

HEMOSTATIC ALTERATIONS FOLLOWING SEVERE DYSBARIC STRESS

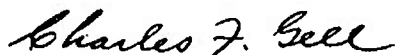
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SUMMARY PAGE

THE PROBLEM

To evaluate the effects of dysbaric stress on the hemostatic (clotting) system in laboratory animals in order to better understand the effects of decompression injury in man.

FINDINGS

A series of hemostatic changes occurred in rats subjected to an inadequate decompression regimen and then observed over a three-day period. An early transient decline in whole blood clotting time, followed by a short-term elevation of plasma fibrinogen one day post-decompression, was detected. A persistent fever was evident by one day after surfacing. Also, platelet numbers were significantly reduced at about two days after experimental treatment. However, partial thromboplastin and prothrombin times were unchanged.

APPLICATION

These experiments indicate that very careful observation should be made over several days after decompression insult to detect symptoms of potentially serious damage which may have occurred even with a fairly normal subjective appearance of the injured individuals.

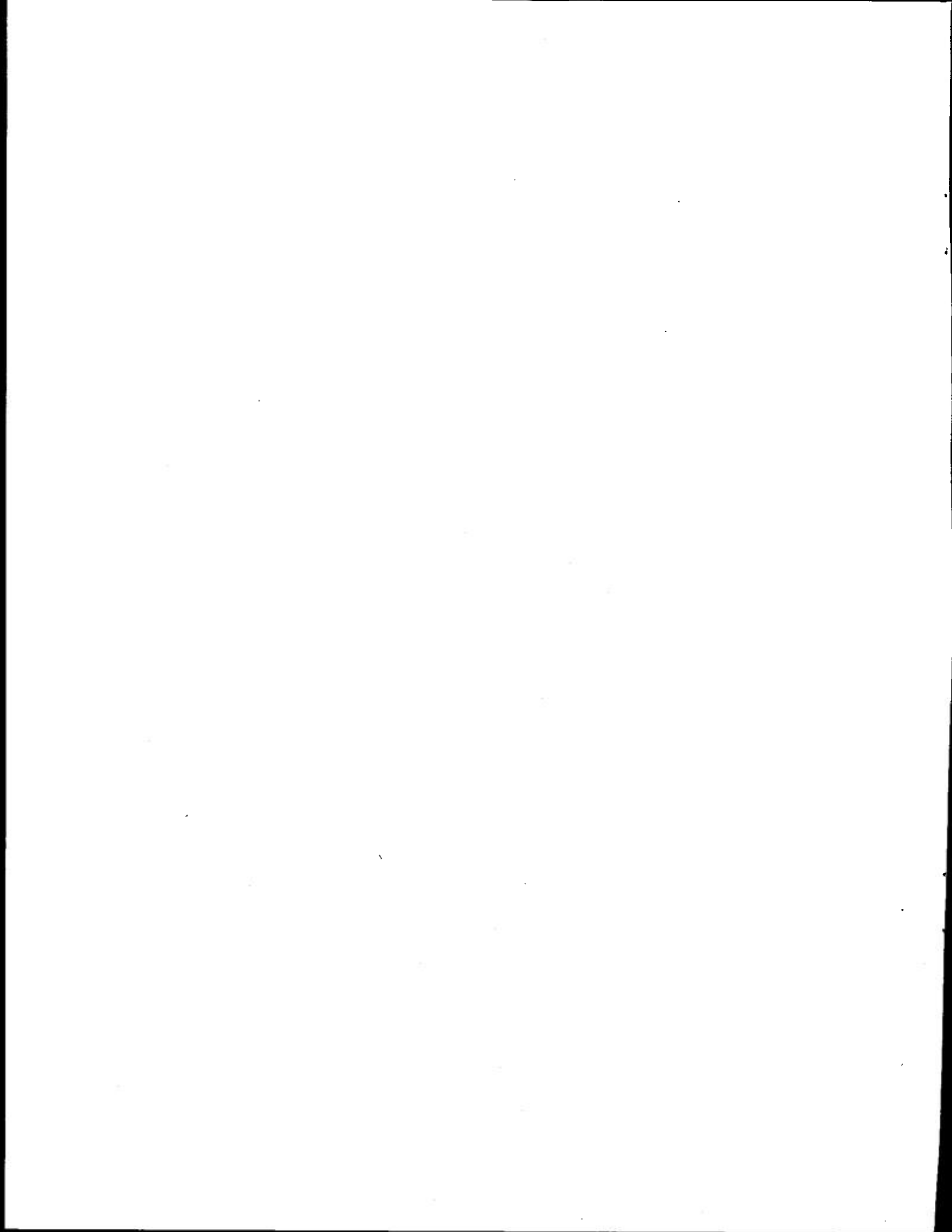
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ABSTRACT

