discarded, hence two extra rats were added to each group as spares. Group 2 is small because there were not enough animals to fill the desired quota. These

groups are numbered identically throughout all subsequent gross experiments. On Day 1 of the experiment, the day of freezing, each animal was marked characteristically on the ear, and initial weights were recorded as on the Data Sheet. The animals of Groups 2, 3, and 5 received their initial subcutaneous injection of Benadryl solution prepared by dilution of the Parke-Davis product containing 10 mg. per cc. to a more convenient strength of 2 mg. per cc. and each animal was given 0.1 cc. for each 100 grams of body weight. By using a tuberculin syringe graduated in hundredths of a cc. it was a simple matter to inject the correct amount just by giving the animal's weight in 1/100's of a cc. The injections were given every three hours for a total of eighteen injections, lasting from half past ten o'clock of Day 1 to half past one o'clock on Day 3, as noted on the Data Sheet. Groups 2 and 3 missed their injection once, at half past seven o'clock on the evening of Day 2 because of a shortage of the drug. The animals were kept in five cages, all the animals of one group in the same cage.

At least one hour after the initial Benadryl injection the animals in Groups 1, 3, 4, and 5 were given subcutaneously from 0.07 cc. to 0.1 cc. of 0.6% nembutal solution. Then, when anaesthetized, one hind leg of each animal in these groups was dipped in a

beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen (at this moment the rat invariably squirmed, in spite of adequate anaesthesia), the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals of Groups 1 and 3 were placed in their cages and the feet were allowed to thaw at room temperature, taking approximately fifteen or twenty minutes to become limp and warm. The animals of groups 4 and 5 were treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then these animals were also returned to their cages and all thirty-six were left quietly alone (except for the three hourly injections of Benadryl) until they wakened from the effects of the nembutal. Observations were made as recorded on the Data Sheets, by the method described above for estimating the degree of frostbite injury under the categories of oedema,

colour change, exudate and slough, and gangrene.

Data Sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. Days 1 and 2 include a record of the three hourly injections. The symbols used are: Grp, group, Gm, grams, q3h, every three hours, O, oedema, C, colour change, E, exudate and slough, G, gangrene, F, freezing, -, means not present or not administered, N, anaesthetic, B, Benadryl injection. All surviving animals were disposed of on Day 10 with chloroform fumes.

Summary Sheet

Following the Data Sheets is a Summary Sheet which in this experiment does two things. Because of the unusually large number of animals per group, the Summary Sheet first summates the values each day by groups, and under I, or Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes apparent together for each group each day. In the lower half of the Summary Sheet the values are interpolated down for a

Data Sheet

Grp	Gm	q3h	O	C	E	G	q3h	O	C	E	G
1	233	-NF--------	3	3	-	-	--------	3	4	2	2
	288	-NF--------	3	2	-	-	--------	3	3	1	2
	284	-NF--------	3	4	1	-	--------	3	4	2	2
	298	-NF--------	3	2	-	-	--------	3	2	1	-
	250	-NF--------	3	1	-	-	--------	3	3	2	-
	317	-NF--------	3	3	-	-	--------	3	3	I	-
	276	-NF--------	3	1	1	-	--------	3	1	2	-
	276	-NF--------	3	3	-	-	--------	3	3	1	1
2	280	B--BBBBBBBB					BB-BBBBB				
	325	B--BBBBBBBB					BB-BBBBB				
	272	B--BBBBBBBB					BB-BBBBB				
	272	B--BBBBBBBB					BB-BBBBB				
	253	B--BBBBBBBB					BB-BBBBB				
3	218	BNFBBBBBBBB	3	1	-	-	BBBBBBBB	3	3	2	-
	326	BNFBBBBBBBB	3	1	-	-	BBBBBBBB	3	4	2	1
	305	BNFBBBBBBBB	3	3	-	-	BBBBBBBB	3	4	2	1
	308	BNFBBBBBBBB	3	3	-	-	BBBBBBBB	3	4	2	1
	272	BNFBBBBBBBB	3	3	-	-	BBBBBBBB	3	4	2	1
	190	BNFBBBBBBBB	3	2	-	-	BBBBBBBB	3	4	2	1
	280	BNFBBBBBBBB	3	3	-	2	BBBBBBBB	3	5	3	5
	188	BNFBBBBBBBB	3	2	-	-	BBBBBBBB	3	5	2	2
4	302	-NF--------	3	1	-	-	--------	3	1	1	-
	260	-NF--------	3	1	-	-	--------	3	1	1	-
	308	-NF--------	3	1	-	-	--------	3	3	1	-
	250	-NF--------	3	1	-	-	--------	3	1	1	-
	315	-NF--------	3	1	-	-	--------	3	1	1	1
	274	-NF--------	3	1	-	-	--------	3	1	1	-
	280	-NF--------	3	1	-	-	--------	3	1	-	-
	280	-NF--------	3	1	-	-	--------	3	1	1	-
5	232	BNF Died									
	275	BNFBBBBBBBB	3	1	-	-	BB-BBBBB	3	2	1	-
	254	BNFBBBBBBBB	3	1	-	-	BB-BBBBB	3	1	1	-
	214	BNFBBBBBBBB	3	1	-	-	BB-BBBBB	3	2	1	-
	268	BNFBBBBBBBB	3	2	-	-	BB-BBBBB	3	3	1	-
	246	BNFBBBBBBBB	3	I	-	-	BB-BBBBB	3	2	2	-
	242	BNFBBBBBBBB	3	1	-	-	BB-BBBBB	3	1	-	-
	196	BNFBBBBBBBB	3	1	-	-	BB-BBBBB	3	2	2	-
Day	1		2					3			

Data Sheet

Grp	Gm	q3h	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
1	216	B	3	4	2	-	215	3	3	2	1	210	3	5	-	3	205	3	3	2	5
	280	B	3	3	1	-	275	3	3	1	-	250	2	5	-	4	260	2	1	-	4
	280	B	3	5	2	-	280	2	5	1	-	270	3	5	-	5	270	3	1	2	5
	280	B	2	3	2	-	260	1	5	-	3	270	2	5	-	5	280	2	1	-	5
	240	B	3	3	1	-	230	3	3	2	-	230	3	5	-	5	225	2	1	-	5
	305	B	3	3	2	-	290	3	4	1	-	285	3	5	-	5	280	3	2	-	4
	265	B	3	4	2	-	260	3	3	2	-	250	2	5	-	4	245	2	1	0	5
	270	B	3	3	1	-	270	3	3	1	-	260	3	3	1	-	255	3	3	-	5
2	290	B					280					280					275				
	330	B					320					320					310				
	260	B					265					260					265				
	270	B					270					270					270				
	250	B					250					245					250				
3	210	B	3	5	2	4	205	2	3	-	4	200	2	3	1	5	200	3	1	-	4
	305	B	3	5	3	1	325	2	3	1	1	280	2	5	-	4	285	2	2	-	4
	290	B	3	5	2	-	280	2	3	2	1	280	2	3	2	2	285	2	2	1	3
	300	B	3	5	2	2	290	2	5	-	4	285	3	5	-	5	265	3	3	2	5
	260	B	3	5	3	-	250	2	3	2	-	250	3	4	3	4	245	3	2	2	5
	175	B	2	3	3	-	175	2	3	1	-	180	1	2	2	-	170	1	2	-	-
	260	B	3	5	3	5	260	2	4	-	5	260	2	5	-	5	260	3	2	-	5
	165	B	2	5	2	2	160	1	5	1	4	170	2	2	1	-	180	3	2	1	5
4	300	-	2	1	-	-	295	1	1	-	-	280	-	1	1	-	280	-	1	-	-
	250	-	1	1	-	-	245	1	1	-	-	250	1	2	1	-	250	-	1	-	-
	310	-	3	3	2	-	300	2	3	1	-	265	1	2	2	-	280	1	1	-	-
	240	-	2	3	-	-	230	1	3	2	-	235	1	3	1	-	225	-	1	1	-
	295	-	2	1	-	1	280	1	-	-	1	290	1	1	-	1	305	1	1	-	1
	275	-	3	3	1	-	265	2	2	2	-	260	1	2	1	-	250	-	1	-	-
	275	-	2	1	-	-	275	1	1	-	-	260	1	1	-	-	260	-	1	-	-
	270	-	2	2	-	-	255	1	1	1	-	250	1	1	-	-	270	1	1	-	-
5	(Dead)																				
	265	B	3	2	1	-	260	2	2	-	-	240	1	2	1	-	235	1	2	-	-
	240	B	3	2	1	-	230	2	3	1	-	235	1	1	1	-	225	1	1	-	-
	220	B	3	3	1	-	220	2	3	1	-	220	1	2	2	-	220	1	3	-	-
	260	B	2	3	-	-	250	1	4	-	-	250	1	4	1	-	240	1	2	-	-
	240	B	2	1	1	-	230	1	1	-	-	235	-	2	1	-	230	1	2	-	-
	230	B	3	1	-	-	235	2	1	-	-	230	-	1	-	-	230	-	1	-	-
	180	B	Died																		

Day	3(cont)	4	5	7	10

Totals Sheet

Grp	Gm	I	Gm	I	I	Gm	I	Gm	I
1	2222	45	2136	66	64	2080	59	2025	91
2	1402		1400			1385		1375	
3	2087	44	1965	86	95	1945	70	1905	85
4	2269	32	2215	42	36	2145	29	2090	27
5	1526	29	1455	42	32	1425	26	1410	22
Day	1	2	3		4	5		7	

Grp	Gm	I
1	2020	75
2	1370	
3	1890	73
4	2110	13
5	1380	16
Day	10	

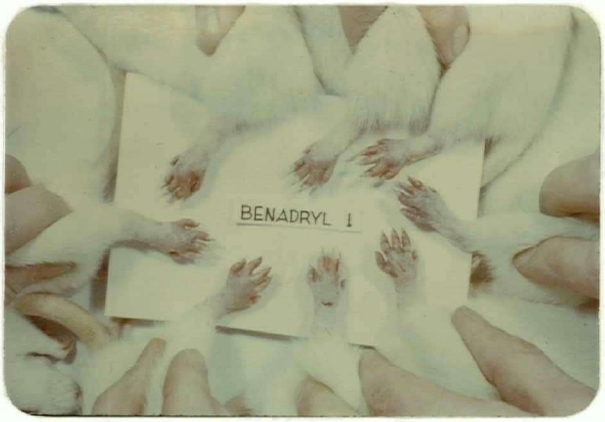
Summary Sheet

Grp	Gm	I	Gm	I	I	Gm	I	Gm	I	Gm	I
1	277	33	267	49	48	260	45	253	69	252	57
2	280		280			277		275		274	
3	261	33	246	64	72	243	52	238	63	236	54
4	284	24	277	30	27	268	21	261	21	264	9
5	254	25	242	37	32	237	26	235	22	230	16
Day	1	2	3		4	5		7		10	

theoretical six animal group, and average weights were obtained by dividing the total weight for the group by the number of animals in the group.

Photographs

On Day 3, at approximately three o'clock in the afternoon, all animals were again anaesthetized with the same dosage of 0.6% nembutal solution, and were photographed in colour. Colour prints of Groups 1, 2, 3, and 4 are attached below, but unfortunately the picture of Group 5 was improperly exposed and did not develop. The feet can be compared with the corresponding entries on the Data Sheets on Day 3.




BENADRYL 2


BENADRYL 3


BENADRYL 4

Graphs

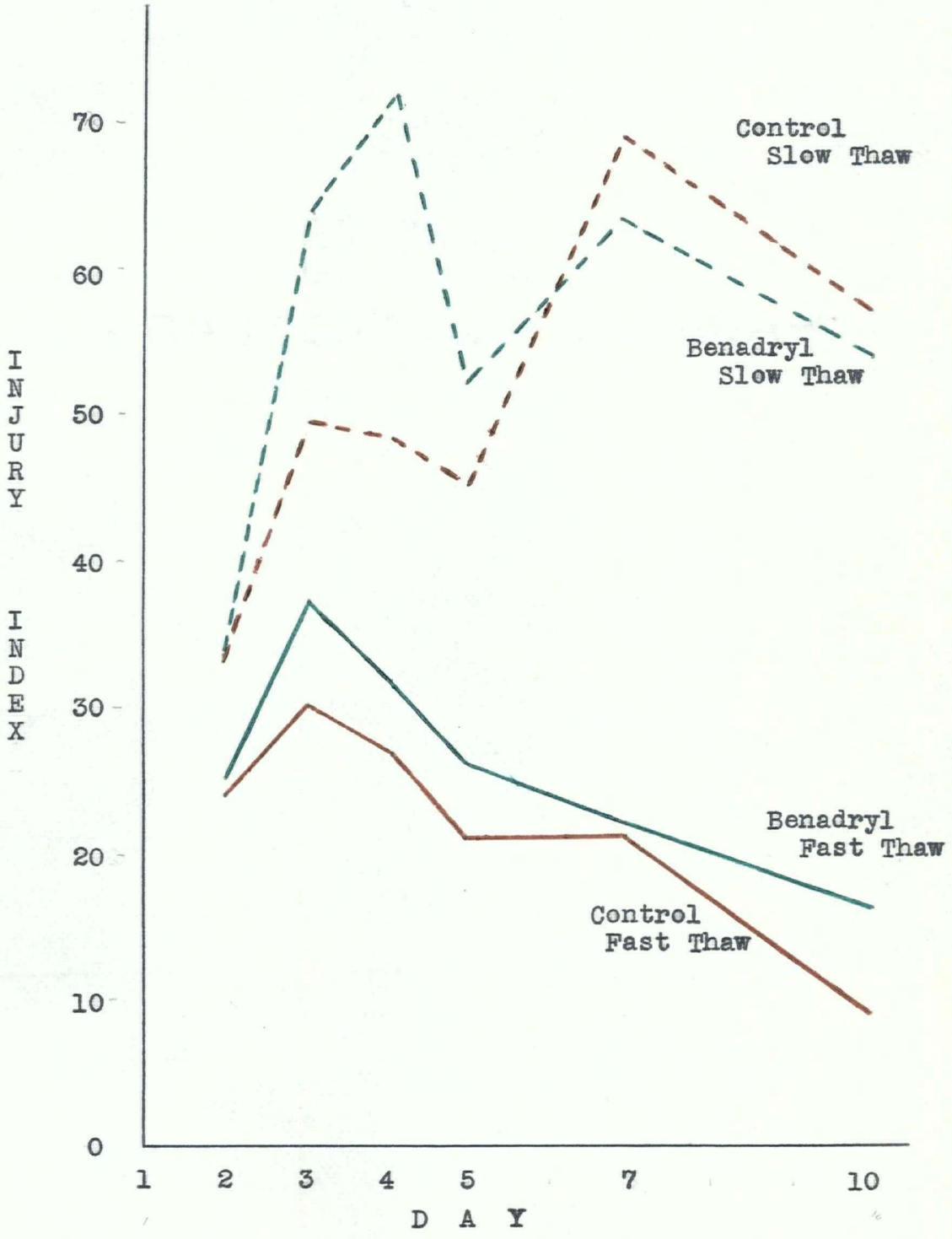
The final interpolated values on the Summary Sheet are plotted on two simple line graphs, the first for the Injury Index changes from day to day, and the second for the corresponding weight changes. These are done in colours for easier reading (except for the Time graphs of the first experiment, these and all other gross graphs have identical colours), but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers.

