Rewarming Following Hypothermia of Two to Twelve Hours *

II. Some Metabolic Effects

BERNARD FISHER, M.D.,** E. J. FEDOR, PH.D., SIL H. LEE, M.D.

From the Department of Surgery, Section of Experimental Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania

IF, in spite of the control of ventricular fibrillation, hypothermia produces profound metabolic and pathologic changes which are not readily reversible upon rewarming, or if the process of rewarming itself results in undesirable sequellae, then serious consideration of hypothermia as a clinical modality must be abandoned. Concern that such might be the case has been aroused by (a) reports of Knocker,[19] Falkmer and Kjellgren,[9] and Sarajas [30, 31] that pathologic changes do result from hypothermia; (b) the findings of Blair, Montgomery and Swan [4] of "acute circulatory collapse" upon rewarming; (c) the mention of Deterling and co-workers [7] that "a metabolic acidosis developed during hypothermia which was not reversed upon rewarming"; (d) the suggestion of Ross [29] that deaths following rewarming might be related to a persistently low cardiac output; and, indeed, (e) the report from this laboratory [11] that as the hours of hypothermia become prolonged, in spite of a steady temperature, the more complex become the physiologic alterations and, perhaps, the more difficult to restore them to normal.

During the past two and one-half years a systematic evaluation of the effects of short and prolonged hypothermia followed by rewarming upon various physiologic parameters has been made by us. Information concerning the liver,[12] cardiovascular system,[10] and general pathology and histochemistry [13] obtained from animals cooled to 23–24° C. for from two to 12 hours and then rewarmed, has already been reported. The purpose of this paper is to present further physiologic data concerning the effects of rewarming after hypothermia. To our knowledge such specific information has not been published.

Methods

Thitry-one healthy mongrel dogs of both sexes, averaging 13 Kg. (28.6 lbs.) in weight, on the standard laboratory diet of Purina, were used in this study. Food, but not water, was withheld for 12 hours prior to induction of hypothermia. Under local anesthesia a catheter was inserted into the right ventricle via the right external jugular vein and pre-cooling control values were obtained. All blood samples were taken from this source. The standard technic and protocol for cooling and rewarming as used by this laboratory were followed. Animals were anesthetized with open drop ether and immersed in a 4° C. water bath. When body temperatures reached 29° C. all animals were removed from the bath and dried. By maintaining them in a thermostatically controlled cold room their temperatures were permitted to drop to 23–24° C., where they were maintained for the duration of the hypothermic period. All dogs breathed spontaneously, positive pressure not being resorted to. For the purpose of uniformity, the length of cooling was de-

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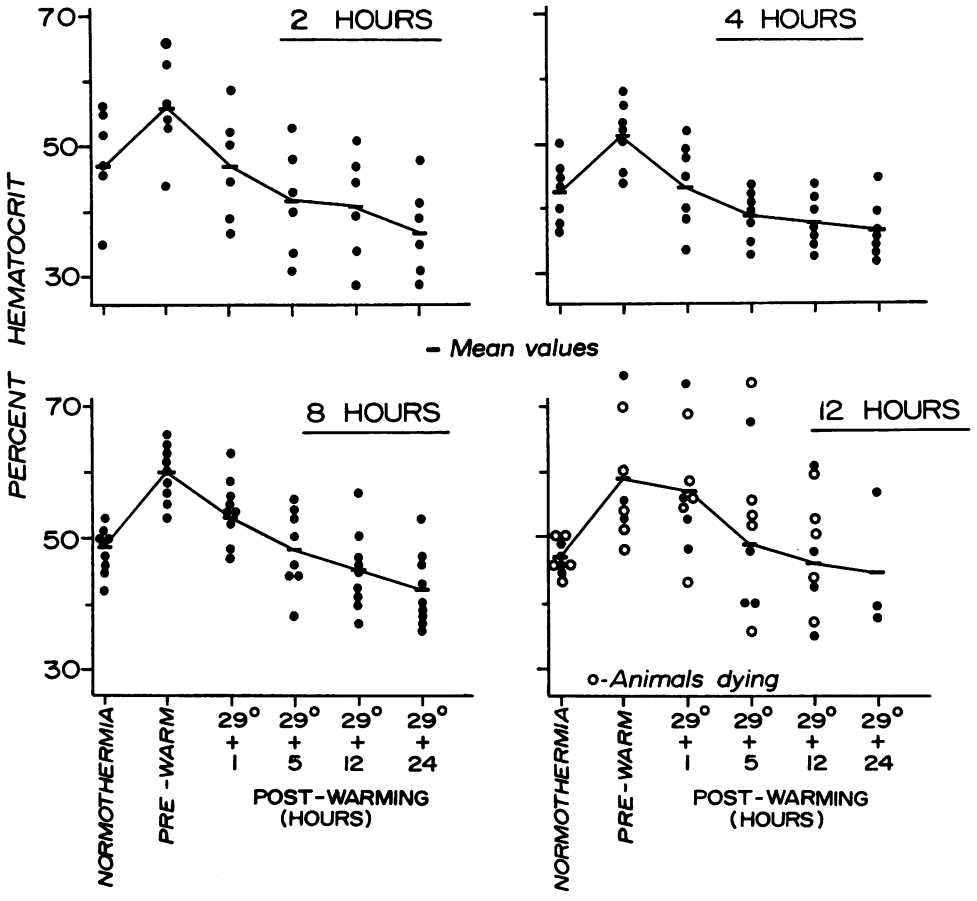