Hemostatic parameters were measured in the blood of mature Sprague-Dawley rats during a three day period following exposure to a compression-decompression schedule designed to produce severe dysbaric stress. The animals were compressed on air to a pressure equivalent to 300 feet of sea water for 30 minutes and stage decompression over a 42 minute interval. Acute decompression stress produced a transient decrease in clotting time. Circulating platelet population was unchanged during the early phase of recovery from severe decompression but it had declined significantly by two days post-surfacing. It then returned to control levels by the end of the observation period. Associated with this thrombocytopenic episode was a tendency toward platelet aggregation. Core temperature measurements indicated the presence of a chronic hyperthermic condition. A significant hyperfibrinogenemia had developed by one day post-dive with a normalization of fibrinogen content occurring during the following two days. No alterations in either prothrombin time or partial thromboplastin time were detected. This animal research has much significance in the biomedical aspects of human diver safety and health.



HEMOSTATIC ALTERATIONS FOLLOWING SEVERE DYSBARIC STRESS

INTRODUCTION

Decompression stress is characterized by alterations in hemostatic mechanisms. Central to this problem is a hemoconcentration¹³ and a depletion of circulating platelets^{1,2,14,16}. In a recent investigation, Jacey and associates¹¹ have described several aspects of the hypovolemia which may occur after completion of an inadequate decompression. It has been suggested by many workers that decompression sickness may initiate a condition similar to disseminated intravascular coagulation (DIC). Hardaway⁶ has defined this condition as an acute, transient coagulation occurring in the flowing blood throughout the vascular tree. The numerous similarities between DIC and decompression sickness have been well documented by Holland⁹.

Hypercoagulation following rapid decompression has been reported in dogs and rabbits by Aggazzotti and in mice, rats and rabbits by Barthelemy.² Philp and co-investigators¹⁷ have demonstrated the existence of platelet thrombi surrounded by aggregated red cells in lungs of rats following severe decompression. In another study, Philp and his group also showed platelet depletion and hemoconcentration in rats exposed to a severe decompression schedule¹⁶.

While considerable documentation exists concerning the changes in hemostatic parameters in acute dysbarism, little is known about the events occurring during a spontaneous recovery from exposure to an inadequate decom-

pression. With this in mind, a study was undertaken to investigate hemostatic alterations during a period of recovery from severe dysbaric stress resulting from decompression insult.

For the purposes of this study, severe dysbaric stress was defined as that stress created by a decompression which is neither safe (100% survival) nor explosive (90-100% death within one hour post-surfacing)¹². By these criteria, the 66% survival rate observed at one hour post-decompression would verify exposure to severe dysbaric stress. Stress-mediated leucocytic changes resulting from the use of this decompression routine has previously been reported¹¹.

METHODS AND MATERIALS

Mature male rats of the Sprague-Dawley strain weighing 530 ± 65 grams were pressurized in compressed air and decompressed according to a schedule designed to produce severe decompression stress¹². The schedule entails pressurization to the equivalent of 300 feet of sea water (FSW) at 60 ft/min with a bottom stay of 25 min. Decompression is accomplished in two stages: 300 FSW to 60 FSW at 20 ft/min with a hold at 60 FSW for 15 min. and 60 FSW to the surface at 4 ft/min.

For each experiment, ten cages of six rats were placed in the outer lock of one of the Naval Submarine Medical Research Laboratory's man-rated chambers (volume 474 ft³). Two cages of control animals were left next to the chamber

for the duration of each dive in order to be exposed to the noises of rushing air. Temperature profiles for this schedule¹² indicated that peaks of chamber temperature always occurred upon reaching 300 FSW (at 5 minutes from the surface) which were then promptly corrected and maintained by subsequent venting and decompression. Heat stress was minimal.

Following completion of the dive, all animals were held in clean cages for varying periods of time with adequate food and water available. All animals were weighed and handled daily. It should be emphasized that only control animals exposed to chamber noise were used.

At the appropriate time just prior to administration of anesthesia, core temperature in each animal was measured with a Yellow Springs Tele-Thermometer. The animals (experimental and control) were then injected intraperitoneally with 40 mg sodium pentobarbital/kg body weight. Blood was collected anaerobically in siliconized glass syringes from the abdominal aorta; and immediately thereafter, 0.5 ml of blood was dispensed into each of 3 siliconized 10 x 75 mm glass test tubes for the determination of clotting time according to a modified Lee-White procedure¹⁹.