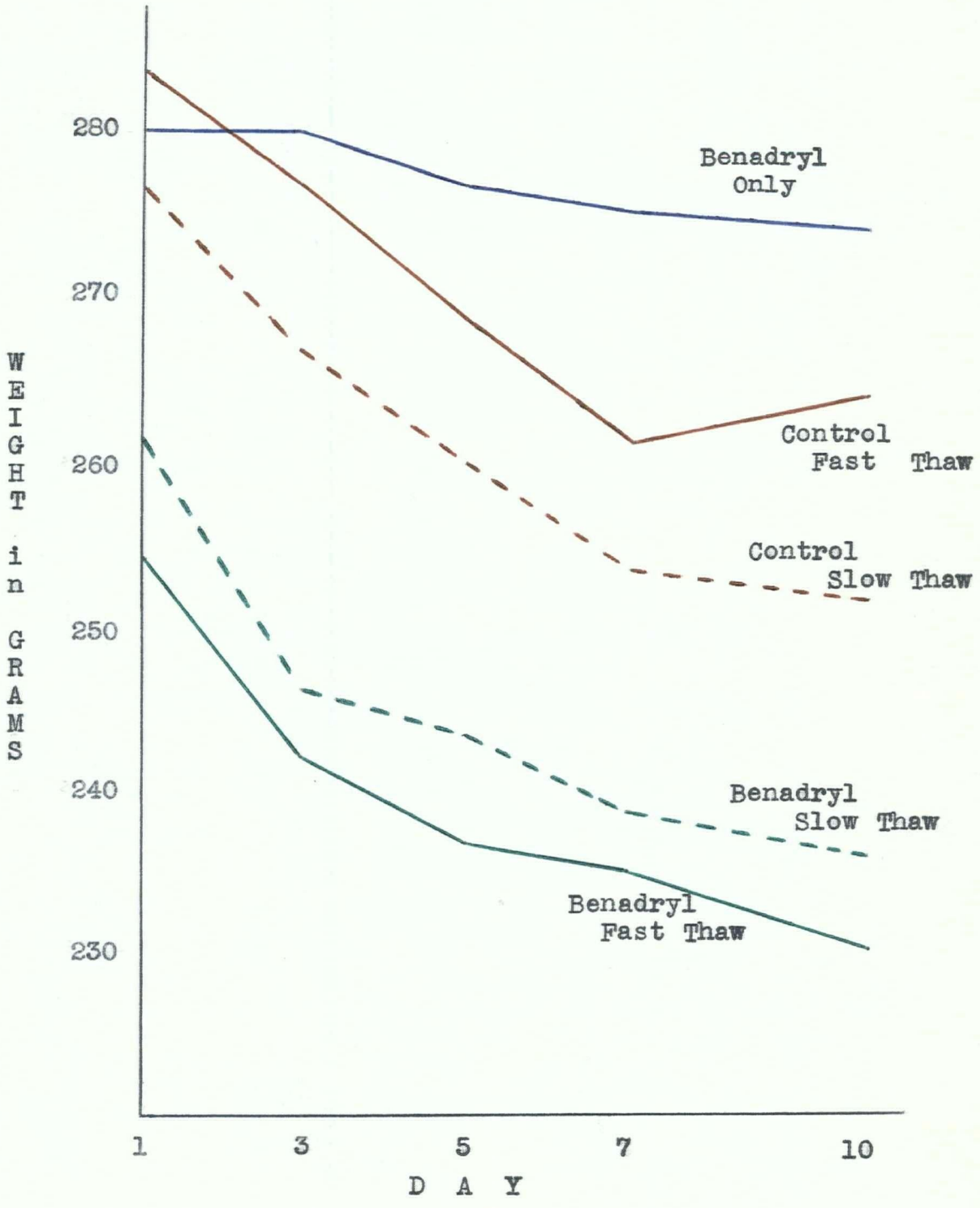
Condition of the Animals

The animals remained in general good health throughout the duration of the experiment. As will be apparent from the Weight graph, the Benadryl treated animals did not lose weight any more than the control animals. The behavior and appearance of the animals did not suffer much.

Discussion of the Observations

It was felt that Benadryl as administered, had a consistently harmful effect on the clinical course of the frostbite lesions as produced in this experiment. This was readily apparent by casual observation of the animals in the various groups, as well as apparent from a study of the data obtained and the graph drawn from it. Although the colour


70
60
50
40
30
20
10
0
INJURY INDEX
Control Slow Thaw
Benadryl Slow Thaw
Benadryl Fast Thaw
Control Fast Thaw
1
2
3
4
5
7
10
D A Y


280
270
260
250
240
230
WEIGHT in GRAMS
Benadryl Only
Control Fast Thaw
Control Slow Thaw
Benadryl Slow Thaw
Benadryl Fast Thaw
1
3
5
7
10
D A Y

photographs are incomplete for group 5, groups 1 and 3 may be compared, and their positions on the Injury graph for day 3 placed (dotted lines).

The previously noted beneficial effects of the rapid thawing at 42°C. was again confirmed, and with Groups 4 and 5, although group 5 was adversely affected by the Benadryl, both groups were in all respects less affected by the frostbite after rapid thawing than were the animals in Groups 1 and 3 which were thawed at room temperature.

All the groups showed a significant average weight loss, except the control group receiving the Benadryl only. This loss was attributed solely to the effects of the freezing injury, both through probably pain, and toxic effects locally.

The fact that an antihistaminic drug adversely affected the course of the lesions produced by a standard frostbite injury suggested both the need for repeating the experiment with another antihistaminic (Chlor-Tripolon) and also the possibility that, if Benadryl antagonizing histamine or a histamine-like substance liberated by the trauma of freezing, a drug like histamine should be investigated for its role in the tissue reaction, and for any possible influence it could have on the course of the frostbite lesions.

Etamon and Hydergine

Experimental Observations with Etamon and Hydergine

The purpose of the experiment here described was to test the effects of a standard cold injury as influenced by parenteral injections of two different drugs. Etamon chloride (tetra-ethyl ammonium chloride) was to be tested at a dosage level of 50 mg. per kilogram body weight administered subcutaneously at three hour intervals, prior to the freezing injury and for forty-eight hours afterwards. Hydergine was to be tested in different animals at a dosage level of 1 cc. per animal administered also subcutaneously at three hour intervals, prior to the freezing injury and for forty-eight hours afterwards. Twenty-four normal adult male Wistar rats were selected, and they were divided into four groups with each group designed as follows:

Group: 4. A group of six animals to serve as a control, to receive a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A group of six animals to receive both the Etamon injections and a freezing injury of twenty seconds duration with rapid thawing in water

at 42°C.

4. Another group of six animals to serve as a control, to receive a freezing injury of twenty seconds duration with rapid thawing at 42°C.
5. A group of six animals to receive both the Hydergine injections and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

The group numbers are kept uniform throughout all the gross experiments, hence explaining the duplication. There will be no ambiguity because the group being discussed will be exactly referred to. On the third day prior to the freezing (Day -3 on the Data Sheets), each animal was marked characteristically on the ear, and initial weights were recorded. On Day 1, the day of the freezing, weights were again recorded and then the animals of both Groups 5 received their initial subcutaneous injections of either Etamon of Hydergine. This was at a quarter of ten o'clock. The respective injections were continued every three hours until a quarter after ten o'clock on Day 3, with no doses midsed. The record of these injections was not put onto the Data Sheets. The animals were

kept in four cages, all animals of one group in the same cage.

At least one hour after the initial injections the animals were given (all animals, that is) subcutaneously from 0.07 to 0.12 cc. of 0.6% nembutal solution. Then when anaesthetized, one hind leg of each animal was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen (at this moment the rat invariably squirmed, in spite of adequate anaesthesia), the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals were then all treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then the animals were returned to their cages and were left quietly alone (except for the three hourly injections) until they wakened from the effects of the nembutal. Observations were made as recorded on the Data Sheets, by the method described above for estimating the degree of frostbite injury

under the categories of oedema, colour change, exudate and slough, and gangrene.

Data Sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. All the data for Etamon is placed on the first, and the data for Hydergine is placed on the second. The symbols used are: Grp, group, Gm, grams, O, oedema, C, colour change, E, exudate and slough, G, gangrene, -, means not present. All surviving animals were disposed of on Day 13 with chloroform fumes.

Summary Sheet

Following the Data Sheets is a Summary Sheet which gives the average weight for each group each day, and under I, or Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes apparent together for each group each day. In view of the fact none of the animals died, no extrapolation was neceasary. As noted on the Summary Sheet, the top half applies to Etamon, and the remainder to Hydergine.

Data Sheet - Etamon

Grp	Gm	Gm	Gm	O	C	E	G	Gm	O	C	E	G
4	168	170	163	3	1	-	-	165	3	2	1	-
	184	193	180	3	2	-	-	186	2	1	-	-
	171	176	166	3	1	-	-	167	3	2	1	-
	175	183	172	3	2	1	-	176	2	2	-	-
	173	191	178	2	2	1	-	182	1	1	-	-
	156	157	151	3	2	-	-	156	3	2	1	-
5	168	180	172	3	2	1	-	173	3	2	1	-
	198	197	199	3	2	1	-	204	3	2	-	-
	180	184	178	3	2	-	-	181	3	2	-	-
	167	169	166	3	1	1	-	168	3	1	-	-
	176	172	170	3	2	1	-	171	3	1	1	-
	174	177	174	3	3	1	-	176	3	2	1	-
Day	-3	1	2					3				

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
4	161	2	2	2	-	161	2	2	2	-	178	1	2	3	-
	184	-	1	-	-	190	-	1	-	-	206	-	-	-	-
	163	2	2	2	-	166	2	2	1	-	175	2	2	3	-
	180	1	2	-	-	182	1	2	-	-	205	-	-	-	-
	179	1	1	-	-	188	-	1	-	-	200	-	-	-	-
	150	2	1	-	-	159	1	2	-	-	182	-	-	-	-
5	168	2	2	-	-	172	2	2	-	-	190	-	-	-	-
	198	2	1	-	-	203	1	2	-	-	218	-	-	-	-
	180	2	2	-	-	182	1	2	-	-	194	1	-	-	-
	163	2	2	1	-	169	1	2	-	-	184	1	-	-	-
	167	2	2	2	1	167	2	2	1	1	189	1	2	-	-
	173	2	3	2	-	176	2	2	-	-	195	-	2	2	-
Day	4					5					13				

Data Sheet - Hydergine

Grp	Gm	Gm	Gm	O	C	E	G	Gm	O	C	E	G
4	330	308	316	3	2	-	-	315	3	2	1	-
	362	342	350	3	2	1	-	352	3	2	1	-
	336	320	329	3	1	-	-	329	3	2	-	-
	268	273	281	3	2	-	-	284	2	2	1	-
	264	257	259	3	2	1	-	260	3	2	-	-
	272	270	275	3	2	1	-	273	3	2	1	-
5	289	282	283	3	2	1	-	281	3	2	1	-
	295	296	295	3	2	1	-	291	3	1	1	-
	279	285	279	3	2	1	-	273	3	1	1	-
	208	203	204	3	3	1	-	202	3	3	-	-
	267	266	263	3	3	-	-	254	3	3	1	-
	297	296	290	3	1	-	-	281	3	2	1	-
Day	-3	1	2					3				

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
4	311	3	3	1	-	311	2	3	1	-	322	2	2	2	-
	346	3	3	2	-	344	3	3	2	-	343	1	2	-	-
	325	2	3	-	-	326	2	2	-	-	343	-	-	-	-
	286	2	2	3	-	282	2	2	3	-	329	1	1	2	-
	258	2	1	1	-	258	2	2	-	-	275	-	-	-	-
	275	2	3	1	-	275	2	3	1	-	285	1	1	-	-
5	273	3	3	1	-	279	2	3	2	-	293	1	2	2	-
	287	3	2	-	-	290	3	1	-	-	298	1	1	-	-
	268	3	2	2	-	269	3	3	2	-	272	2	2	3	-
	193	3	3	1	-	201	3	3	1	-	221	1	2	1	1
	261	3	2	1	-	257	3	3	2	-	285	1	2	2	1
	275	3	1	1	-	279	3	2	2	-	303	1	2	-	-
Day	4					5					13				

Summary Sheet

Etamon

Grp	Gm	Gm	Gm	I	Gm	I	Gm	I
4	171	178	168	29	172	27	170	21
5	177	180	177	35	179	31	175	30
Day	-3	1	2		3		4	

Grp	Gm	I	Gm	I
4	174	19	191	13
5	178	23	196	9
Day	5		13	

Hydergine

Grp	Gm	Gm	Gm	I	Gm	I	Gm	I
4	305	295	301	32	302	33	300	37
5	273	271	271	35	264	35	265	36
Day	-3	1	2		3		4	

Grp	Gm	I	Gm	I
4	299	35	316	15
5	263	41	279	26
Day	5		13	

Graphs

The values on the Summary Sheet are plotted directly on the two simple line graphs, the first for Etamon and the second for Hydergine. Weight and Injury Index changes are plotted against the days. The graphs had to be foreshortened between Days 5 and 13 in order to contain them within one page at the scale chosen. The graphs are done in colours for easier reading (except for the Time graphs of the first experiment, these and all other gross graphs have identical colours), but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers.

Condition of the Animals

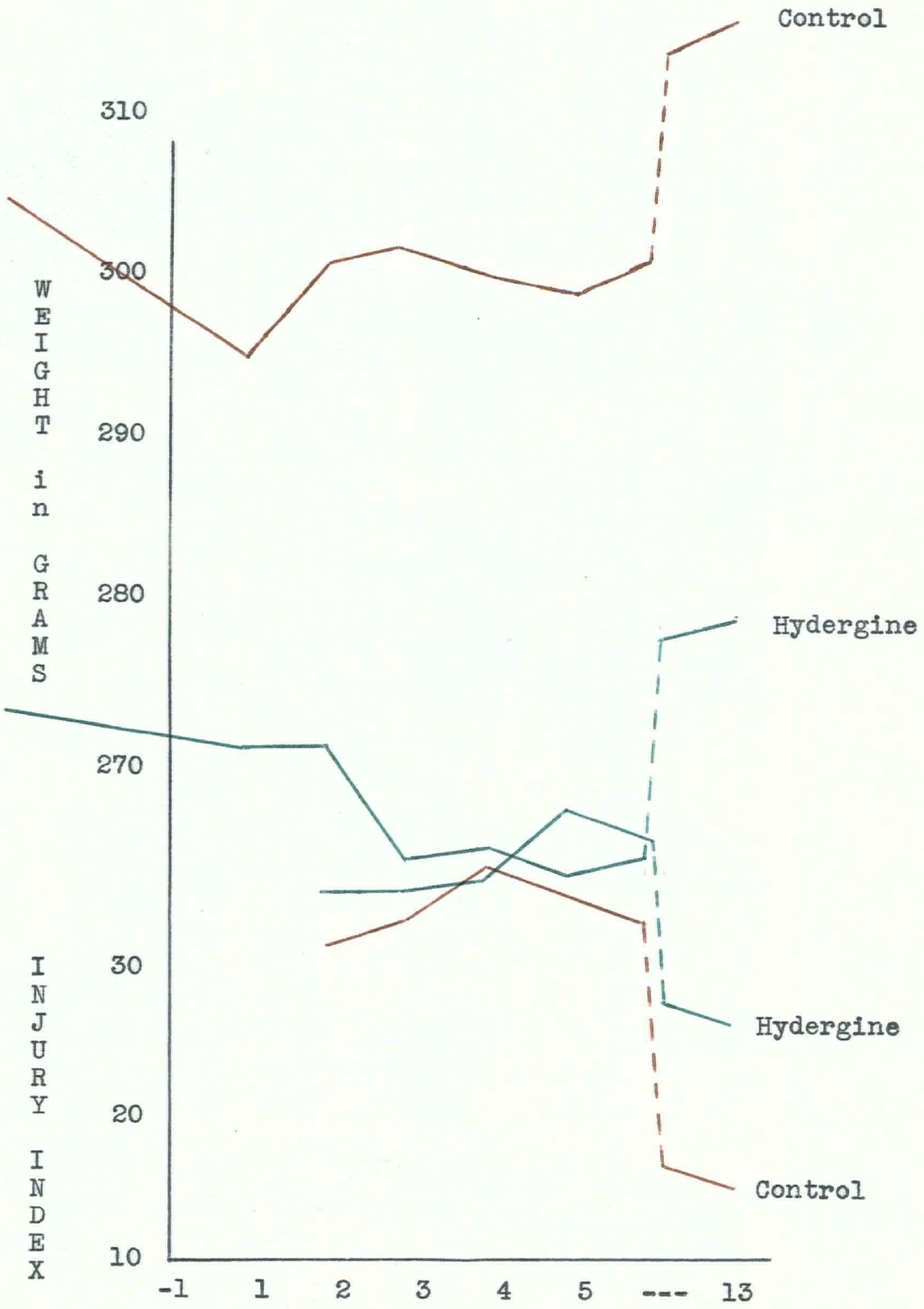
The animals remained well throughout the duration of the experiment. Initially the Etamon had a subduing effect on the behaviour of the rats, but later they became normally active. The Etamon injections were somewhat painful, causing squealing each time the material was injected. The Hydergine had no apparent effect on the animals receiving it.

Discussion of the Observations

The impression clinically was that Etamon had no influence on the course of the frostbite injury, and that Hydergine worsened the course of the injury.