FIG. 1. Hematocrit—Two to 12 hours of hypothermia followed by rewarming.

termined from the time of removal from the bath. Just enough ether was subsequently administered to prevent shivering. Six animals were cooled two hours, seven for four hours, nine for eight hours and nine for 12 hours. Prewarming samples were taken and return to normothermia was effected by placing animals in a 40° C. water bath. Following rewarming, blood samples were taken from the unanesthetized dogs one, five, nine or 12 and 24 hours after their body temperatures had returned to 29° C. Ninth hour observations were taken from animals cooled two and four hours, while the twelfth hour sample was collected from eight and 12 hour hypothermia dogs. These times will subsequently be referred to as 29° + one hour, 29° + five hours, etc. Upon removal from the warm water, animals were dried, returned to their cages and permitted water and milk.

Hematocrits were determined by centrifugation of blood in Wintrobe tubes for 30 minutes at 3,000 r.p.m.; plasma and whole blood by the copper sulphate technic of Phillips, Van Slyke and co-workers; [27] coagulation time by a modified three-tube Lee-White method; and prothrombin time by the use of Simplastin®. A Coleman flame photometer was used for the determination of serum Na and K; and accepted analytical methods were used for estimation of serum chloride,[26] calcium,[20] phosphate,[16] and sugar.[25] Blood samples for CO_2 were col-

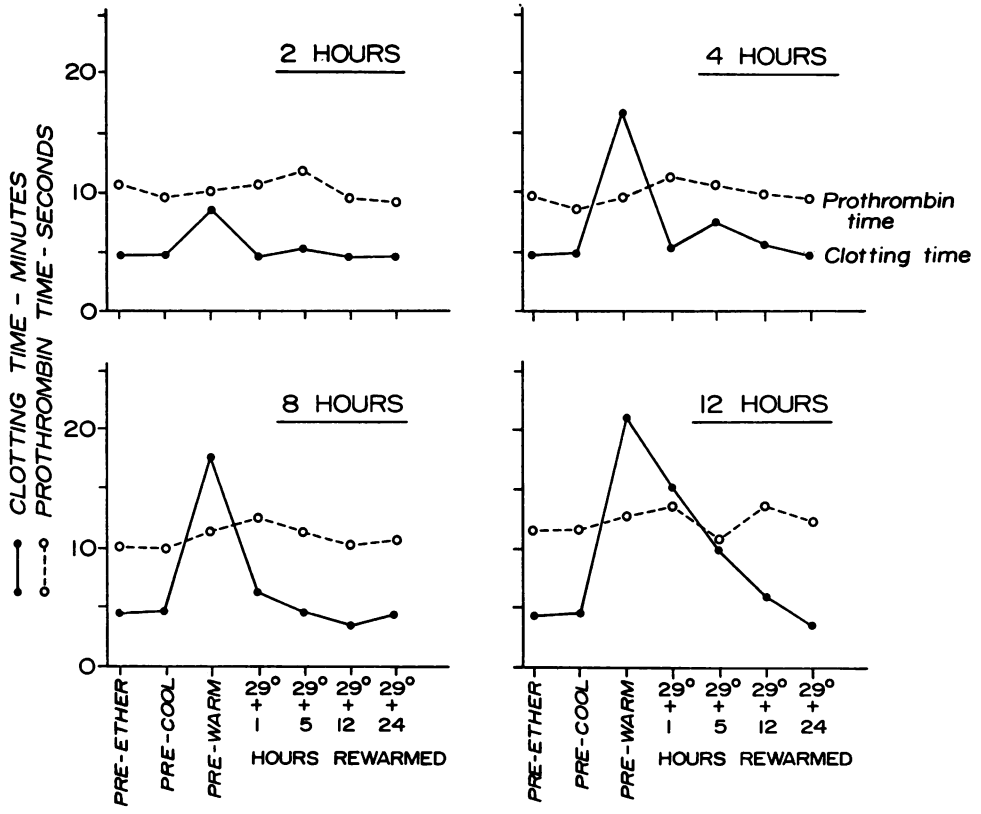

FIG. 2. Clotting and prothrombin times—rewarming after two to 12 hours of hypothermia.

lected under oil, immediately centrifuged and the plasma drawn off under oil. Blood pH was obtained at room temperature using a Beckman pH meter; blood lactates by the method of Barker and Summerson; [3] pyruvates by that of Friedemann and Haugen; [17] and amino acids by Albanese and Irby.[1]

Approximately 25 ml. of blood was removed at each sampling, which was replaced by an equal volume from a donor animal.