Without delay, 2 ml aliquots of whole blood were also transferred to glass tubes containing 0.2 ml 5% sodium citrate and 20 μ l heparin (1000 units/cc), respectively. Blood and anticoagulants were mixed by several gentle inversions.

Prothrombin time and partial thromboplastin time were estimated

using citrated blood. The remaining hemostatic parameters were measured utilizing heparinized blood with the exception of fibrinogen levels which were determined in heparinized plasma. Platelet and platelet aggregates were counted according to standard hematological methods^{19,23}. Prothrombin time was measured with the Simplastin kit (General Diagnostics Corp.) while partial thromboplastin time was quantitated employing the Fibrosystem reagent package (Beckman Dickenson Corp.). Plasma fibrinogen estimations were performed according to a modification of the procedure of Rice and Muesse¹⁹.

RESULTS

Although many investigators studying the effects of decompression stress have grouped depressurized animals according to the severity of symptoms, no attempt was made to classify our experimental animals on the basis of degree of injury. The following data presents all animals treated in each particular series.

The responses of several clotting parameters after severe decompression and a 3-day recovery period are shown in Table 1. A transient decrease in clotting time of almost two minutes was noted at one hour post-surfacing. Clotting times had normalized by one day post-decompression, remaining at this value for the duration of the observation period. No alteration in either prothrombin time or partial thromboplastin time was detected at any stage of the experiment.

While acute decompression stress had no discernible effects on the

Table 1. Clotting Parameters in Rat Blood Following Severe Decompression Stress

		Control	POST DECOMPRESSION			
			1 Hour	1 Day	2 Days	3 Days
Clotting Time Minutes	Mean	7.34	5.42*	7.33	7.39	6.90
	SEM	.49	.48	.59	.68	.41
	N	33	28	30	25	29
Platelet Count <u>Cells x 10³</u> mm ³	Mean	878.3	826.1	876.5	771.4 *	897.7
	SEM	25.8	35.6	23.7	39.6	48.1
	N	58	35	57	38	39
Platelet Clumping Clumps per 1/5000 mm ³	Mean	7.4	8.5	6.9	10.4	8.7
	SEM	1.1	1.3	.8	1.3	1.1
	N	27	26	26	19	29
Prothrombin Time Seconds	Mean	13.1	13.0	12.6	12.9	13.1
	SEM	.2	.1	.2	.1	.1
	N	33	26	28	26	27
Partial Thromboplastin Time Seconds	Mean	14.1	14.3	14.2	14.4	13.8
	SEM	.2	.4	.2	.2	.2
	N	32	25	29	26	28

* Statistically significant at the 5% level or better.

circulating platelet population immediately following the dive, on the second day post-surfacing a marked decrease in cell number occurred. This was followed by a return to a normal count by the end of the observation. Coinciding with the significant drop in the circulating platelet population on the second day of the observation period, there was a tendency toward a higher number of platelet clumps, although not attaining statistical significance.

The results of studies of core temperature and fibrinogen levels following severe decompression are illustrated in Figure 1. Core temperature was markedly elevated at one day post-decompression with the hyperthermia persisting for the duration of the observation period. Fibrinogen concentrations rose significantly one day after the dive and declined gradually during the next two days of recovery from severe dysbaric stress.

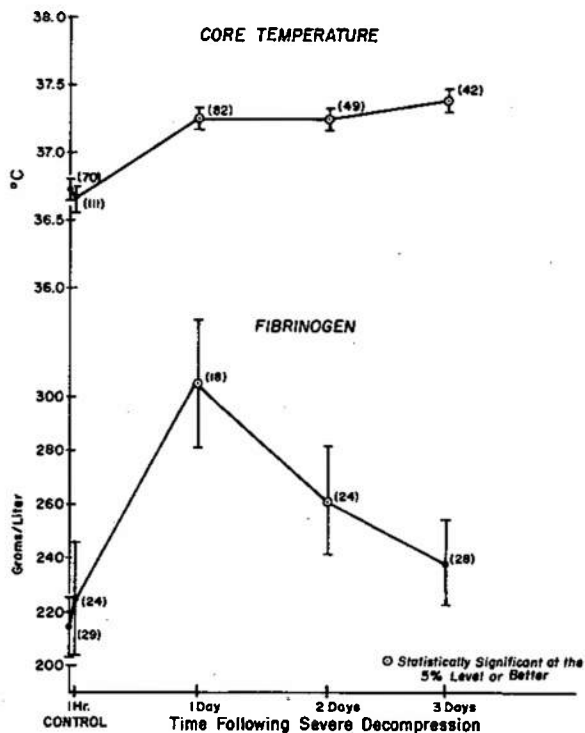


Fig. 1. Responses of Core Temperature and Plasma Fibrinogen Levels During Recovery from Dysbaric Stress in Rats. Numbers in () equal number of animals in each group.