Etamon
Control
190
180
170
WEIGHT
30
20
10
INJURY INDEX
Control
Etamon
-1
1
2
3
4
5

13


Control
310
300
290
280
270
WEIGHT in GRAMS
Hydergine
30
20
10
INJURY INDEX
Hydergine
Control
-1
1
2
3
4
5

13

On the Etamon graph the Injury Index line for the Etamon treated group remained slightly above the control line, later crossing it. However with the Hydergine treated Index line there is a later divergence.

Noteworthy is the fact that both control Injury Index lines are similar, which they should be in that similar groups, Groups 4, were both treated identically. If the mean of these two red lines were drawn in, the resulting line would only serve to accentuate the differances described above.

The weight graphs run essentially parallel for all groups.

Apresoline and Chlor-Tripolon

Experiments with Apresoline and Chlor-Tripolon

The purpose of the experiment here described was to test the effects of a standard cold injury as influenced by parenteral injections of two different drugs. Apresoline was to be tested at a dosage level of 2 mg. per animal administered subcutaneously at three hour intervals, prior to the freezing injury and for forty-eight hours afterwards. Chlor-Tirpolon was to be tested in different animals at a dosage level

of 5 mg. per animal administered also subcutaneously at three hour intervals, prior to the freezing injury and for forty-eight hours afterwards. Eighteen normal adult nonpregnant female Wistar rats were selected, and they were divided into three groups of six animals each, with each group designed as follows:

Group: 4. A group of six animals to serve as a control, to receive a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A group of six animals to receive both the Apresoline injections and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A group of six animals to receive both the Chlor-Tripolon injections and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

The group numbers are kept uniform throughout all the gross experiments, hence explaining the duplication. There will be no ambiguity because the group being discussed will be exactly referred to. On Day 1 of the experiment, the day of freezing, each animal was marked

characteristically on the ear, and initial weights were recorded as on the Data Sheet. The animals of both Groups 5 received their appropriate initial injections at ten o'clock and again at one o'clock. At three o'clock all animals received by intraperitoneal injection an anaesthetizing dose of 0.6% nembutal solution of 0.075 cc. Then when anaesthetized, one hind leg of each animal was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen (at this moment the rat invariably squirmed, in spite of adequate anaesthesia), the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals were then all treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then the animals were returned to their cages and were left quietly alone (except for the three hourly injections) until they wakened from the effects of the nembutal. Observations were made as recorded on the

Data Sheets, by the method described above for estimating the degree of frostbite injury under the categories of oedema, colour change, exudate and slough, and gangrene. The animals were kept in three cages, all animals of one group in the same cage. On Day 14 all animals were disposed of with chloroform fumes.

Data Sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. Days 1, 2, and 3 include a record of the three hourly injections. The symbols used are: Grp, group, Gm, grams, q3h, every three hours, O, oedema, C, colour change, E, exudate and slough, G, gangrene, -, means not present or not administered, F, freezing injury, A, Apresoline injection, C, Chlor-Tripolon injection.

Summary Sheet

Following the Data Sheets is a Summary Sheet which gives the average weight for each group each day, and under I, or Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes apparent together for each group

Data Sheet

Apresoline

Grp	Gm	q3h	Gm	O	C	E	G	q3h	Gm	O	C	E	G	q3h
4	155	--F----------	141	3	2	-	-	-----	145	3	1	-	-	--
	158	--F----------	143	3	1	-	-	-----	141	2	1	-	-	--
	153	--F----------	134	3	2	-	-	-----	131	3	2	-	-	--
	133	--F----------	145	3	4	-	-	-----	146	3	4	-	-	--
	131	--F----------	140	3	4	1	-	-----	143	3	4	1	-	--
	132	--F----------	145	3	2	-	-	-----	150	3	2	1	-	--
5	163	AAFAAAAAAAAAA	153	3	1	-	-	AAAAA	152	3	1	-	-	AA
	159	AAFAAAAAAAAAA	146	3	1	-	-	AAAAA	146	3	1	-	-	AA
	162	AAFAAAAAAAAAA	148	3	1	-	-	AAAAA	151	3	1	-	-	AA
	167	AAFAAAAAAAAAA	157	3	1	-	-	AAAAA	156	2	1	-	-	AA
	163	AAFAAAAAAAAAA	151	3	1	-	-	AAAAA	147	3	1	-	-	AA
	161	AAFAAAAAAAAAA	144	3	1	-	-	AAAAA	149	3	1	-	-	AA
Day	1		2						3					

Chlor-Tripolon

Grp	Gm	q3h	Gm	O	C	E	G	q3h	Gm	O	C	E	G	q3h
5	140	CCFCC---CCCCC	132	2	3	-	-	----C	125	1	5	-	-	CCh
	132	CCFCC---CCCCC	121	3	3	-	-	----C	115	3	3	-	1	CC
	175	CCFCC---CCCCC	161	3	3	-	-	----C	155	3	4	-	-	CC
	189	CCFCC---CCCCC	171	3	2	1	-	----C	162	3	2	2	-	CC
	151	CCFCC---CCCCC	142	3	2	-	-	----C	137	3	3	1	-	CC
	136	CCFCC---CCCCC	126	3	2	-	-	----C	120	3	3	-	-	CC
Day	1		2						3					

Data Sheet

Apresoline

Grp	Gm	O	C	E	G	GM	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
4	155	2	1	-	-	158	1	1	-	-	160	-	2	-	-	163	-	1	-	-
	153	1	1	-	-	157	-	1	-	-	164	-	1	-	-	167	-	-	-	-
	148	2	2	-	-	153	1	2	-	-	159	1	2	-	-	159	1	1	-	-
	146	3	4	-	-	152	3	4	-	-	158	2	2	2	6	158	1	1	1	6
	147	3	3	-	-	153	3	3	1	-	155	2	2	-	2	159	1	2	-	2
	150	3	1	1	-	157	2	1	-	-	162	1	2	2	-	162	2	2	4	-
5	155	2	1	-	-	162	1	1	-	-	174	1	2	2	-	181	1	2	1	-
	145	2	1	-	-	150	1	2	2	-	169	1	2	1	-	159	1	2	2	-
	156	2	1	-	-	158	2	2	-	-	173	1	2	1	-	174	1	1	1	-
	161	1	1	-	-	163	-	1	-	-	180	-	1	-	-	180	-	-	-	-
	159	1	1	-	-	153	1	-	-	-	165	-	1	-	-	163	-	1	-	-
	149	1	1	2	-	155	1	1	2	-	168	1	2	2	-	168	1	2	2	-
Day	4					5					9					11				

Chlor-Tripolon

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
5	121	-	5	-	-	123	-	5	-	5	125	1	1	-	7	130	-	-	-	7
	107	2	1	-	6	108	2	1	2	7	122	2	1	-	7	123	-	-	-	7
	145	3	2	-	1	148	2	1	-	4	152	1	1	2	6	154	1	-	-	6
	152	2	2	1	5	156	2	1	2	5	175	1	1	-	6	182	-	-	-	6
	128	2	3	2	1	134	3	4	1	1	150	1	1	-	6	153	1	1	-	6
	112	1	5	2	3	112	1	5	1	6	113	1	1	-	7	122	-	-	-	7
Day	4					5					9					11				

Data Sheet

Apresoline

Grp	Gm	O	C	E	G
4	166	-	1	-	1
	164	-	-	-	-
	164	-	1	-	-
	143	1	1	-	6
	142	2	2	-	2
	135	1	2	-	-
5	198	1	1	-	-
	166	1	2	-	-
	187	1	2	-	-
	186	-	-	-	-
	169	-	-	-	-
	178	1	2	1	1

Day 14

Chlor-Tripolon

Grp	Gm	O	C	E	G
5	134	1	1	-	7
	135	2	1	-	7
	175	2	1	2	6
	194	1	1	-	6
	162	2	1	1	6
	125	2	2	-	7

Day 14

Summary Sheet

Apresoline

Grp	Gm	Gm	I	Gm	I	Gm	I	Gm	I	Gm	I
4	144	141	34	143	33	147	27	155	23	160	29
5	163	150	24	155	23	153	17	157	17	172	20
Day	1	2		3		4		5		9	

Chlor-Tripolon

Grp	Gm	Gm	I	Gm	I	Gm	I	Gm	I	Gm	I
5	154	142	35	136	40	128	49	130	61	139	54
Day	1	2		3		4		5		9	

Apresoline

Grp	Gm	I	Gm	I
4	161	25	152	20
5	171	18	181	13
Day	11		14	

Chlor-Tripolon

Grp	Gm	I	Gm	I
5	144	42	154	59
Day	11		14	

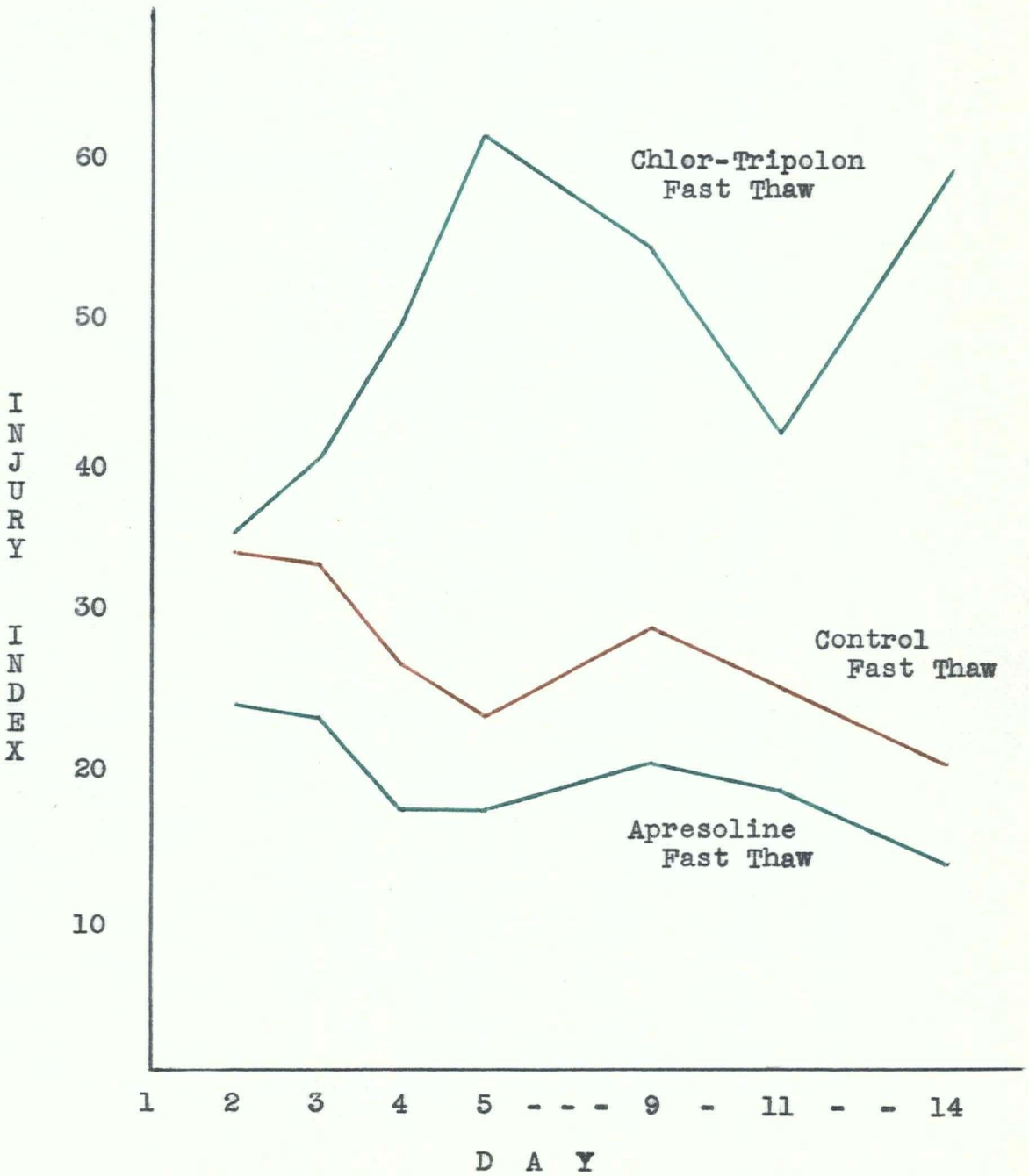
each day. In view of the fact that none of the animals died, no extrapolation was necessary.

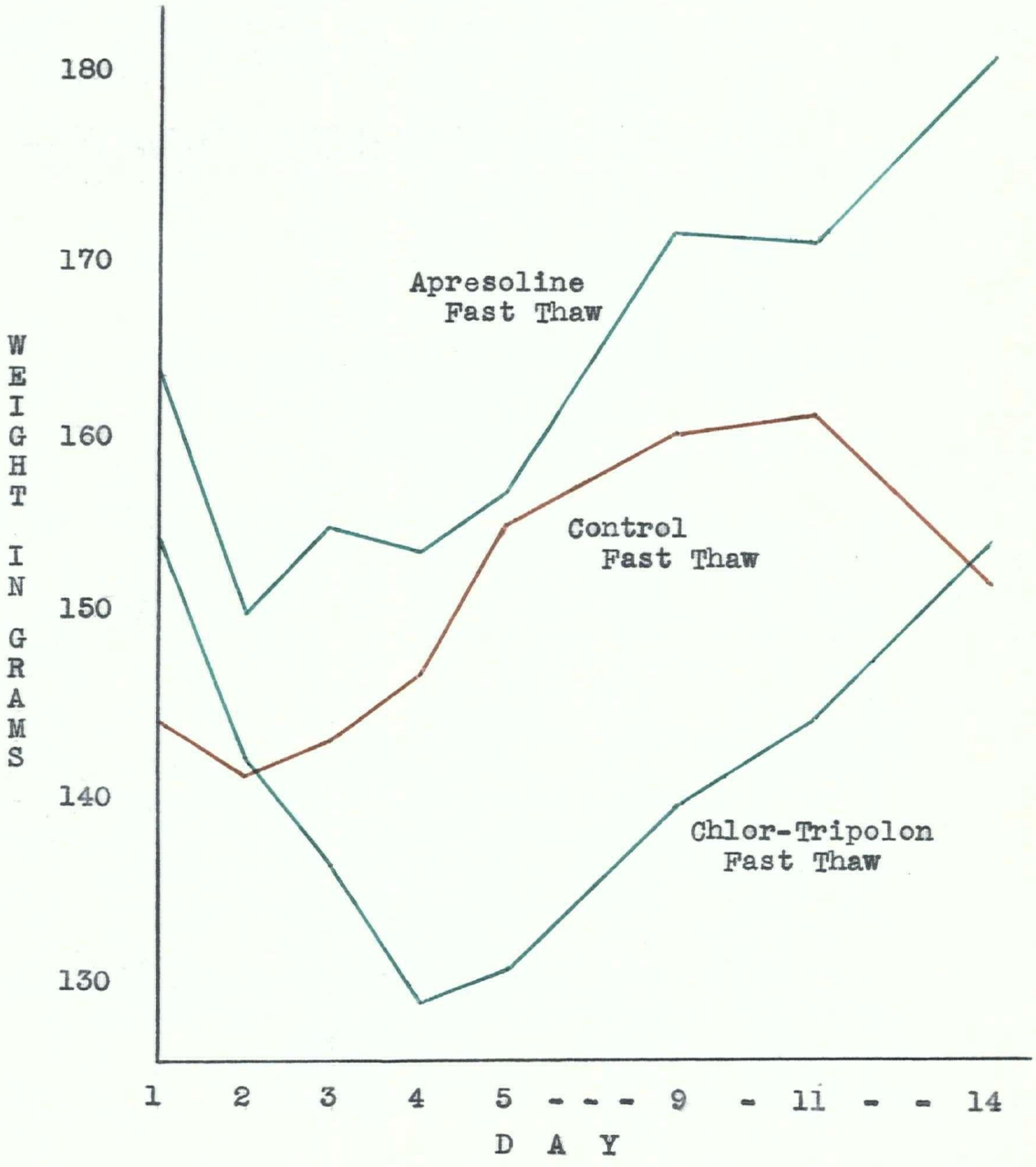
Graphs

The values on the Summary Sheet are directly plotted onto the two simple line graphs, the first being for Injury Index changes by days, and the second for weight changes also by days. The graphs had to be foreshortened between Days 5 and 14 in order to contain them within one page at the scale chosen. The graphs are done in colours for easier reading (except for the Time graphs of the first experiment, these and all other gross graphs have identical colours), but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers.