All values obtained in the post-warming period are compared with samples taken from the unanesthetized animal before cooling. Data was subjected to statistical analysis utilizing Fisher's t test.[14] A p value of $< .01$ was considered significant when comparing any two groups.

Results

Of the 31 animals used in this study, all of the two, four and eight hour cooled dogs survived following rewarming, while five of the nine hypothermic for twelve hours died within the first 24 hours after returning to normothermia. A complete analysis of the fate of the last 100 animals cooled in this laboratory has been presented elsewhere.[10] Death occurred in only two of 47 animals following rewarming after up to eight hours of hypothermia, whereas, nine of 18 died after rewarming following 12 hours of cooling.

By 29° + one hour the body temperature had not yet quite returned to normal, the average for all groups being 36.7° C. At 29° + five hours all animals were back to the pre-cooling temperature level.

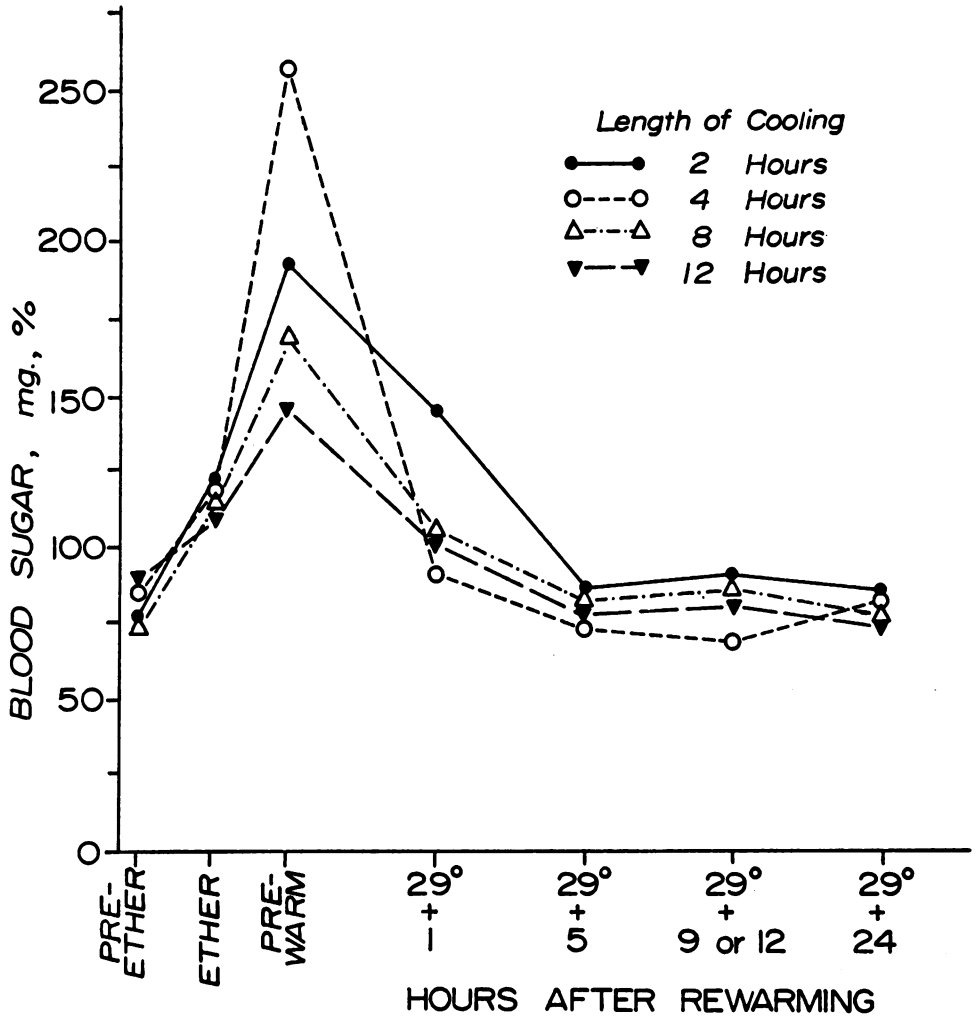

FIG. 3. Blood sugar—two to 12 hours of hypothermia followed by rewarming.