DISCUSSION

Several recent investigations in the field of dysbaric research have focused on the problems of hemostatic alterations following compression-decompression procedures. The Canadian group at Downsview¹⁵ noted decreases in circulating platelets in human subjects with and without symptoms of dysbarism after a 3.5 hour decompression from an exposure to 10 ATA compressed air for 10 min. It was felt by these authors that the "possibility of hypercoagulation occurring post-decompression is realistic." A 37% reduction in platelet count together with a hypercoagulable

state was also reported by Sicardi²⁰ in 3 divers surfacing from a 650 FSW dive. Bennett and Gray³ detected a fall in platelets in 2 subjects who underwent a continuous 10.5 day decompression after a sojourn at 1500 FSW in a helium-oxygen atmosphere.

In contrast to the results of the experiments described above, Bonin and co-workers⁴ collected evidence which indicated no alterations in hemostatic parameters in volunteers pressurized to 10 ATA on a He-O₂-N₂ mixture for 2 hours and then subjected to a borderline decompression regimen.

The significant factor in the above studies in contrast to our own, is that only the blood sample was collected per subject before the dive and after decompression, usually soon after reaching the surface. Animal experiments for studying hemostatic changes following compression-decompression have also generally followed the single pre-dive/post-dive sampling protocol.¹⁵

Philp and co-investigators¹⁵ have discussed some possible factors affecting the changes in platelet population seen soon after decompression. These include: (1) decreased production, i.e., decreased megakaryopoiesis; (2) active sequestration by organs such as the spleen; (3) increased utilization in the repair of vascular endothelium; and (4) the formation of platelet aggregates with subsequent trapping in the microcirculation. Based on the results of their hemostatic studies, these authors concluded that the last two factors were probably responsible for the depletion of circulating platelets seen soon after decompression. More recently,

Warren and associates²² concluded that platelet adhesion to the interface combined with endothelial damage accounted for the drop in platelet counts seen in decompression situations, even when the mechanical blockage of the vasculature by gas bubbles is not significant. One can only speculate concerning the changes which might have been detected had the blood sampling been extended over a period of several days.

While a marked depression in clotting time was noted in our experiments at one hour post-surfacing, no acute changes in circulating platelet population were seen. However, by the second post-decompression day, a statistically significant decline in platelet number occurred which was normalized by the end of the observation period (day three). Although platelet aggregation was not altered statistically, a tendency toward an increased aggregation occurred concomitantly with the existence of the thrombocytopenic state. Similar results (i. e., a lack of early changes) were found by Martin and Nichols¹⁴. Moreover, they reported maximal platelet depletion 3 days after a compressed air dive to 100 FSW for 1 hour with a 122 minute decompression. In both of these experiments, cell depletion was not evident until long past the acute post-decompression stage. We attach considerable significance to the fact that similar results were obtained using either the very severe decompression schedule of our experiments or the mild schedule of Martin and Nichols¹⁴.

Furnishing support for our observation, a shortening of coagulation time was noted by Aggazzotti¹ in 12 of 17 animals after exposure to pressures

varying from 6 to 11 atmospheres. Alterations in coagulation in mice, rats and rabbits were also reported by Barthelemy² following rapid decompression. In addition, Hartmann⁷ demonstrated changes in clotting factor parameters in dogs compressed and decompressed.

To facilitate discussion of possible hemostatic alterations resulting from dysbaric injury a schematic diagram depicting the interaction of the intrinsic and extrinsic pathways of coagulation is shown in Figure 2. Partial thromboplastin time, clinically, is a screening procedure for those factors involved in the intrinsic system of coagulation. These include those elements involved in the generation of plasma thromboplastin but not platelet function or Factor VII. One stage prothrombin time is the preferred test of the extrinsic pathway of coagulation including Factors I, II, VII, X, and V, but not Factor III, tissue thromboplastin^{5,23}.

Although fibrinogen (Factor I) was elevated at one day post-decompression, no changes were observed during the acute phase (1 hour) when the coagulation abnormality was demonstrated. It should be noted that hyperfibrinogenemia is not associated with coagulopathies^{21,23}.