Condition of the Animals

The animals receiving Apresoline were apparently quite unaffected by the drug. However, the Chlor-Tripolon quite adversely affected the rats receiving it. As will be noted from the Data Sheets, quite a few of the injections with Chlor-Tripolon had to be missed because of serious illness in the animals. However none expired, and all recovered to an apparently normal state after the Chlor-Tripolon was discontinued.


60
50
40
30
20
10
INJURY INDEX
Chlor-Tripolon
Fast Thaw
Control
Fast Thaw
Apresoline
Fast Thaw
1 2 3 4 5 - - - 9 - 11 - - 14
D A Y


180
170
160
150
140
130
WEIGHT IN GRAMS
Apresoline
Fast Thaw
Control
Fast Thaw
Chlor-Tripolon
Fast Thaw
1 2 3 4 5 - - - 9 - 11 - - 14
D A Y

Discussion of the Observations

There was only one untreated control group used in this experiment, but it behaved in a typical manner, and the Injury Index line graph for this group is comparable with the similar graph in other experiments. Generally speaking Apresoline favourably influenced the behaviour of the injured feet of those animals receiving it. This was the general impression clinically, and the Apresoline Injury Index graph bears this out. However, the Chlor-Tripolon treated animals were made very much worse after the freezing injury. This reaction is much greater than it was for Benadryl. It is possible the general effects the Chlor-Tripolon had on the animals could have influenced the healing of the injured feet.

The weight graph confirms the fact that the Chlor-Tripolon had an adverse effect generally on the animals receiving it.

Rutin

Experimental Observations with Rutin

The purpose of the experiment here described was to test the effects of a standard cold injury as

influenced by rutin administered orally. Thirty normal adult male Wistar rats were selected for the test and they were divided into five groups of six animals each, with each group designed as follows:

Group: 1. A control group to receive a freezing injury of twenty seconds duration with slow thawing at room temperature.

2. Another control group to receive only the oral rutin medication.

3. A group to receive both the rutin and a freezing injury of twenty seconds duration with slow thawing at room temperature.

4. A control group to receive only a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A group to receive both the rutin and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

As noted previously, these groupings are the same throughout all gross experiments. On Days -9, -7, and

-5, being the ninth, seventh, and fifth days prior to freezing, all animals in groups 2, 3, and 5 received 100 cc. of water containing 100 mg of rutin (two ground up 50 mg. compressed rutin tablets). Each animal was in a separate cage, each receiving its own individual water supply containing the rutin. On Day -4, being the fourth day before the freezing, the concentration of the rutin solution was increased so that each animal of Groups 2, 3, and 5 received each day 100 mg. of rutin contained in 35 cc. This was administered on each of Days -4, -3, -2, and -1, and of course the same solution was given daily afterwards until the animals were disposed of on day 6. No record was kept of the precise amount of rutin consumed by the animals receiving it. There was a little wastage, and several of the animals left some of their solution each day. However, it was felt that it could be safely claimed each animal took at least 50 mg. of rutin per day, and many took as much as 90 or 95 mg. Although no record was kept of the fact, it was noted that the animals were somewhat consistent in whether or not they drank up all their rutin solution each day. All animals in Groups 1 and 4 were kept six to a cage, that is, one cage for each of these two groups. All animals were weighed on Days 1, 3, and 5.

On Day 1, the day of the freezing, the animals of Groups 1, 3, 4, and 5 were given subcutaneously from 0.1 to 0.125 cc. of 0.6% nembutal solution. Then when the animals were anaesthetized, one hind leg of each animal in these groups was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen (at this moment the rat invariably squirmed, in spite of adequate anaesthetic), the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals of Groups 1 and 3 were placed in their cages and the feet were allowed to thaw at room temperature, taking approximately fifteen or twenty minutes to become limp and warm. The animals of groups 4 and 5 were treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then these animals were also returned to their cages and all thirty were left quietly alone until they wakened from the effects of the nembutal.

Observations were made as recorded in the Data Sheet, by the method described before for estimating the degree of frostbite injury under the categories of oedema, colour change, exudate, slough, and gangrene.

Data Sheet

This table is fairly self-explanatory, being a simple tally by animals in groups, and by days, Dayl being the day of freezing. It is necessarily as compact as possible. The symbols used are: Grp, group, Gm, grams, O, oedema, C, colour change, E, exudate and slough, G, gangrene, F, freezing, -, means not present.

Summary Sheet

Following the Data Sheet is a Summary Sheet which gives the average weight for each group each day, and under I, or Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes apparent together for each group each day. Except for Group 2, where of course no freezing was performed, and no Index number will appear. Because no animals expired no extrapolation was necessary.

Data Sheet

Grp	Gm	O	C	E	G	Gm	O	C	E	G	O	C	E	G	Gm	O	C	E	G	O	C	E	G
1	230	3	2	2	-	210	2	2	2	-	3	3	3	1	220	3	3	2	1	3	2	-	1
	270	3	1	1	-	255	3	1	2	-	3	3	3	-	245	2	3	3	-	2	2	3	-
	280	2	3	2	2	250	3	2	1	2	3	3	3	1	250	3	3	3	1	3	3	1	2
	255	3	2	2	-	230	3	1	2	-	3	3	3	-	210	2	1	1	2	2	1	-	3
	245	3	2	2	-	225	2	1	2	-	3	3	3	-	220	3	3	2	-	3	3	-	-
	260	3	2	2	-	240	3	1	1	-	2	2	2	-	240	2	2	2	±	1	2	-	-
2	240					235									240								
	235					200									225								
	250					205									235								
	240					230									235								
	255					245									255								
	200					200									215								
3	215	2	2	2	-	210	2	1	2	-	3	2	3	-	210	2	2	2	-	2	2	1	-
	240	2	2	2	-	230	2	2	2	1	3	3	2	1	235	2	3	3	2	2	3	2	3
	235	3	2	1	-	200	2	2	2	1	3	2	1	1	210	3	2	2	1	1	1	1	-
	195	2	1	1	-	190	2	1	2	-	2	2	2	-	180	1	1	1	-	1	1	1	-
	210	2	1	1	-	215	2	1	1	-	1	2	2	-	215	1	1	1	-	1	1	1	-
	235	2	1	2	-	205	2	2	2	-	2	2	2	-	225	1	1	1	-	1	1	1	-
4	190	3	2	2	-	185	1	1	1	-	2	2	2	-	190	2	2	2	-	2	2	-	-
	215	2	2	2	2	205	1	1	-	2	1	1	-	2	210	1	1	-	2	1	1	-	2
	240	3	1	1	-	230	2	1	2	-	2	1	2	-	220	2	2	1	-	1	2	2	-
	265	3	1	-	-	260	1	1	-	-	1	2	2	-	270	-	1	-	-	1	1	-	-
	230	2	2	2	-	215	1	2	2	-	1	1	-	-	220	1	2	1	-	1	2	-	-
	245	3	1	1	-	240	1	1	-	-	1	1	1	-	245	-	2	2	-	1	2	1	-
5	250	2	1	-	-	225	1	-	-	-	-	1	-	-	230	-	1	-	-	-	-	-	-
	235	1	1	-	-	210	-	-	-	1	-	1	-	1	205	-	-	-	1	-	-	-	1
	230	2	1	-	-	210	2	2	2	-	2	2	2	-	210	1	2	2	-	1	2	2	-
	245	2	1	-	-	215	-	-	-	-	-	-	-	-	215	-	-	-	-	-	-	-	-
	205	2	1	1	-	180	2	1	1	-	2	1	2	-	175	2	2	2	-	1	1	2	-
	245	1	1	-	-	230	1	-	-	-	1	1	-	-	250	-	-	-	-	-	-	-	-

Day 1 2 3 4 5 6

Summary Sheet

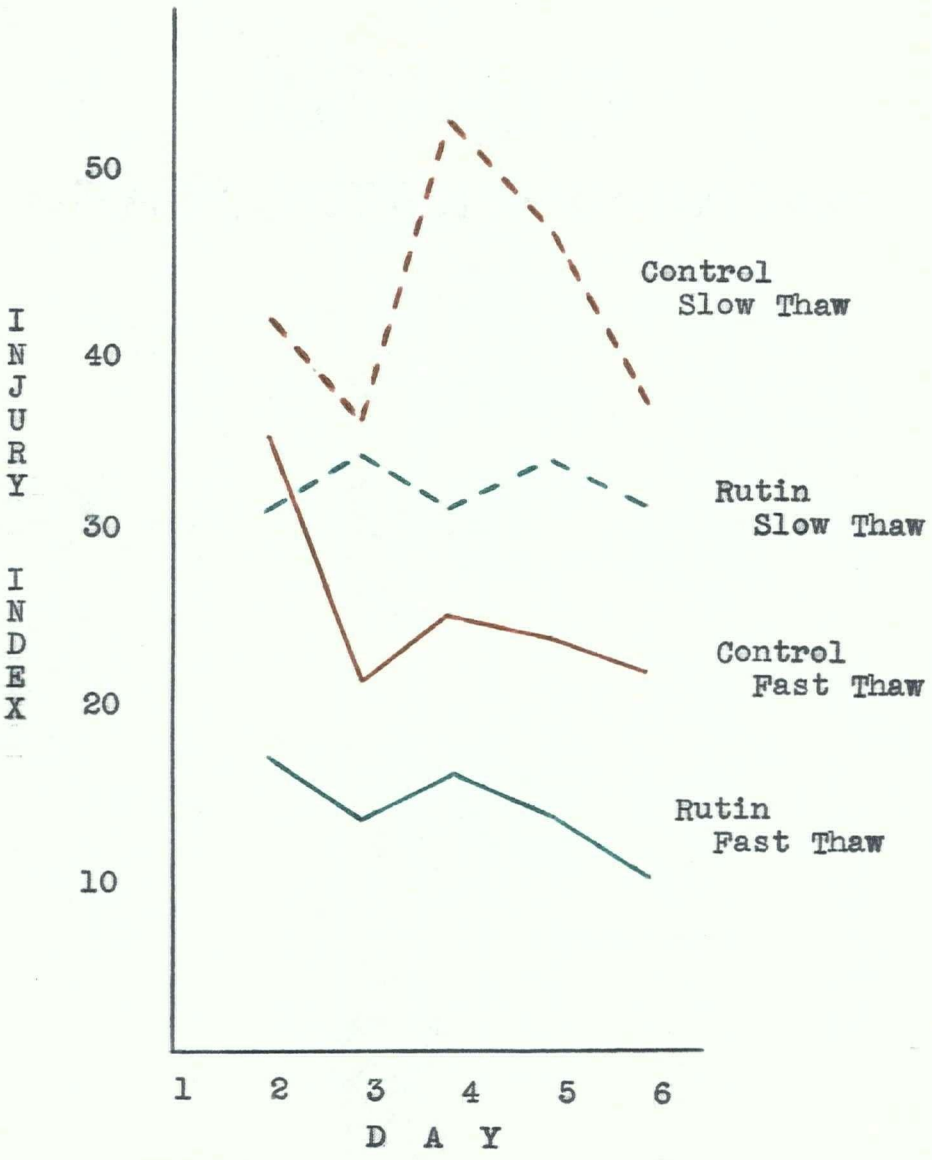
Grp	Gm	I	Gm	I	I	Gm	I	I
1	257	42	235	36	53	231	47	37
2	237		237			234		
3	222	31	208	34	31	212	33	31
4	231	35	223	21	25	216	24	22
5	235	17	228	13	16	214	13	10
Day	1	2	3		4	5		6

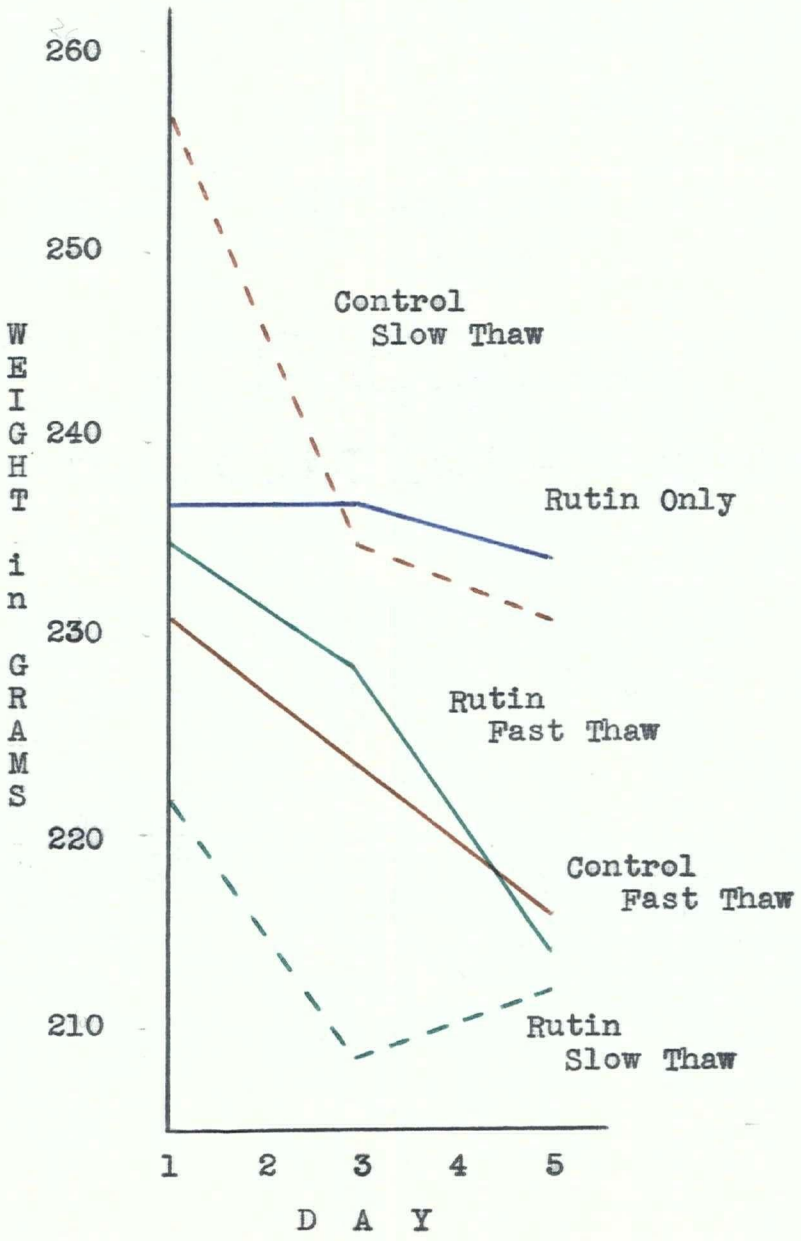
Graphs

The values appearing on the Summary Sheet above were plotted directly onto two simple line graphs, the first for the Injury Index changes from day to day, and the second for the weight changes. These are done in colours for easier reading (except for the Time graphs of the first experiment, these and all succeeding gross graphs have identical colours, and of course, all are on the same scale), but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers.

Condition of the Animals

All animals remained in excellent condition for


INJURY INDEX
50
40
30
20
10
1
2
3
4
5
6
D A Y
Control
Slow Thaw
Rutin
Slow Thaw
Control
Fast Thaw
Rutin
Fast Thaw


260
250
240
230
220
210
WEIGHT in GRAMS
Control
Slow Thaw
Rutin Only
Rutin
Fast Thaw
Control
Fast Thaw
Rutin
Slow Thaw
1
2
3
4
5
D A Y

the duration of the experiment. They drank their rutin solution readily.

Photographs

On Day 4, after again anaesthetizing the animals with 0.1 to 0.125 cc of 0.6% nembutal solution, all the feet were photographed in colour as indicated by the colour prints below. It is interesting to compare the appearance of these feet with the values recorded for Day 4 on the Data Sheet, and also with the Injury Index graph for Day 4.


RUTIN 3


RUTIN 4


RUTIN 5

Discussion of Observations

It was felt both clinically and through observation of the Data Sheet and graphs that rutin as administered had a consistently beneficial effect on the clinical course of frostbite lesions as produced in this experiment. This consisted in decreased development of oedema, less colour change, less skin slough, and less gangrene. The Injury Index graph lines are well and consistently separated.

The previously noted beneficial effect of rapid thawing in water at 42°C. was again confirmed; and with rutin, the animals receiving the drug were in all respects less affected by the injury than those not receiving the drug, although both groups were rapidly thawed.

Rutin as administered appeared to have no adverse effects on the animals receiving it.