Hematocrit (Fig. 1). It has been demonstrated repeatedly that upon cooling an increase in hematocrit value occurs and that as the hypothermia is prolonged the value becomes progressively greater.[11]

In this group of experiments the two and four hour cooled animals demonstrated a 16 per cent mean increase in hematocrit, a 20 per cent mean rise after eight hours of hypothermia, and 24 per cent after 12 hours. Following rewarming there was a prompt return to the control hematocrit in five of the animals cooled two hours. In one animal, which had a hematocrit of 55 before cooling, the 29° + one hour hematocrit was 59. It had been 66 before rewarming. With the four hour cooled animals as 29° + one hour there was no statistically significant difference in the hematocrit values (43 ± 6) from the controls (42 ± 3). By 29° + five hours in both the two and four hour groups the mean hematocrit values were below those of the controls, and although no statistically significant difference existed (p values 0.4 and 0.1) nine of the 13 animals in these two groups

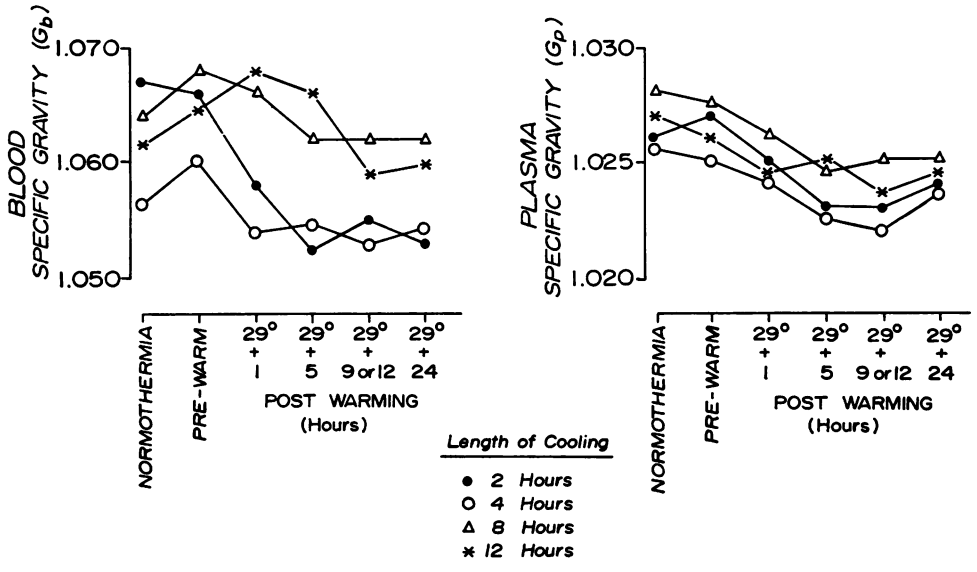

FIG. 4. Plasma and whole blood specific gravity—two to 12 hours of hypothermia followed by rewarming.

showed a lower hematocrit than before cooling. Practically the identical pattern was present at 29° + nine hours. By 29° + 24 hours this decreasing hematocrit was more evident, the p value for the two hour cooled group approaching significance (0.05) and that for the four hour group indicating a statistically significant difference (< 0.01).

Following eight hours of hypothermia and rewarming, the 29° + one hour values had not returned to normal in eight of nine animals, the p value of < 0.01 demonstrating a significant increase above the controls at this time. By 29° + five hours there was no difference from the controls and, as with the two and four hour groups, the values continued to decrease so that by 29° + 24 hours the hematocrit value for the group was 42 ± 5.4, while the controls were 48 ± 3.3, the p value being < 0.01.

After 12 hours of cooling, even by 29° + five hours all hematocrits had not returned to the pre-cooling level. This was true particularly for animals that failed to survive. The hematocrits in these animals for the most part remained higher until death than in those that survived, although one of the latter had a persistently high hematocrit, it being 49 before cooling, 75 before rewarming, and 57 at 29° + 24 hours.

Clotting and Prothrombin Times (Fig. 2). As we have demonstrated previously,[11] the longer the hypothermia the more prolonged becomes the clotting time. Again, in these experiments this was found to be so. Following rewarming all animals cooled for only two hours were back to the control level by 29° + one hour, where they remained during the entire period of observation. After four hours of hypothermia this return to normal was almost as rapid—five of seven animals returning to the pre-cooled level. Clotting times for animals cooled eight hours were slightly prolonged in five of the nine animals at 29° + one hour, but by 29° + five hours were back to normal. After 12 hours of cooling, clotting times in many instances remained increased until 24 hours after rewarming.

No significant increase in the prothrombin time of any group resulted from cooling, not even in those hypothermic for 12 hours. Our previous report emphasized that pro-