Heyder and Tappan⁸ in a series of separate experiments utilizing the same decompression table employed in this work, studied electrolyte dynamics following exposure of rats to severe dysbaric stress. Interestingly enough ionized calcium (Factor IV) was increased during the first hour and significantly decreased during the remainder of the period of observation. Currently,

INTERACTION OF INTRINSIC AND EXTRINSIC PATHWAYS
OF COAGULATION

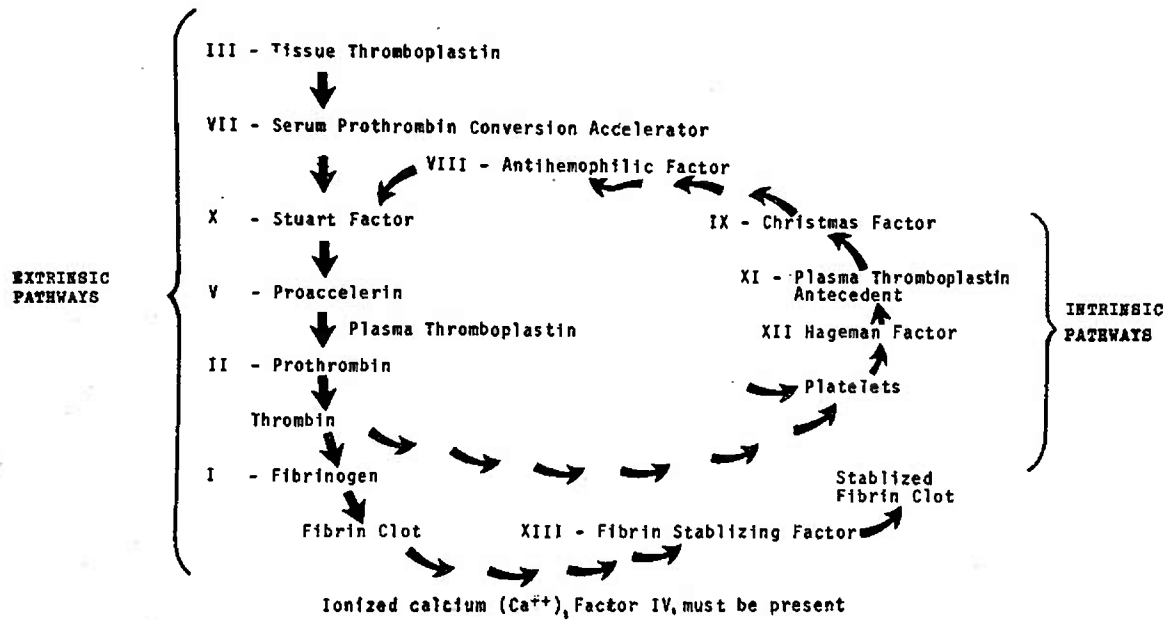


Fig. 2. Schematic Showing Coagulation Pathways.

hypercalcemia is believed to have no clinical significance in clotting defects^{5,23}. The lack of change in both partial thromboplastin time and prothrombin time during the three day period of observation following severe decompression, especially during the first hour, coupled with the non-applicability of fibrinogen and calcium changes, would indicate that the origin of the clotting defects lies elsewhere than in those factors discussed above.

Although further studies are required to adequately define the site of the observed clotting defects, the acceleration of clotting time seen following trauma, as suggested by the work of Innes and Sevitt¹⁰ may be due to the entry into the circulation of thromboplastin (Factor III) from injured tissue. Vascular tissue damage following severe decompression is well documented^{17,22}.

The picture that seems to be emerging from this series of studies designed to explore hemostatic alterations following exposure to inadequate decompression suggests a situation analogous in many ways to that reported by Innes and Sevitt¹⁰ in injured patients. The sequelae outlined by these authors in patients that had undergone some sort of traumatization is as follows: (1) the first few hours are characterized by an acceleration of clotting time which is followed by a normalization of this parameter; (2) a thrombocytopenia occurs within one to three days; (3) plasma fibrinogen levels, usually rise within 24 hours. The similarities suggest a generalized response to non-specific injury generated by the inadequate decompression procedure utilized in these experiments. Hyperfibrinogenemia is well documented in trauma states, surgical as well as accidental, and seems

to result from overproduction by the liver^{10,21}. The persistent hyperthermia recorded after the acute decompression period may also be taken as evidence of an inflammatory response to nonspecific tissue insult.

These findings demonstrate that inadequate decompression can result in an early clotting defect followed by the development of a latent syndrome of biochemical and hematologic abnormalities.

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