All of the animals on the average in each group showed a significant weight loss, except for the control group receiving rutin only. The role that limited fluid intake played in this weight loss was not assessed.

Note:

The senior medical student who was helping with the project part time performed the actual freezing of

the feet, and while he froze them more or less uniformly throughtout the rutin series, his degree of injury was only about eighty percent as severe as the injury used elsewhere in the experiments. Although it is easy to write about a standard cold injury, the actual performance of the same is afflicted with many minor variations.

Ascorbic Acid

Experimental Observations with Ascorbic Acid

The purpose of the experiment here described was to test the effects of a standard cold injury as influenced by parenteral injections of ascorbic acid at a dosage level of 50 mg. administered subcutaneously in 1 cc. amounts twice a day for five days preceding the freezing injury and for five days afterwards. Forty-two normal adult nonpregnant female Wistar rats were selected, and they were divided into seven groups of six animals each, with each group designed as follows:

Group: 1. A control group to receive a freezing injury of twenty seconds duration with slow thawing at room temperature.

2. A group to receive only the ascorbic

acid injections as outlined.

3. A group to receive both the ascorbic acid injections and a freezing injury of twenty seconds duration with slow thawing at room temperature.
4. A group to receive a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.
5. A group to receive both the ascorbic acid injections and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.
6. A control group for microscopic observation to receive no ascorbic acid.
7. A group for microscopic observation to receive the ascorbic injections as outlined.

These groups, with the exception of Groups 6 and 7 are numbered identically throughout all gross experiments. Note the microscopic work was not performed as planned on these last two groups, but they were carried along until Day 3, and their weights were included in the Data Sheets as controls. On the fifth day before the freezing, Day -5, each animal was marked characterist-

ically on the ear, and initial weights were recorded as on the Data Sheets. The animals of Groups 2, 3, 5, and 7 all received their initial subcutaneous injections of ascorbic acid at nine o'clock. The injections were continued at nine o'clock both morning and evening until nine o'clock in the morning of Day 3, with the injections being missed once, the evening dose on Day -1. Daily weights were recorded as on the Data Sheets. On Day 1, the day of the freezing, the animals of Groups 1, 3, 4, and 5, at a quarter past ten o'clock, were anaesthetized with an intraperitoneal injection of 0.075 to 0.1 cc of 0.6% nembutal solution. Then, when anaesthetized, one hind leg of each animal in these groups was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen (at this moment the rat invariably squirmed, in spite of adequate anaesthetic), the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals of Groups 1 and 3 were placed in their cages and the feet were allowed to thaw at room temperature, taking approximately

fifteen or twenty minutes to become limp and warm. The animals of groups 4 and 5 were treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then these animals were also returned to their cages and all animals were left quietly alone until they wakened from the effects of the nembutal. Observations were made as recorded in the tables, by the method described above for estimating the degree of frostbite from the categories of oedema, colour change, exudate and slough, and gangrene. The animals of Groups 6 and 7 were destroyed on Day 3 and the remainder on Day 8, all by chloroform fumes.

Data Sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. There is included a record of all ascorbic acid injections. The symbols used are: Grp, group, Gm, grams, bid, twice a day,

Data Sheet

Grp	Gm	Gm	bid	Gm	bid	Gm	bid	Gm	bid	Gm	bid
1	190	191	--	193	--	193	--	193	--	196	-F-
	192	195	--	198	--	198	--	198	--	198	-F-
	172	176	--	177	--	180	--	181	--	180	-F-
	219	217	--	221	--	221	--	227	--	230	-F-
	195	199	--	204	--	204	--	205	--	201	-F-
	206	208	--	212	--	210	--	212	--	212	-F-
2	200	197	CC	200	CC	201	CC	205	C-	201	C-C
	199	194	CC	189	CC	195	CC	201	C-	196	C-C
	208	209	CC	215	CC	220	CC	218	C-	217	C-C
	203	205	CC	212	CC	212	CC	216	C-	205	C-C
	190	198	CC	198	CC	201	CC	203	C-	194	C-C
	208	214	CC	219	CC	217	CC	219	C-	206	C-C
3	215	225	CC	225	CC	232	CC	233	C-	227	CFC
	220	220	CC	224	CC	231	CC	225	C-	226	CFC
	206	209	CC	212	CC	210	CC	210	C-	212	CFC
	212	217	CC	221	CC	225	CC	229	C-	229	CFC
	220	222	CC	229	CC	233	CC	229	C-	233	CFC
	203	209	CC	212	CC	220	CC	224	C-	225	CFC
4	204	204	--	208	--	210	--	213	--	218	-F-
	218	215	--	220	--	222	--	224	--	226	-F-
	200	201	--	201	--	202	--	201	--	203	-F-
	185	187	--	190	--	195	--	193	--	193	-F-
	208	211	--	209	--	210	--	212	--	215	-F-
	219	219	--	221	--	220	--	227	--	232	-F-
5	201	201	CC	206	CC	205	66	206	C-	206	CFC
	209	214	CC	221	CC	229	CC	233	C-	230	CFC
	204	204	CC	206	CC	200	CC	203	C-	205	CFC
	198	194	CC	199	CC	202	CC	200	C-	199	CFC
	200	206	CC	210	CC	211	CC	214	C-	210	CFC
	190	195	CC	192	CC	194	CC	192	C-	196	CFC
6	219	220	--	216	--	226	--	226	--	230	---
	200	200	--	200	--	200	--	199	--	203	---
	216	214	--	211	--	211	--	217	--	218	---
	185	186	--	182	--	191	--	189	--	189	---
	174	174	--	178	--	179	--	186	--	186	---
	182	186	--	187	--	191	--	186	--	188	---
7	210	214	CC	217	CC	214	CC	213	C-	212	C-C
	206	210	CC	207	CC	219	CC	218	C-	217	C-C
	200	205	CC	206	CC	203	CC	196	C-	199	C-C
	195	198	CC	201	CC	212	CC	207	C-	210	C-C
	193	195	CC	196	CC	196	CC	196	C-	196	C-C
	192	196	CC	199	CC	207	CC	203	C-	196	C-C
Day	-5	-4		-3		-2		-1		1	

Data Sheet

Grp	Gm	O	C	E	G	bid	Gm	O	C	E	G	bid	Gm	O	C	E	G	Gm
1	199	4	3	1	-	--	196	4	3	2	-	--	194	3	3	2	-	185
	199	4	3	1	-	--	199	4	3	2	-	--	202	3	4	2	-	195
	180	4	3	2	-	--	181	4	4	1	-	--	179	3	4	1	-	175
	228	4	3	2	-	--	223	4	3	2	-	--	221	3	2	1	-	220
	200	4	3	2	-	--	201	4	3	2	-	--	196	3	3	1	-	190
	217	4	3	2	-	--	218	4	3	2	-	--	211	3	3	2	-	206
2	203					CC	204					CC	206					208
	199					CC	200					CC	202					202
	223					CC	214					CC	215					210
	199					CC	187					CC	183					176
	186					CC	177					CC	171					164
	198					CC	189					CC	181					174
3	227	4	3	2	-	CC	220	4	4	2	-	CC	217	3	3	2	-	214
	230	4	3	2	-	CC	221	3	4	2	-	CC	222	1	4	-	1	221
	217	4	3	2	-	CC	213	3	4	2	-	CC	213	1	5	1	2	216
	228	4	3	2	-	CC	219	3	4	2	-	CC	221	2	5	1	1	218
	235	4	3	2	-	CC	223	4	4	2	-	CC	226	4	3	1	-	224
	227	4	3	2	-	CC	217	4	3	2	-	CC	211	3	3	2	-	205
4	217	4	1	2	-	--	211	4	1	-	-	--	211	3	2	1	-	212
	225	4	1	2	-	--	219	4	1	1	-	--	220	3	2	2	-	215
	203	4	1	2	-	--	195	3	2	-	-	--	193	3	1	2	-	193
	187	3	3	-	-	--	183	3	3	-	-	--	176	3	3	-	-	184
	214	4	1	1	-	--	206	4	1	1	-	--	195	2	1	-	-	199
	232	4	3	1	-	--	226	4	3	1	-	--	204	3	3	1	-	217
5	204	4	2	2	-	CC	202	3	3	3	-	CC	201	3	3	2	-	198
	236	4	2	1	-	CC	226	3	2	1	-	CC	223	3	3	2	-	220
	209	4	3	1	-	CC	202	3	2	-	-	CC	203	3	3	-	-	198
	204	4	1	2	-	CC	203	3	2	-	-	CC	203	3	2	1	-	208
	215	4	1	1	-	CC	210	3	1	1	-	CC	214	2	2	1	-	211
	202	3	2	-	-	CC	198	3	2	2	-	CC	199	2	2	2	-	205
6	235					--	231					--						
	204					--	204					--						
	208					--	221					--						
	195					--	188					--						
	192					--	191					--						
	194					--	196					--						
7	214					CC	211					CC						
	223					CC	223					CC						
	202					CC	200					CC						
	216					CC	222					CC						
	202					CC	196					CC						
	202					CC	202					CC						

Day 2 3 4 5

Data Sheet

Grp	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
1	3	4	2	-	195	3	3	1	4	196	3	3	3	3	194	3	3	1	4
	3	4	2	-	199	3	4	2	4	198	2	2	3	6	196	2	3	2	6
	3	4	2	-	178	2	4	1	2	174	2	4	3	3	171	2	3	3	6
	3	4	2	1	219	3	4	2	5	219	2	3	5	5	212	2	3	2	6
	3	4	1	-	192	3	4	2	1	194	3	5	3	5	193	2	3	3	6
	3	4	2	-	199	3	4	2	5	201	3	4	3	5	204	3	3	3	5
2					214					220					215				
					205					205					200				
					219					220					210				
					167					161					148				
					156					154					154				
					164					157					151				
3	3	4	2	-	210	2	5	1	4	207	2	5	2	5	210	2	2	3	8
	1	4	-	7	211	-	-	-	9	210	1	3	2	9	212	2	2	1	9
	1	4	1	6	218	1	5	1	7	214	3	3	1	8	214	2	2	0	9
	2	4	-	3	214	2	3	2	7	208	3	3	2	8	214	3	3	2	9
	3	4	2	-	221	3	3	2	4	217	3	3	3	5	220	3	3	3	5
	3	4	2	-	203	3	3	3	6	203	3	3	3	6	200	3	3	2	7
4	3	3	2	-	215	2	2	1	-	215	2	3	3	-	213	1	3	2	-
	3	3	3	-	221	3	3	2	-	220	3	3	2	-	211	2	3	3	-
	3	1	2	-	202	2	-	2	-	199	2	1	2	-	197	2	2	2	-
	3	4	1	-	193	2	4	2	2	189	3	3	2	2	188	2	3	2	3
	2	1	1	-	204	1	1	2	-	202	2	2	2	-	202	2	2	1	-
	3	3	3	-	219	3	3	3	-	213	3	3	3	-	211	3	3	3	-
5	3	2	3	-	202	3	2	3	-	205	3	2	3	-	196	3	3	3	-
	3	2	3	-	217	3	3	3	-	216	3	3	3	-	214	3	3	3	-
	3	2	-	3	208	3	2	-	3	208	3	3	2	3	210	2	2	1	3
	2	2	1	-	209	2	2	-	-	207	2	2	1	-	212	1	2	1	-
	2	2	1	-	218	2	2	-	-	219	1	2	1	-	214	2	2	2	-
	2	2	2	-	211	1	2	2	-	203	1	3	2	-	208	1	3	2	-
Day	5(Cont)				6					7					8				

O, oedema, C, colour change, E, exudate and slough, G, gangrene, C, ascorbic acid injection, F, freezing injury, -, means not present or not administered.

Summary Sheet

Following the Data Sheets is a Summary Sheet which gives the average weight for each group each day, and under I, or Injury Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes apparent together for each group each day. Since no animals expired there was no extrapolating required.