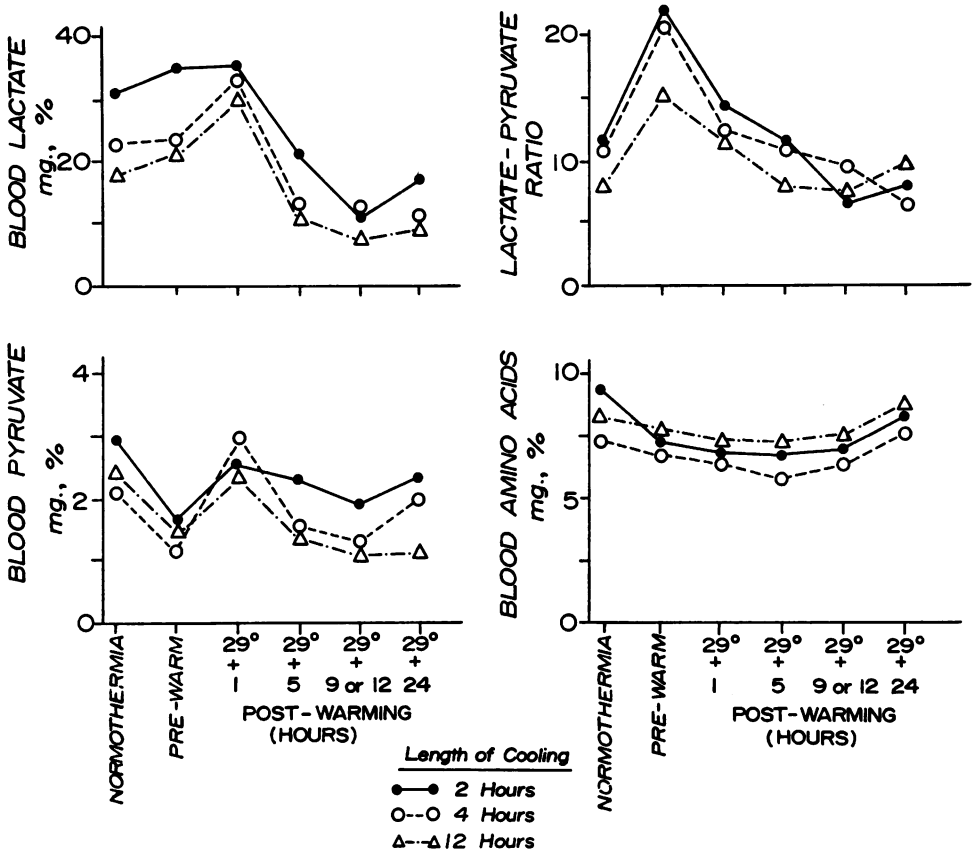

FIG. 5. Blood lactate, pyruvate, L-P ratio and amino acids—two to eight hours of hypothermia followed by rewarming.

nounced changes did not occur until after 14 or more hours of hypothermia. Following rewarming, animals cooled two hours showed no deviation in this value from the normal. Return to normothermia after four, eight and 12 hours of cooling was accompanied by a slight prolongation of this value. All were normal in the four and eight hour groups by 29° + nine and 12 hours, having been increased by 15 and 25 per cent, respectively, at 29° + one hour. Three of the four animals cooled 12 hours and surviving rewarming demonstrated prolongation until 24 hours after rewarming. This lengthening of prothrombin time in all animals dying was greater after rewarming.

Blood Sugar (Fig. 3). In all groups, before rewarming an increased blood sugar was observed. Part of this increase, as reported previously by us,[11] was due to the effects of ether anesthesia. Upon rewarming, all but the two hour group showed a mean value at 29° + one hour which was not significant from the pre-cooling one. The mean increase for the two hour group was due to one animal having a blood sugar of 210 mg. per cent. At 29° + five hours and thereafter blood sugars of all animals in all groups were within the normal range.

Plasma and Whole Blood Specific Gravity (Fig. 4). No significant difference in plasma specific gravity before rewarming from the precooling values was observed, thus agreeing with our previous report[11] and that of D'Amato.[6] At 29° + one hour of

TABLE 1. *Blood pH—Two to 12 Hours of Hypothermia Followed by Rewarming*

Pre-Cooling	Hours Cooled	Pre-Warming	29° + 1 hr.	29° + 5 hrs.	29° + 9 or 12 hrs.	29° + 24 hrs.
7.45 ±0.04	2	7.13 ±0.03	7.32 ±0.03	7.39 ±0.04	7.40 ±0.02	7.34 ±0.05
7.41 ±0.07	4	7.13 ±0.21	7.26 ±0.08	7.46 ±0.05	7.44 ±0.05	7.45 ±0.07
7.53 ±0.09	8	7.31 ±0.07	7.48 ±0.12	7.59 ±0.11	7.60 ±0.12	7.51 ±0.07
7.52 ±0.06	12	7.32 ±0.07	7.43 ±0.05	7.51 ±0.11	7.55 ±0.04	7.60 ±0.08

rewarming no alteration from normal was seen. The 29° + five and nine or 12 hour observations showed a progressive decrease in plasma specific gravity in practically all animals in all groups. This lowered value persisted in 50 per cent of all animals even at 29° + 24 hours.

Whole blood specific gravity increased significantly upon cooling in all but the two hour animals, where it remained unchanged. By 29° + one the two and four hour groups had mean values below the pre-cooling levels, whereas the eight and 12 hour groups were still elevated. These and subsequent observations followed the changes in hematocrit so that by 29° + 12 hours whole blood specific gravity was below the pre-cooling level in all groups.

The increase in hematocrit value occurring during hypothermia has been attributed to either capillary leakage or to differential plasma trapping with a decrease in circulating blood volume. The exact mechanism has not been proven definitely. Whatever the cause, it would seem from this data that in short periods of hypothermia (up to four hours) prompt return of plasma volume and circulating blood volume might be expected. Information concerning cardiac output upon rewarming [10] would substantiate this.

Lactates, Pyruvates and Amino Acids (Fig. 5). It has been well documented [21, 32] that in shock, due either to hemorrhage, tumbling or tourniquets, the metabolism of pyruvic, lactic and amino acids shows abnormalities. There occurs an elevation in both lactic and pyruvic acid, but a greater increase in the former, thus resulting in a rise in the L/P ratio. This shift toward anaerobic carbohydrate metabolism is due to anoxia, particularly as a result of peripheral circulatory failure. The blood amino acid nitrogen likewise increases. This is ascribed to hepatic anoxia and an impairment of amino acid metabolism. To further elucidate the adequacy of the circulation during hypothermia and following rewarming, these values were estimated in animals cooled from two to eight hours. Following cooling no significant change in blood lactates occurred even after up to eight hours of hypothermia. Animals in all groups demonstrated a significant decrease in blood pyruvate, resulting in an increase in L/P ratio, this value being approximately twice the upper limit of normal. After rewarming, at 29° + one hour lactates in the four and eight hour animals showed a slight increase, while those cooled two hours failed to show a change. Mean values for pyruvates were normal in the two and eight hour groups and slightly increased in the four hour animals due to one member of this group having a value of 5.1 mg. per cent. L/P ratios at 29° + one hour were at the upper limits of normal. Subsequent observations at 29° + five, nine or 12 and 24

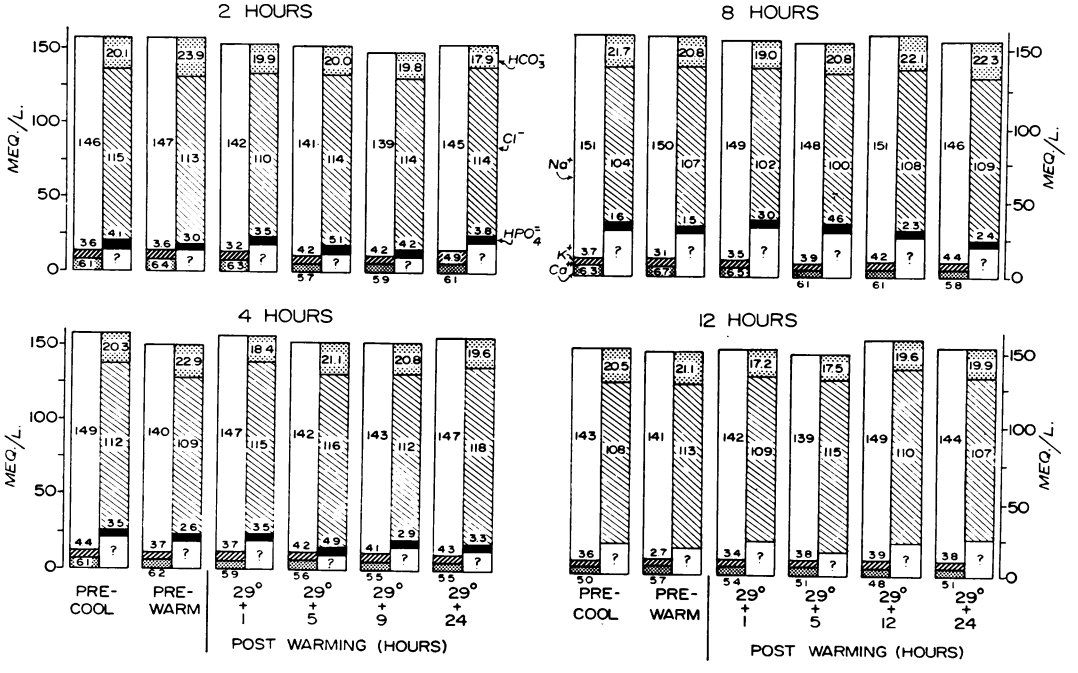

FIG. 6. Electrolyte changes—two to 12 hours of hypothermia followed by rewarming (mean values).

hours revealed normal L/P ratios, lactates and pyruvates being for the most part below the control values.

Blood amino acids were unchanged by cooling and rewarming. No significant differences from the controls were observed at any of the sampling times.

Acid Base Balance. The high pH before cooling (Table 1), as previously commented upon,[11] was due to the hyperventilation of animals occurring when they were restrained. Also, as mentioned before, most animals upon cooling demonstrated a significant drop in pH, this being to lower levels in the two and four hour cooled group where the pH was not as high as before cooling. Following rewarming there was a prompt return to normal pH, there being no statistical difference between the control values and any of the observations made.