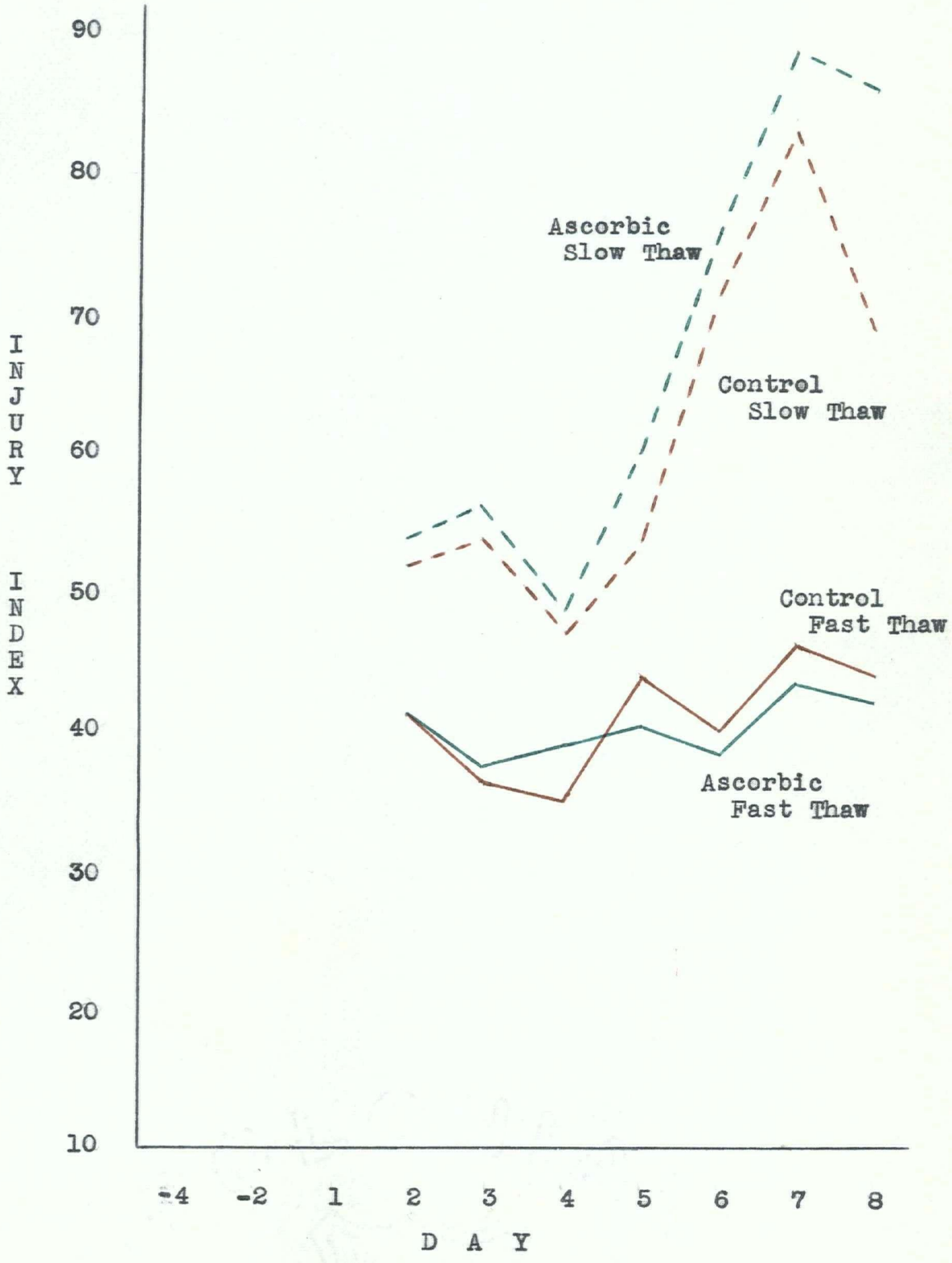
Graphs

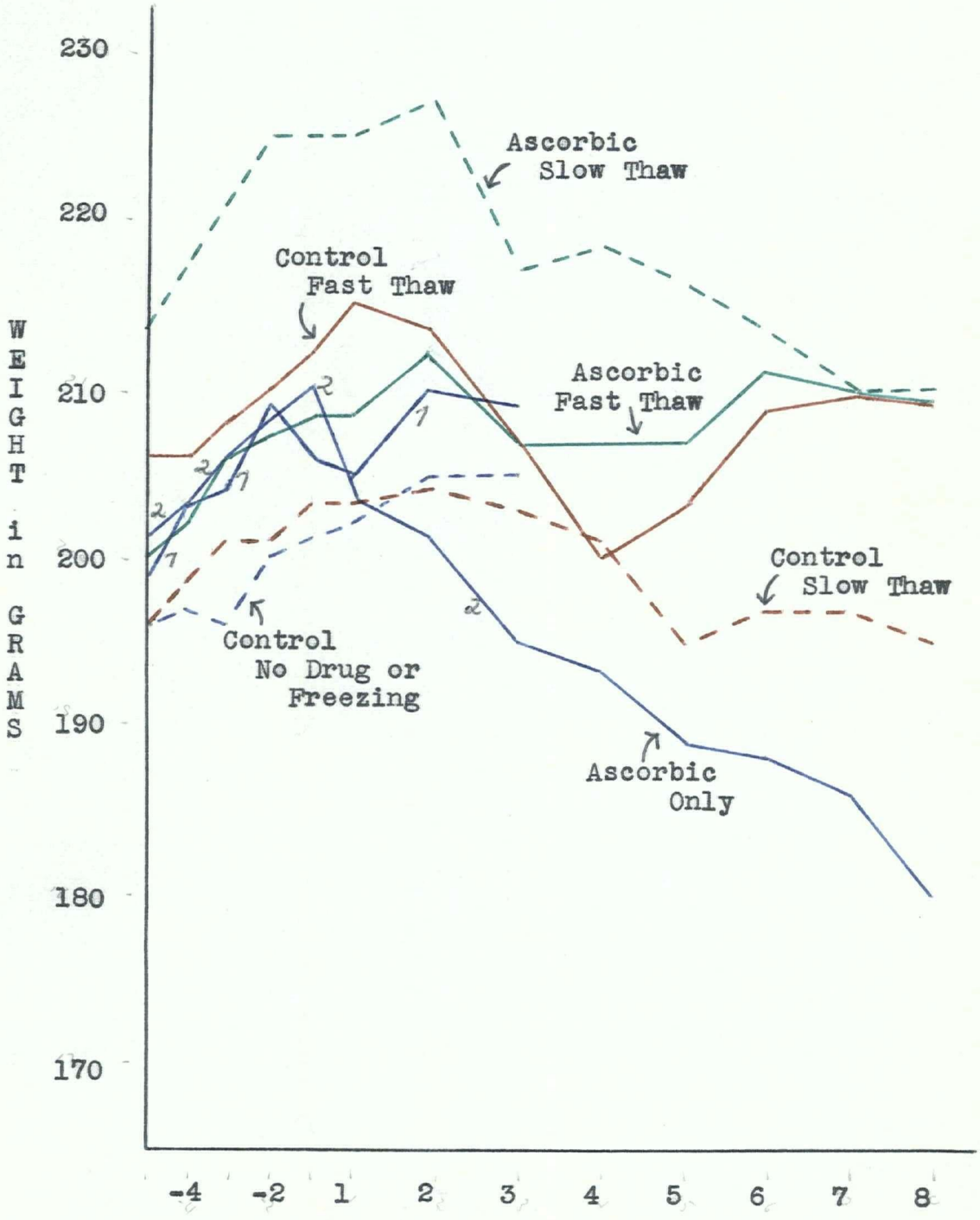
The values on the Summary Sheet are plotted directly onto two simple line graphs, the first for the day to day Injury Index changes, and the second for the corresponding weight changes. These are done in colours for easier reading (the same colours are used throughout all gross graphs), but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers. In the case of the weight graph, two shorter lines are included for Groups 6 and 7. It has, because of crowding, been necessary to indicate Group numbers on a few of the crossing lines.

Summary Sheet

Grp	Gm	Gm	Gm	Gm	Gm	Gm	Gm	I	Gm	I
1	196	198	201	201	203	203	204	52	203	54
2	201	203	206	208	210	203	201		195	
3	213	217	221	225	225	225	227	54	217	56
4	206	206	208	210	212	215	213	41	207	36
5	200	202	206	207	208	208	212	41	207	37
6	196	197	196	200	201	202	205		205	
7	199	203	204	209	206	205	210		209	
Day	-5	-4	-3	-2	-1	1	2		3	

Grp	Gm	I	Gm	I	Gm	I	Gm	I	Gm	I
1	201	47	195	54	197	71	197	83	195	79
2	193		189		188		186		180	
3	218	48	216	60	213	76	210	89	210	86
4	200	35	203	44	209	40	206	46	204	44
5	207	39	207	40	211	38	210	43	209	42
Day	4		5		6		7		8	


90
80
70
60
50
40
30
20
10
INJURY INDEX
Ascorbic Slow Thaw
Control Slow Thaw
Control Fast Thaw
Ascorbic Fast Thaw
-4
-2
1
2
3
4
5
6
7
8
D A Y


230
220
210
200
190
180
170
WEIGHT in GRAMS
-4
-2
1
2
3
4
5
6
7
8
Ascorbic
Slow Thaw
Control
Fast Thaw
Ascorbic
Fast Thaw
Control
Slow Thaw
Control
No Drug or
Freezing
Ascorbic
Only

Condition of the Animals

The ascorbic acid injections as performed with the dosage used caused a skin slough at the site of injection. The skin over the deposit of the drug would turn black within twenty-four to thirty-six hours, and by the second day a circular piece of skin and fur would drop out, leaving a clean circular defect. This must be attributable to a strictly local effect, with no generalized reactions. Except for the pain of the injection material, the animals seemed otherwise unaffected by the drug. There is no clear-cut tendency toward weight changes apparent from the graphs that would indicate a general adverse effect. The control group, Group 2, receiving only ascorbic acid injections does show a marked weight loss that could be attributed only to the injections.

Discussion of Observations

Ascorbic acid as administered caused little influence on the course of the lesions after the standard cold injury as performed. In the case of slow thawing groups, the ascorbic acid line is at all times slightly higher than the control, and is also quite parallel to it except on Day 8 when there is diversion. It is doubtful if this difference is significant. In the case of the rapid thaw groups

the lines cross, but generally remain close together. So little effect did the ascorbic acid have clinically that it was decided useless to perform the microscopic experiments, and as mentioned the animals of Groups 6 and 7 were destroyed on Day 3.

The Injury graph gives a dramatic demonstration of the value of rapid thawing in water at 42°C. as contrasted with thawing at room temperature. The control graphs are not quite typical of what usually happend. This would indicate a slightly more severe freezing injury than is usually performed.

Histamine

Experimental Observations with Histamine

The purpose of the experiment here described was to test the effects of a standard cold injury as influenced by parenteral injections of histamine at a dosage level of 10 mg. per kilogram body weight administered subcutaneously at three hour intervals beginning immediately prior to the freezing injury and continuing for forty-eight hours afterwards. In view of the fact an effective dosage of histamine was unknown, this experiment was in the nature of a trial run, and accordingly, because animals were scarce,

a varied collection of rats were used, with almost no selection possible. There were three groups of six animals, each group containing an adult normal male Wistar, two normal adult male Hoodeds, and three normal adult nonpregnant female Hoodeds. Although each group was a mixture of breeds, each group was fairly similar to each other. The groups were designed as follows:

Group: 2. A control group to receive only the histamine injections as outlined.

4. A control group also to receive a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A test group to receive both the histamine injections as outlined, and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

These groups have the same numbering as similar groups throughout all the gross experiments. Daily weights were taken after each animal was characteristically marked on the ear, and recorded on the Data Sheets. On Day 1, the day of the freezing, the animals of

Groups 2 and 5 received their initial subcutaneous injection of histamine, and an hour and a half later, at half past ten o'clock, theanimals of Groups 4 and 5 were anaesthetized with 0.1 cc. of 0.6% nembutal solution administered subcutaneously. When anaesthetized, one hind leg of each animal in these groups was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen, the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals were then treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then the animals were returned to their cages, and were left quietly alone (except for the three hourly injections of histamine) until they wakened from the effects of the nembutal. Observations were made as recorded on the Data Sheets, by the method described above for estimating the degree of frostbite

injury under the categories of oedema, colour change, exudate and slough, and gangrene.

Data Sheets

These tables are fairly self-explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. Days 1 and 2 include a record of the three hourly injections. The symbols used are: Grp, group, Gm, grams, q3h, every three hours, O, oedema, C, colour change, E, exudate and slough, G, gangrene, W, Wistar, H, Hooded, m, male, f, female, H, histamine injection, -, means not present or not administered, F, freezing injury. All surviving animals were disposed of on Day 8 with chloroform fumes.

Summary Sheet

Following the Data Sheets is a Summary Sheet which gives the average weight for each group each day, and under I, or Injury Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes together for each group each day. Note that several values had to be obtained by extrap-

Data Sheet

Grp		Gm	Gm	Gm	Gm	q3h	Gm	O	C	E	G
2	Wm	210	219	229	229	H-HHHHHHHHH	225				
	Hm	232	236	238	235	H-HHHHHHHHH	230				
		232	230	233	233	H-HHHHHHHHH	226				
	Hf	193	177	190	193	H-HHHHHHHHH	193				
		152	139	149	145	H-HHHHHHHHH	146				
		162	151	166	170	H-HHHHHHHHH	165				
4	Wm	214	219	219	223	-F---------	220	4	1	2	-
	Hm	223	216	226	225	-F---------	219	4	2	2	-
		180	180	193	198	-F---------	187	4	1	1	-
	Hf	158	160	136	157	-F---------	153	4	3	2	-
		159	163	140	157	-F---------	153	4	1	2	-
		157	160	137	159	-F---------	158	4	1	1	-
5	Wm	177	177	179	184	HFHHHHHHHHH	182	4	2	1	-
	Hm	133	126	129	127	HF (Died)					
		159	158	169	171	HFHHHHHHHHH	167	4	2	2	-
	Hf	160	157	156	155	HFHHHHHHHHH	150	2	2	2	-
		150	150	154	154	HFHHHHHHHHH	147	4	1	2	-
		132	137	141	145	HFHHHHHHHHH	138	4	1	-	-
Day		-4	-3	-1	1		2				

Grp	q3h	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
2	HHHHHHHH	232					233					234				
	HHHHHHHH	227					230					239				
	HHHHHHHH	224					233					239				
	HHHHHHHH	186					193					194				
	HHHHHHHH	141					147					147				
	HHHHHHHH	169					169					174				
4	--------	218	4	1	2	-	211	3	1	2	-	209	3	1	2	-
	--------	220	4	2	2	-	221	3	2	1	-	225	2	2	-	-
	--------	190	3	2	1	-	202	1½	1½	1	-	203	1	1	1	-
	--------	157	3	3	2	-	160	3	3	2	-	157	3	2	3	-
	--------	156	4	1	2	-	163	3	2	1	-	156	3	2	2	-
	--------	160	4	2	1	-	166	3	2	1	-	164	3	2	3	-
5	HHHHHHHH (Dead)	176	3	2	1	-	183	3	2	2	-	184	3	2	3	-
	HHHHHHHH	166	3	3	1	-	171	3	3	2	-	178	3	2	3	-
	HHHHHHHH	150	2	3	2	-	151	2	3	3	-	152	2	4	3	-
	HHHHHHHH	145	3	1	2	-	147	3	1	2	-	152	3	2	3	-
	HHHHHHHH	134	3	1	1	-	137	3	2	2	-	134	2	2	1	-
Day	2 (Cont)	3					4					5				

Data Sheet

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
2	239					242					245				
	233					237					240				
	239					244					244				
	197					195					200				
	151					150					150				
	172					175					180				
4	205	2	1	2	-	203	2	2	3	-	266	2	2	2	-
	222	2	2	3	-	221	2	2	3	-	222	2	2	3	-
	208	1	2	2	-	207	1	2	3	-	215	1	2	1	-
	167	2	3	2	-	163	2	3	2	-	167	2	3	2	-
	157	2	3	2	-	159	2	2	-	-	161	3	2	3	-
	160	3	3	3	-	165	3	3	3	-	167	3	3	3	-
5	186	2	3	-	-	187	2	3	1	-	187	2	3	2	-
	(Dead)														
	181	2	2	2	-	188	2	3	1	-	193	2	3	2	-
	153	2	3	2	6	157	2	3	3	6	152	2	3	2	7
	157	3	2	2	-	147	3	3	3	-	134	2	3	3	-
	144	2	2	2	-	141	2	3	3	-	(Died)				
Day	6					7					8				

Summary Sheet

Grp	Gm	Gm	Gm	Gm	Gm	I	Gm	I	Gm	I
2	197	192	201	201	198		197		201	
4	182	183	175	187	182	43	184	43	187	36
5	156	156	160	156	157	40	154	37	158	43
Day	-4	-3	-1	1	2		3		4	

Grp	Gm	I	Gm	I	Gm	I	Gm	I
2	205		205		207		210	
4	186	36	187	40	186	40	200	41
5	160	46	164	44	164	52	161	54
Day	5		6		7		8	

olation in order to make up for the animals that expired. There is, of course, no Injury Index for Group 2.

Graphs.

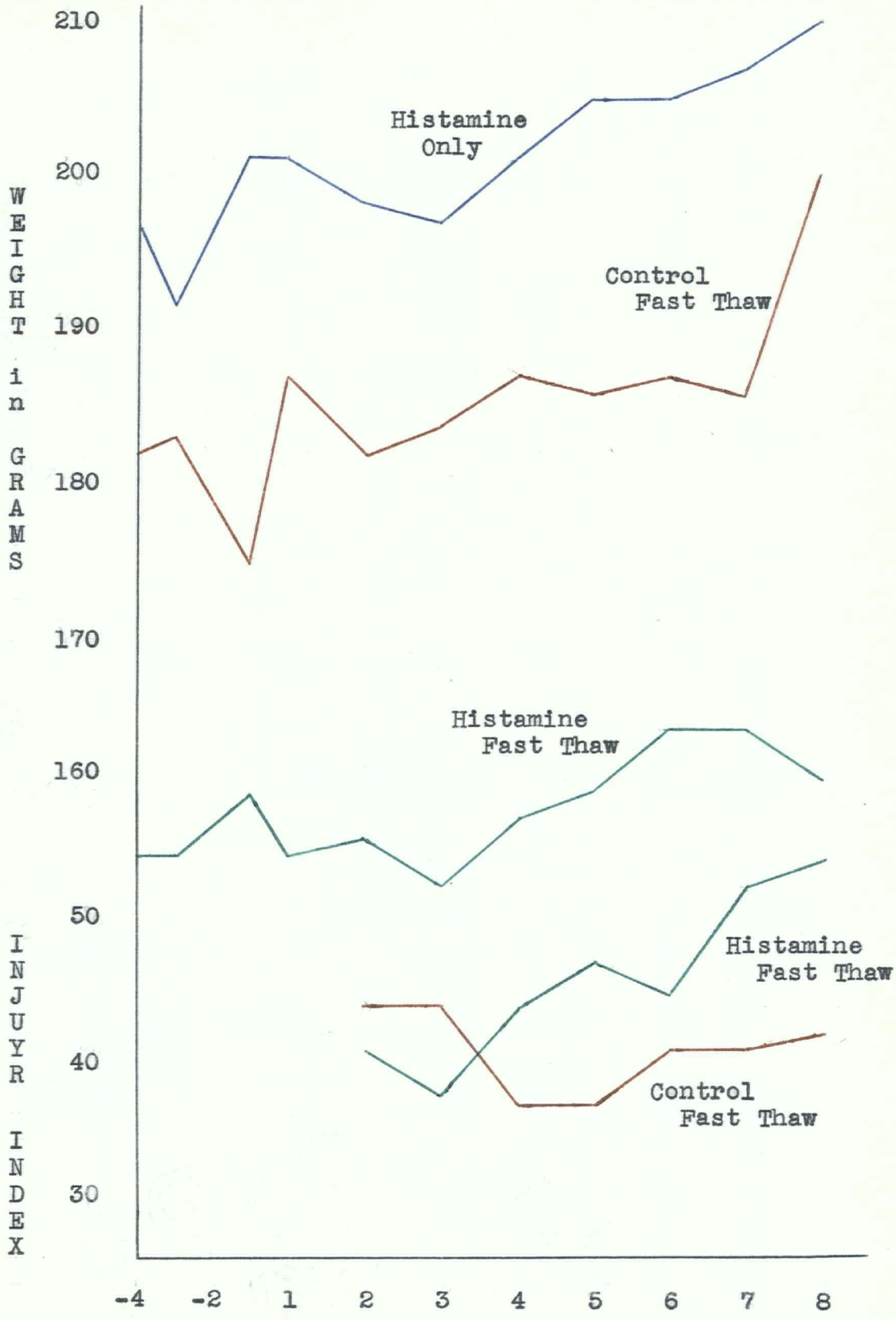
The values on the Summary Sheet are plotted directly on the simple line graph. The upper part of the graph deals with the weight changes from day to day, and there is for this part three lines. The lower part contains the Injury Index changes. These are done in colours for easier reading, but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers. The same colours and scale hold for all gross graphs.