In so far as CO_2 is concerned (Fig. 6), no striking changes occurred during cooling or rewarming. The occasionally observed decrease in CO_2 (2 hour cooled animals at 29° + 24 hours, 17.9 mEq/L) with a normal or elevated pH after rewarming can be explained by the hyperventilation of the animal during sampling. Twelve hour cooled animals that did not survive showed showed similar pH and CO_2 values.

Electrolytes (Fig. 6) *Serum sodium:* Consistent with our previous report,[11] no significant alteration resulted from cooling. Likewise, rewarming failed to produce a change. Only one animal, cooled for two hours, had a marked decrease in serum sodium at 29° + nine hours, at which time it was 127 mEq/L, having been 143 mEq/L before cooling. At 29° + 24 hours it was 150 mEq/L.

Serum potassium: No significant alterations in this value from the normal were observed following rewarming in any of the animals. Serum potassium levels remained quite constant throughout the entire observation period.

Serum calcium: In support of our previous findings no change in serum calcium occurred during cooling and, as with potas-

sium, rewarming likewise produced no change.

Chlorides and phosphate: No alteration in chlorides was observed in any of the groups either upon cooling or rewarming. Serum phosphorous likewise showed no difference from the controls.

The electrolyte pattern in animals dying did not vary from the survivors.

Discussion

The exact status of clinical hypothermia is still a disputed issue. During recent years, in addition to its use in cardiac surgery,[22, 33] it has been employed in numerous general surgical procedures; [8] in the management of the poor risk patient undergoing surgery; [2, 23] and in neurosurgical problems.[18] Universal acceptance has been inhibited by the fear of ventricular fibrillation and by the possible undesirable sequellae resulting from the hypothermia and subsequent rewarming. It has always been felt in this laboratory that if cooling produced pathologic and physiologic changes which were irreversible upon rewarming, or if rewarming itself caused damage, then, even should ventricular fibrillation be controllable or preventable, this modality had little future clinically. In order to evaluate this problem, we have, over the last few years, employed a standard cooling experiment which has purposely been made more extreme than is ordinarily employed in the human so as to more clearly define the safe boundaries of hypothermia in so far as depression of temperature and length of cooling are concerned, it being assumed that if six or eight hours of hypothermia at 23–24° C. are innocuous, then one hour at 29 or 30° C., as used in the human, should be safer still.[19] This, of course, remains to be proved. Ether has always been used by us as the anesthetic because depth of anesthesia could be more accurately controlled than following agents injected parenterally and because there was no respiratory depression. It is to be realized fully that differences in results reported by investigators may well be due to variation in anesthesia, experimental design, etc. It behooves the critical analyst of this subject to take these variables into consideration before establishing conclusions.

As mentioned previously, recent studies would indicate that pathologic changes result from hypothermia and that the reported depletion of hepatic glycogen and increased lipid deposition in the liver, adrenals and renal tubules,[19] droplet formation in hepatic cells,[9] degenerative changes in the central portions of the hepatic lobules,[30] and miliary necrosis in the myocardium [31] are due to anoxia attendant with hypothermia. Because of these reports a histologic re-evaluation using routine oversight and appropriate histochemical technics was made and the results reported.[13] It was concluded that hypothermia, even after many hours, under the conditions of our experiments did not produce consistent or significant morphologic alterations of vital organs as seen by routine staining technics. Using more specialized methods, changes such as increased deposition of lipid and depletion of glycogen in peripheral portions of hepatic lobules and in myocardium were noted. Likewise, succinic dehydrogenase appeared decreased in peripheral portion of hepatic lobules during cooling and failed to return to normal in the rewarming period. Hepatic alkaline phosphatase was increased in cell cytoplasm following rewarming in 50 per cent of the animals studied. Even so, these alterations noted occurred only after prolonged periods of hypothermia (4 hours at the earliest), and they could not be explained on the basis that hypothermia was attendant with hypoxia. Further, it was impossible to relate cause of death during hypothermia to any of these changes.

This laboratory likewise has evaluated some of the physiologic effects of hypothermia and rewarming upon the liver.[12] It was concluded: 1) that the pattern of biliary secretion after six hours of hypo-

thermia was no different from that following one hour of open drop ether; 2) that only after six hours of hypothermia was a decrease in in-vitro oxygen consumption of the liver slice obtained, and that even after 12 hours of hypothermia did rewarming result in a prompt return to normal Qo_2; 3) that following rewarming even after prolonged hypothermia was there a prompt return of hepatic blood flow; and 4) that aside from a depletion of glycogen, which did not return to normal upon rewarming, the composition of the liver remained unchanged.