Condition of the Animals

Two of the female Hooded rats of Group 5 were puny and did not survive the full course of the experiment. Several of the other animals appeared sickly, but this was considered quite truthfully to be the fault of the animals themselves, rather than caused by the demands of the experiment.

Discussion of Observations

As just mentioned the animals used were rather a scrubby lot. They did so poorly generally that it would not be significant to derive any positive conclusions from them. However, it will be noted that the


210
200
190
180
170
160
50
40
30
WEIGHT in GRAMS
INJUYR INDEX
Histamine Only
Control Fast Thaw
Histamine Fast Thaw
Histamine Fast Thaw
Control Fast Thaw
-4
-2
1
2
3
4
5
6
7
8

Injury Index line for histamine (solid green) is higher than for the control. It may not be possible to state the histamine influenced the course of the frostbite lesions adversely, but it is quite true that there was no beneficial effect.

As mentioned at the start, this experiment was in the nature of a trial run. It obviously needed repeating with a more suitable group of animals.

Histamine

Experimental Observations with Histamine (repeat)

The purpose of the experiment here outlined was to repeat the effects of a standard cold injury as influenced by parenteral injections of histamine at a higher dosage level of 50 mg. per kilogram body weight administered subcutaneously at three hour intervals beginning immediately prior to the freezing injury and continuing for forty-eight hours afterwards. Eighteen normal adult maleWistar rats were selected and they were divided into three groups of six animals designed as follows:

Group: 2. A control group to receive only the histamine injections as outlined.

4. A control group also to receive a

freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

5. A test group to receive both the histamine injections as outlined, and a freezing injury of twenty seconds duration with rapid thawing in water at 42°C.

These groups have the same numbering as similar groups throughout all the gross experiments. Each animal was marked characteristically on the ear, and initial weights were recorded as on the Data Sheet. The animals of Groups 2 and 5 received their initial subcutaneous injection of histamine, and an hour and a half later, at half past nine o'clock in the evening, the animals of Groups 4 and 5 were anaesthetized with 0.1 cc of 0.6% nembutal solution administered intraperitoneally. When anaesthetized, one hind leg of each animal in these groups was dipped in a beaker of ether through which carbon dioxide gas was bubbling to bring the temperature down to -20°C. The foot was immersed up to the hock (just below the fur line), and as soon as the skin blanched, indicating the foot was completely frozen

the time was counted so that the leg was immersed and frozen for exactly twenty seconds by stop watch. The animals were then treated by rapid thawing, whereby the frozen feet were immersed immediately at the expiry of the twenty seconds into water maintained at exactly 42°C. The feet were left in the warm water for approximately one minute, until thoroughly thawed and warm. Then the animals were returned to their cages, and were left quietly alone (except for the three hourly injections of histamine) until they wakened from the effects of the nembutal. Observations were made as recorded on the Data Sheet, by the method described above for estimating the degree of frostbite injury under the categories of oedema, colour change, exudate and slough, and gangrene.

Data Sheets

These tables are fairly self explanatory, being simple tallies by animals in groups, and by days, Day 1 being the day of freezing. They are necessarily as compact as possible. Days 1, 2, and 3 include a record of the three hourly injections. The symbols used are: Grp, group, Gm, grams, q3h, every three hours, O, oedema, C, colour change, E, exudate and slough, G, gangrene, H, histamine injection,

Data Sheet

Grp	Gm	q3h	Gm	O	C	E	G	q3h	Gm	O	C	E	G	q3h
2	248	H-HHHH	241					HHHHHHHHH	232					HH
	313	H-HHHH	296					HHHHHHHHH	291					HH
	232	H-HHHH	229					HHHHHHHHH	219					HH
	213	H-HHHH	214					HHHHHHHHH	206					HH
	244	H-HHHH	235					HHHHHHHHH	227					HH
	206	H-HHHH	211					HHHHHHHHH	207					HH
4	243	-F----	234	4	1	-	-	---------	224	2	2	-	-	--
	278	-F----	279	4	1	1	-	---------	265	3	2	-	-	--
	249	-F----	247	4	2	1	-	---------	233	3	2	1	-	--
	205	-F----	208	4	2	1	-	---------	195	3	2	1	-	--
	305	-F----	295	4	1	1	-	---------	301	3	2	-	-	--
	234	-F----	227	4	1	1	-	---------	230	3	2	1	-	--
5	188	HFHHHH	191	3	2	1	-	HHHHHHHHH	184	3	2	1	-	HH
	219	HFHHHH	217	4	1	1	-	HHHHHHHHH	209	4	2	1	-	HH
	239	HFHHHH	247	4	1	1	-	HHHHHHHHH	230	4	2	1	-	HH
	229	HFHHHH	234	4	2	1	-	HHHHHHHHH	223	3	2	1	-	HH
	261	HFHHHH	253	4	1	-	-	HHHHHHHHH	245	3	2	1	-	HH
	209	HFHHHH	200	4	2	1	1	HHHHHHHHH	192	3	1	1	-	HH
Day	1		2						3					

Grp	Gm	O	C	E	G	Gm	O	C	E	G	Gm	O	C	E	G
2	237					241					241				
	296					294					293				
	225					227					230				
	218					224					223				
	239					243					244				
	215					224					224				
4	233	1	1	-	-	228	1	1	1	-	232	1	1	-	-
	271	1	1	-	-	267	1	1	-	-	269	1	1	-	-
	241	3	2	1	-	236	2	2	1	-	244	1	2	-	-
	203	3	3	1	-	201	2	2	2	-	196	2	2	1	-
	290	2	2	-	-	300	1	1	-	-	291	1	1	-	-
	225	3	3	2	-	228	3	3	2	-	224	3	3	2	-
5	188	3	2	2	-	192	3	2	3	-	189	3	3	3	-
	213	3	2	2	-	214	3	2	2	-	216	3	2	2	-
	231	3	2	2	-	192	3	2	2	-	234	3	3	3	-
	222	3	2	1	-	231	2	3	2	-	230	2	3	-	-
	245	2	2	2	-	246	2	3	2	-	247	2	3	3	-
	194	3	2	1	-	199	3	2	2	-	198	2	2	1	-
Day	4					5					6				

-, means not present or not administered, F, freezing injury. All animals were disposed of on Day 6 with chloroform fumes.

Summary Sheet

Following the Data Sheet is a Summary Sheet which gives the average weight for each group each day, and under I, or Injury Index, gives the total for the injury changes occurring for that group that day. As described before, this concept of an Injury Index is just an arbitrary number obtained by adding all the pathological changes together for each group each day. In as much as no animals died, no extrapolation was required to complete the groups for six animals.

Graphs

The values on the Summary Sheet are plotted directly on the simple line graph. The upper part of the graph deals with the weight changes from day to day, and there is for this part three lines. The lower part contains the Injury Index changes. These are drawn in colours for easier reading, but each line is otherwise clearly labelled by the nature of the injury and treatment rather than by group numbers. The same colours and scale hold for all gross graphs.

Summary Sheet

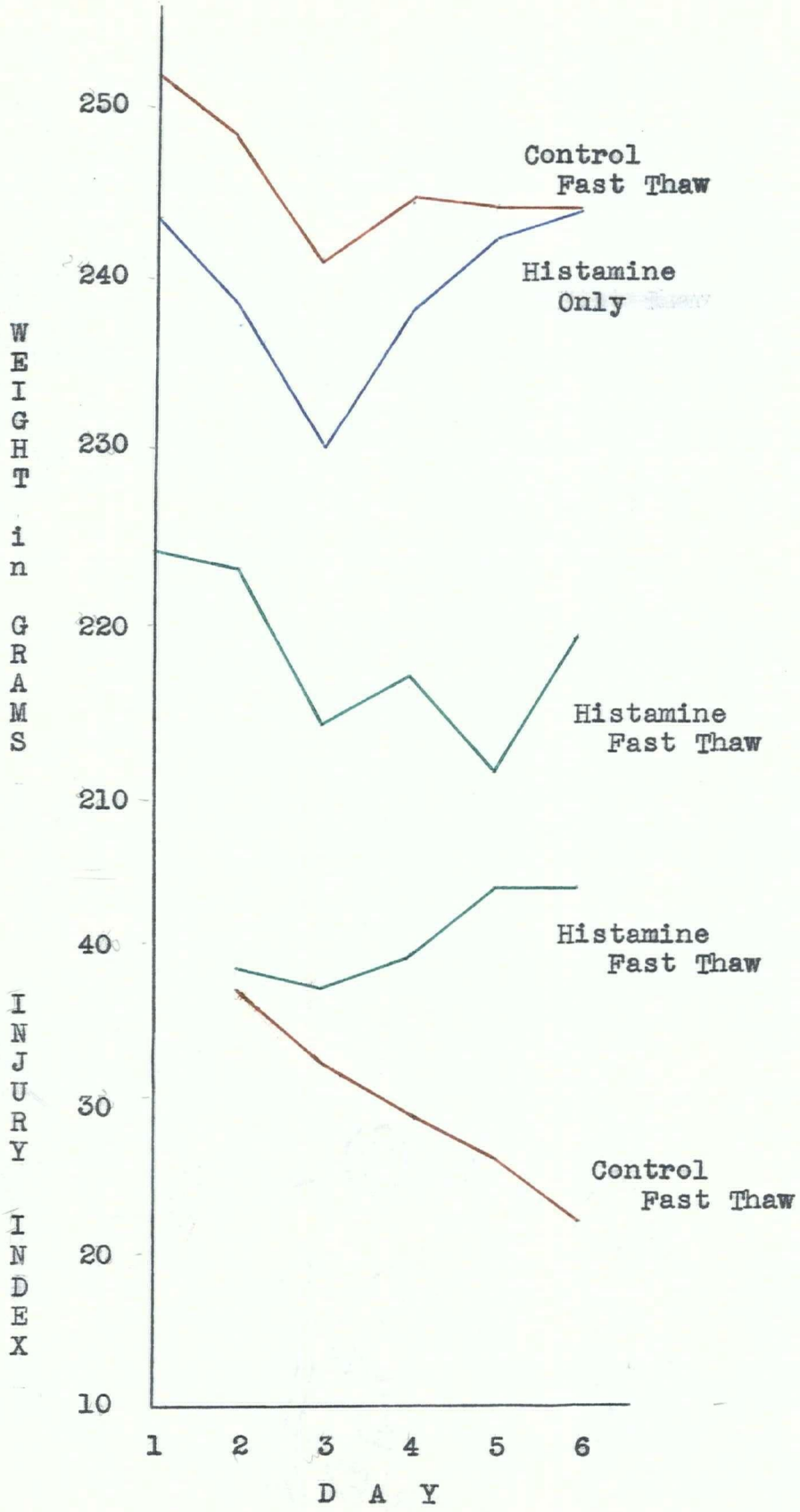
Grp	Gm	Gm	I	Gm	I	Gm	I	Gm	I	Gm	I
2	243	238		230		238		242		243	
4	252	248	37	241	32	244	29	243	26	243	22
5	224	223	38	214	37	217	39	212	43	219	43
Day	1	2		3		4		5		6	

Condition of the Animals

The present selection of rats, being a more mature group than the animals used in the last experiment survived excellently, and were in no way adversely affected by the histamine injections.

Discussion of Observations

In view of the fact a further experiment with histamine with a better group of animals continued to give diverging Injury Index graph lines, with the line for the histamine on top, all adds significance to the contention that histamine, as administered, adversely influences the course of frostbite lesions as produced in this experiment.


250
240
230
220
210
40
30
20
10
WEIGHT in GRAMS
INJURY INDEX
Control
Fast Thaw
Histamine
Only
Histamine
Fast Thaw
Histamine
Fast Thaw
Control
Fast Thaw
1
2
3
4
5
6
D A Y

VII Significance of Data

There is no intention of analyzing statistically in this section of the thesis all of the data obtained so far. Such would be a tremendous task, quite beyond the capabilities of the writer, and certainly not justified because of the very subjective nature of the observations, especially of the microscopic part. However, conclusions have been made from the data which do not have obvious justification, and it is proposed to study a few selections of the data to determine whether or not they are significant. In this way the remainder of the data may be related to the analyzed material.

Threshold Epinephrine Response

The main purpose behind the preliminary microscopic experiments in Part III was to provide a standard for microscopic observation of the mesoappendix vasculature that could be related to all subsequent observations. Accordingly, the data obtained must be considered and the Standard Error (S.E.) calculated.

On page 49 we have a summary of data which is here recorded, to the nearest millionths dilution. X refers to each individual number, $\bar{X}$ is the mean, Σ, the sum, $\bar{x}-x$ the deviation of each number from the mean, $(\bar{x}-x)^2$ the square of this deviation.

Data	x	$\bar{x}-x$	$(\bar{x}-x)^2$
	5	.4	.16
	4	.6	.36
	5	.4	.16
	5	.4	.16
	5	.4	.16
	4	.6	.36
	4	.6	.36
	5	.4	.16
$\bar{x}$	4.6		$\sum$1.88

$$S.E. = \sqrt{\frac{\sum(\bar{x}-x)^2}{n(n-1)}} = .183$$

This being less than 2.0 suggests a very close relationship between the values, and the fact that twice the Standard Error is only .366, also far less than 2.0 indicates such a relationship even more strongly.

Further along, in the same section, at pages sixty and sixty-one we have a comparison of the threshold epinephrine response between normal controls and animals made hypertensive with compound F. Here we have two sets of figures recorded to the nearest millionths of dilution:

Control	Test
5	18
5	18
5	18
7	18
4	20
10	
5	

The difference between these sets is highly significant because there is absolutely no overlap of any of the numbers, and if none of the data overlap, the values

for twice the significant error on either side of the mean for both groups could not possibly overlap either.

Procaine Microscopically

In the microscopic experiments using topical procaine, the first data obtained provides two sets of figures, as copied below from page 70, the numbers indicating the time in seconds for the onset of vascular stasis:

	x	$\bar{x}-x$	$(\bar{x}-x)^2$	
Control	45	49	2401	
	210	116	13456	
	30	64	4096	
	90	4	16	
$\sum$	375		$\sum$ 19969	
$\bar{x}$	94			S.E. = 40.8
Test	20	152	23104	
	210	38	1444	
	90	82	6724	
	90	82	6724	
	450	278	77284	
$\sum$	860		$\sum$ 115280	
$\bar{x}$	172			S.E. = 75.9

These Standard Errors are exceedingly large, and the spread on either side of the mean for the control group would be from 53.2 to 130.8, and for the test group from 96.1 to 247.9 There is too much overlap for there to be any real significant difference between the two groups of data, and any significance is only slightly possible.

However, we find more favourable data for procaine

parenterally. The summary table on pages 82 and 83 gives the following figures, also for time for onset of stasis in seconds:

	x	$\bar{x}-x$	$(\bar{x}-x)^2$	
Control	45	10	100	
	30	5	25	
	45	10	100	
	30	5	25	
	30	5	25	
	30	5	25	
Σ	210		Σ 300	
$\bar{x}$	35			S.E. = 3.16
Test	60	10	100	
	30	40	400	
	180	110	12100	
	60	10	100	
	40	30	900	
	50	20	400	
Σ	420		Σ 14000	
$\bar{x}$	70			S.E. = 21.6

The spread of the Standard Error about the mean for the control group is 31.84 to 38.16, and for the test group it is from 48.4 to 91.6. It will be noted that there is no overlapping in these two ranges, which suggests a probable significant difference between the two groups. However, the same spread using twice the Standard Error is for the control groups from 28.68 to 41.32 and for the test group it is from 26.8 to 113.2. Here we notice the range for the test values embraces the spread for the controls. This greatly reduces the chances of the difference being significant, although a definite difference is still possible.

The data thus far examined indicates the trend in the experiments involving the microscopic observation.

Time and Rate of Thawing

Concerning the gross experiments, we have first the time and rate of thawing data to consider. The graphs and the summary sheet are but two expressions of the same summarized data (see pages 209 and 210). What has to be determined is whether any two graphs are significantly different from each other. To settle this we must first establish whether any two points on the graph lines in question on the same day are significantly different.