Studies related to the cardiovascular system following rewarming [10] confirmed the findings of "rewarming shock" as described by Blair, Montgomery and Swan [4] only in so far as prolonged hypothermia was concerned (after 8 hours of cooling). Following two hours of cooling at 23–24° C. there was a prompt return to the pre-cooling level of cardiac index, left ventricular work, and other parameters related to the cardiovascular system. With prolongation of hypothermia this readjustment was less prompt and, in fact, 50 per cent of the animals cooled 12 hours failed to survive following rewarming.

The results presented in this paper add further physiologic information as to what happens following rewarming and are in agreement with our other findings. In general, two or four hours of hypothermia followed by rewarming are attendant with a rather prompt readjustment to normal, and as the hours of cooling are extended, return to normothermia is not always, even for many hours after, associated with a return to pre-cooling levels of many of the parameters studied.

The prompt return of the hematocrit to normal after shorter periods of hypothermia, followed by a continued drop over the first 24 hours after rewarming, and the changes observed in plasma and whole blood specific gravity, would suggest a rapid readjustment of plasma and whole blood volume with even an increase following rewarming. Recent studies by us [15] related to blood volume would indicate that this is so, adding further confirmation to our failure to observe "rewarming shock" following relatively short hypothermia. They are contrary to the suggestions of Blair *et al.*[4] that perhaps the reason for the circulatory shock following rewarming, seen by these investigators, was "a failure of homeostatic mechanisms to release blood from reservoirs into the effective circulation. . . ."

It was of interest to see that there was a prompt return of the clotting time to normal after rewarming following up to four hours of cooling. This has been a concern to those using hypothermia in surgery. Eight and 12 hours of hypothermia required a longer time to return to the pre-cooling level. The findings of no change in prothrombin time in short hypothermia and only slight transient rise in this value following rewarming in the groups cooled for longer periods of time add confirmation to our findings concerning the innocuous effects of cooling upon the liver, as does the failure of alteration of blood amino acids by cooling and rewarming.

Failure to find an increase in blood lactic acid or a rise in the lactate pyruvate ratio following rewarming would indicate an adequate circulating blood volume and, together with previous reports [10] related to coefficient of oxygen utilization, arterial-venous oxygen differences and other parameters, would suggest that prompt readjustment of circulation following rewarming is the rule after hypothermia.

The finding of a normal lactate but decreased pyruvate during cooling is difficult to explain with assurance, but perhaps may be due to a shift in the equilibrium between pyruvate and lactate as a result of the interference of cold with certain of the enzymes, or due to an accumulation of diphosphopyridine nucleotide and lactic acid dehydrogenase.

Following rewarming there was a prompt

return of blood pH, contrary to the findings of Deterling and associates.[7] In their experiments sodium pentobarbital was used as the anesthetic agent, muscle relaxants were administered and respirations were controlled. The increase in pH is compatible with the essentially normal CO_2 and lack of increase in fixed acid.

Evidence reported by others relating to physiologic parameters not evaluated by this laboratory supports this and all of the other experimental data reported by us that hypothermia of moderate duration produces no residual ill effects upon rewarming. Insofar as the physiology of the nervous system is concerned, it has been shown that the decreased cerebral blood flow, decline in cerebral metabolism, decrease in brain volume, diminution of intracranial pressure, etc., attendant with hypothermia, are all reversible on rewarming with no undesirable sequelae.[5, 28] The effect of hypothermia on renal hemodynamics in man and animal has been evaluated.[24] Glomerular filtration and renal blood flow returned to about 75 per cent of the control levels within 24 hours of rewarming.

Summary and Conclusions

This laboratory has concerned itself with the pathologic and physiologic effects of hypothermia and of rewarming, endeavoring, in particular, to define the time limits for cooling beyond which alterations become so profound that they are not readily reverted to control level upon return to normothermia. The purpose of this paper is to supplement the information already published concerning this problem.

Under the conditions of these experiments it was found that:

1. Animals cooled as long as four hours (23–24° C.) and then rewarmed had a prompt return of hematocrit to pre-cooling control levels. It took progressively longer for this to occur in animals cooled eight and 12 hours. Hematocritis in all groups continued to fall below normal as observations were continued. Blood and plasma specific gravity demonstrated the same pattern. The significance of this is discussed.

2. The prolonged clotting time occurring in hypothermia returns to normal simultaneously with rewarming in animals cooled two and four hours, but remains prolonged for a longer time in animals cooled eight and 12 hours, being increased in some animals in the latter group for as long as 24 hours. No significant changes occurred in prothrombin time aside from some transient increases during rewarming in some of the longer cooled animals.

3. Rewarming after hypothermia of two to 12 hours was not attendant with significant change in electrolytes, acid base balance, blood sugar and amino acids or lactate/pyruvate ratio.

From these findings, and other data reported, there is nothing to suggest that hypothermia or rewarming following hypothermia of approximately six hours duration is attendant with changes that would make one seriously consider abandoning this modality for clinical use if the dangers of fibrillation could be eliminated.

Acknowledgment

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