On Day 2, the Injury Index value for Group 3 was 39 and for Group 6 it was 48 (page 209). These are two points relating the influence of the rate of thaw in a freezing injury of sixty seconds (broken blue lines). The data sheet for Day 2 (page 206) relative to the animals of these groups gives us, by summing the injury values for each animals in turn the following data:

	x	$\bar{x}-x$	$(\bar{x}-x)^2$
Group 3	7	.5	.25
	6	.5	.25
	7	.5	.25
	7	.5	.25
	6	.5	.25
	6	.5	.25
$\sum$	39		$\sum$ 1.50
$\bar{x}$	6.5		S.E.= .7

	x	$\bar{x}-x$	$(\bar{x}-x)^2$
Group 6	9	1	1
	9	1	1
	9	1	1
	8	0	0
	8	0	0
	5	3	9
$\sum$	48		$\sum$ 12
$\bar{x}$	8		

S.E. = .63

The spread for twice the Standard Error about the mean gives a range for Group 3 of from 5.8 to 7.2 and for Group 6 of from 7.37 to 8.63. The fact that there is no overlap of even twice the Standard Error is very strongly suggestive that there is a significant difference between the two groups. S uch a conclusion would apply to all the data of this experiment provided we compare only the lines of the same colour or only solid or dotted lines. Comparing a solid line of one colour with a dotted line of another colour would lead to meaningless comparisons. For instance, a comparison of the five second freeze graph with slow thaw against the twenty second freeze graph with fast thaw (blue broken and red solid lines on page 210) would involve two variables, duration of freezing and rate of thaw.

Rutin

As a further example of significant data, the graphs for rutin will be considered. On the Data Sheet, page 274, it will be seen that no animals died. The data

for Groups 1 and 3 on Day 6 gives two sets of injury values by animals as below:

	x	$\bar{x}-x$	$(\bar{x}-x)^2$	
Group 1	6	0	0	
	7	1	1	
	9	3	9	
	6	0	0	
	6	0	0	
	3	3	9	
$\sum$	37		$\sum$ 19	
$\bar{x}$	6			S.E. = .8
Group 3	5	.5	.25	
	10	5.5	30.25	
	3	1.5	2.25	
	3	1.5	2.25	
	3	1.5	2.25	
	3	1.5	2.25	
$\sum$	27		$\sum$ 39.50	
$\bar{x}$	4.5			S.E. = 1.2

The spread about the mean for Group 1 given by the Standard error gives a range of from 5.2 to 6.8 and for Group 3 the corresponding range would be from 3.3 to 5.7. Here there is some overlap which does reduce the significance of the difference considerably, but still a difference in the behaviour of the two groups on the particular day in question is likely. Only one aspect of the complete graph has been examined, and this aspect was the most unlikely appearing comparison, taken on Day 6 when the graph lines in question were fairly close together. On Day 4 the same two graphs are furthest apart. Taking the data from page 274 for these two groups, Groups 1 and 3 on Day 4 we have again injury totals by animals as follows:

	x	$\bar{x}-x$	$(\bar{x}-x)^2$	
Group 1	10	1	1	
	9	0	0	
	10	1	1	
	9	0	0	
	9	0	0	
	6	3	3	
Σ	53		Σ 11	
$\bar{x}$	9			S.E. = .6
Group 3	8	1	1	
	9	2	4	
	7	0	0	
	6	1	1	
	5	2	4	
	6	1	1	
Σ	41		Σ 11	
$\bar{x}$	7			S.E. = .6

Here the spread on either side of the mean given by the Standard Error gives for Group 1 a range of 8.4 to 9.6 and for Group 3 a range of from 6.4 to 7.6. Here we see no overlap which indicates a significant difference although examination of the same spread provided through the more exacting standard of using twice the Standard Error gives a range for Group 1 of 7.8 to 10.2 and for Group 3 of from 5.8 to 8.2. This reduces the probability of the difference being significant, but does not necessarily cancel out the significance. Hence, incorporating both these analyses into the graphs for slow thaw frostbite lesions permits us to say it is likely but not entirely probable that rutin results in a significantly less severe course over the time interval studied.

Ascorbic Acid

Then, at the other extreme, as in the case with ascorbic acie, we have to consider whether the corresponding graphs are similar or not. The dotted lines on page 290, as are the solid lines, are running quite parallel to each other. It is almost apparent, without calculating standard error that the medication has not made the slightest difference on the course of the frostbite lesions. However, the other comparison is made even more significant, that rapid thawing results in an obviously decreased severity in the course of frostbite lesions.

Conclusion

This brief examination of the data as has been done will serve to strengthen the validity of the conclusions arrived at in the final section of this thesis. All the data certainly could not have been analyzed, nor could the more elaborate statistical methods dealing with the degree of probability be applied. At best we can only speak with rough generalities.

VIII Conclusions Reached

This investigation was an indirect attempt to determine whether a specific selection of ten medications would or would not influence favourably the vascular responses in the frozen extremities of the rat. An indirect approach was considered necessary because it was not considered practical to observe the vessels in the extremities. As has been outlined at the beginning, the vascular bed of the mesoappendix was selected as the site for the microscopic studies. Any observations made at the mesoappendix portion of the vascular system had to be compared with the gross effects at the site of original interest, the extremities.

This whole investigation was at all times a most interesting one to be working on. Much experience was gained in the microscopic examination of vascular reactions. Each of the preparations studied required at least half an hour. Such experience certainly provided a different concept of the vascular system than had been entertained previously by the writer.

The threshold epinephrine response test of Zweifach was duplicated with some high degree of reliability, which was very grafifying in view of the fact such work

was the writer's first experience with microtechnique of this nature, and also the vasotropic effect of compound F was corroborated with Warner's findings, which was a source of keen satisfaction to us both.

As far as the influence that the various medications had on the vascular system of the mesoappendix after localized freezing was concerned, it is firstly important to remember that all the drugs tested were expected to have some influence. Such influence as was noted was observed according to very highly subjective impressions. These impressions could not be proven to be statistically significant. All the microscopic experiments resulted in the same conclusion: any differences noted in behavior of the circulation due to treatment by one of the ten drug agents were of doubtful significance. Because of this it was decided that the technique as employed for observing the effect of localized cold injury on the vascular bed of the mesoappendix was a very poor tool for investigating cold injury.

In regard to the gross experiments more definite findings were found:

1. As expected, the rate of thawing a frozen extremity was a significant factor in determining the degree of injury that freezing would produce. Warm thawing in water at 42°C. was the method of rapid thawing selected.

2. The duration of the frozen state also bore a direct relationship to the severity of the resulting lesions, up to a maximum, of course, of total gangrene and subsequent amputation by the animal.

3. Procaine was found to be very toxic in the dosage level administered, and on a repeat experiment it was found to provide no significant benefit.

4. Priscoline provided no significant benefit grossly.

5. Benadryl provided no significant benefit. It was suggested but not proved that Benadryl might actually have a detrimental effect on the course of frostbite lesions.

6. Etamon and Hydergine provided no significant benefit.

7. Apresoline provided some possibly significant benefit, but Chlor-Tripolon as administered made the course of the frostbite injury significantly more severe.

8. Rutin has been considered already on page 313, and it was felt that rutin had a probably significant benefit on the course of the frostbite lesions.

9. Ascorbic acid as administered had absolutely no influence whatever on the course of the frostbite inju ry.

10. Histamine was found to have a probably significant harmful effect.

The attempt to follow the course of a frostbite injury on a time basis was made throughout all the gross experiments, and it was found quite practical to present the day by day changes on a simple line graph. However to accomplish this one a numerical basis, the arbitrary concept of an Injury Index was introduced. The actual determination of an all inclusive Index evaluation presented some difficulties, but a workable solution was found and this was employed throughout all the gross experiments.

By way of apparatus, the equipment for the microscopic examination of the rat mesoappendix was constructed according to Zweifach's directions, and the cold point for controlled hypothermia of Hass and Taylor was made. This equipment was found to work quite satisfactorily.

Summary of Conclusions

1. Usefulness of the threshold epinephrine response test for vasotropic substances was confirmed.

2. The vasculature of the rat mesoappendix was observed in respect to its reactions to a standard localized freezing injury. Procaine, Priscoline, Benadryl, Etamon, Hydergine, Apresoline, Chlor-Tripolon, Rutin, Ascorbic acid, and Histamine failed to influence these reactions significantly.

3. A dynamic graphical method was described

for recording gross frostbite lesions grossly, and the arbitrary concept of an Injury Index was introduced.

4. Procaine, Priscoline, Benadryl, Etamon, Hydergine, and Ascorbic acid were found to have no significant effect on the course of frostbite lesions grossly.

5. Chlor-Tripolon and Histamine probably had a significant adverse effect.

6. Apresoline, and especially Rutin probably had a beneficial effect.

7. The value of rapid thawing in water at 42°C. was consistantly confirmed.

APPENDIX

Equipment for Hass and Taylor's Cold Point Apparatus

1. Cylinder of carbon dioxide gas at 1,000 pounds pressure.

2. Freezing attachment for Spencer carbon dioxide microtome, complete with copper tube connection. Scientific Supplies Co. Ltd., Cat. No. 50, p.630, Item No. 38756.

3. Hypodermic needles as in Becton, Dickinson and Company (B.-D.) Cataloque for May, 1951.

Item LNR 19 G 3" page 26.

Item LNRS 15 G 1½" page 26.

4. Connectors:

Luer-Lok hose end connector, H/468L, Page 33

Adams Luer-Lok connector with side arm adapter, 429A, page 36.

5. Labour required:

(1) Attach H/468L to freezing attachment

(2) Solder 429A at proper site on shaft of No.19 needle.

Chemicals Used

Water, ordinary laboratory single distilled from city supply (chlorinated), sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, sodium

dihydrogen phosphate, glucose, ash-free gelatin, all as ordinary C.P. laboratory reagents as available without special ordering.

Procaine hydrochloride, Winthrop-Stearns, as Novocain powder in five gram bottles.

Priscoline, Ciba Company Limited brand of 2-benzyl-imidazoline hydrochloride (benzazoline) available in rubber capped vials of 10 cc., each cc. containing 25 mg.

Benadryl hydrochloride, Parke, Davis and Co. Ltd. brand of diphenhydramine hydrochloride available in 10 cc. Steri-vials each cc. containing 10 mg.

Etamon Chloride, Parke, Davis and Co. Ltd. brand of tetraethylammonium chloride available in 20 cc. Steri-vials with 100 mg. per cc.

Hydergine, Sandoz (Canada) Ltd. brand of equal proportions each of dihydroergocornine, dihydroergocristine, and dihydroergokryptine, available in 1 cc. ampoules containing 0.3 mg. of the mixture.

Apresoline Hydrochloride, Ciba Company Ltd. brand of 1-hydrazinophthalazine hydrochloride, available in 1 cc. ampoules containing 20 mg. in boxes of 5 ampoules.

Chlor-Tripolon, Schering Corporation Limited brand of chlorprohpenpyridamine maleate, obtained as the pure powder.

Rutin, Parke, Davis and Co. Ltd., obtained as 50 mg. compressed tablets in bottle of 500.

Ascorbic acid and Histamine phosphate obtained in 1 gram vials from British Drug Houses.

Hydrocortone, Merck and Co. Ltd. brand of hydrocortisone acetate (compound F) available as the saline suspension in sterile rubber-capped vials containing 25 mg. per cc.

Epinephrine, Connaught Medical Research Laboratories 1:1000 solution of epinephrine hydrochloride, available in 30 cc. sterile rubber-capped vials.

Nembutal, Abbott Laboratories Limited, Item No. 8612. 100 cc. rubber diaphragm capped vial for veterinary use containing 60 mg. per cc.

Ringer-Locke Solution

Sodium chloride	36 gms.
Potassium chloride	1.68
Calcium chloride	.96
Sodium bicarbonate	.80
Water	4000 cc.
Glucose	8 gms.
Gelatin	40

All solid ingredients except gelatin were weighed out carefully and dissolved in the water. The gelatin was dissolved in a 1000 cc only, heated until completely dissolved, then filtered under suction and added to the rest of the solution. Usually the sodium bicarbonate was not weighed out, but when the solution was mixed, a

dropper full of methyl red indicator was added to the completed solution, and then sodium bicarbonate was slowly added until the first change to red.

References Cited

(1) Orr, K.D. and Fainer, D.C.,
Cold Injury in Korea During Winter of 1950-51,
Medicine 31:177-220,(may)1952

(2) Webster, D.R. and Bigelow, W.G.,
Injuries Dueto Cold, Frostbite, Immersion Foot, and Hypothermia,
Canad.M.A.J., Civil Defence Issue, 67: 534-538,(Dec)1952.

(3) Ferrer, M.Irene, ed.,
Trans. First Conference, Cold Injury, June 4-5, 1951,
Josiah Macy Jr. Foundation, New York, 1952.

(4) Shumacker, H.B.Jr., and Lempke, R.E.,
Recent Advances in Frostbite,
Surgery 30:873-904,(Nov)1951

(4a) Editorial,
Frostbite,
J.A.M.A. 148:940-941,(Mar.15)1952.

(5) Laufman, H.,
Profound Accidental Hypothermia,
J.A.M.A. 147:1201-1212,(Nov.24)1951.

(6) Larrey, D.J.,
Memoirs of Military Surgery and Campaigns of the French Armies,
Trans. by Hall, R.W.
Baltimore, J.Cushing, 1914, vol.II, 156-164.

(7) Warner, D.L.,
Capillary Changes in Rats Rendered Hyper-Tensive by the Administration of Compound F.
Unpublished Graduating Thesis, Faculty of Medicine, University of British Columbia, April, 1954.

(8) Hass, G.M. and Taylor, C.V.,
A Quantitative Hypothermal Method for the Production of Local Injury of Tissue,
Arch. Path. 145:563-580, (May)1948.

(9) Griffith, J.Q. and Farris, E.J., eds.,
The Rat in Laboratory Investigation,
J.B. Lippincott, Philadelphia, 1942.

(10) Beckman, H.,
Pharmacology in Clinical Practice,
W.B. Saunders, Philadelphia, 1953, p.208.

(11) Hurley, L.A., Roberts, J.E., Buchanan, A.R., and
Tillquist, G.,
Preliminary Investigations of the Value
of the Dihydrogenated Alkaloids of Ergot
in the Treatment of Experimental Frostbite,
Surgery, Gynecology, and Obstetrics, 92:
303-308,(Mar)1951.

(12) Bluntschli, H.J. and Goetz, R.H.,
The Effect of Ergot Derivatives on the
Circulation in Man With Special Reference
to Two New Hydrogenated Compounds (Dihydro-
ergotamine and Dihydroergocornine),
Am. Heart J., 35:873-894,1948.

(13) Rothlin, E.,
Pharmacology of the Natural and Dihydro-
genated Alkaloids of Ergot.
Bull. Acad. Suiss. Sc.M., 2:249-273,1946-47.

(14) Zweifach, B.W.,
Indirect Methods for Regional Blood Flow:
I, Microscopic Observations of Circulation
in Rat Mesoappendix and Dog Omentum: Use in
Study of Vasotropic Substances, in
Potter, V.R., ed., Methods in Medical Re-
search, 1:131-139,1948, Year Book Pub. Chicago.

(15) Knisely, M.H.,
Method of Illuminating Internal Organs,
Anat. Record, 64:499,1936.

(16) Knisely, M.H.,
Improved Fused Quartz Illumination,
Anat. Record, 58:73,1934, and 71:503,1938.

(17) Knisely, M.H.,
Microscopic Observations on Transillumined
Spleens,
Proc.Soc.Exp.Biol.&.Med., 32:212,1934.

(18) Knisely, M.H., in
Handbook of Microscopical Technique,
McClung, C.E., ed., p.632-642.

(19) Chambers, R.,
Vasomotion in Hemodynamics of Blood
Capillary Circulation.
Ann. N.Y. Acad. Sc., 49:549-552,(May)1948.

(20) Chambers, R., and Zweifach, B.W.,
Topography and Function of Mesenteric
Capillary Circulation.,
Am. J. Anat. 75:173,(Sep)1944.

(21) Chambers, R.,
Blood Capillary Circulation Under Normal
Conditions and in Traumatic Shock,
Nature, London, 162:835-837,(Nov)1948.

(22) Chambers, R. and Zweifach, B.W.,
Functional Activity of the Blood Capillary
Bed, With Special Reference to Visceral
Tissue.
Ann. N.Y. Acad. Sc., 46:683-695,(Sep)1946.

(23) Luyet, in
An Outline of General Physiology,
Heilbrunn, L.V.,
Saunders, Philadelphia, 3rd ed., 1952,
p.491.