DISCUSSION
Shock is a very dangerous condition of general body collapse which can rapidly occur as the result of stresses of severe traumatic injuries, burns, major surgery, massive hemorrhage etc. The fundamental defect in shock is failure of effective blood flow and hence impaired transport of vital materials in the blood to the organs and tissues. It has been noted that there is a depletion of ascorbic acid and cholesterol in various stress conditions such as burns, effect of high altitude, effect of toxins (Dugal and DesMarias, 1949; Tepperman et al., 1947; Kuchel and Mitchell, 1936; Ghosh, 1939). The use of ascorbic acid in the treatment of shock has been repeatedly suggested in many papers over last thirty years. Ungar (1942) was able to prevent traumatic shock and death of guinea pigs by an injection of 100 mg or more ascorbic acid per kg of body weight and reported that ascorbic acid increased their resistance to trauma and improved their survival. There is a considerable medical literature on cold temperatures and their effect on the ascorbic acid in the body. Dugal et al., (1947) reported that rats which were exposed for long periods to freezing temperatures, but which were able to adjust to these low temperatures, had large increases in the ascorbic acid levels in different tissues whereas those rats unable to
adjust to the cold environments had decreased levels. Dugal and Fortier (1952) also showed that ascorbic acid increases cold resistance among monkeys. However, no tests have been conducted to see whether resistance of human to cold temperatures could be improved by ascorbic acid. Santome and Gomez (1963) showed that hemorrhagic shock induces a highly significant decrease in adrenal ascorbic acid in dogs used as experimental animals in spite of higher levels in blood. The higher blood level is likely to be an artifact because the liver synthesized ascorbic acid rapidly under the stress and poured it in the diminished volume of blood. The low level of the adrenal ascorbic acid is a direct effect of the prevailing stress condition.

Physical stress such as occurs during surgical operation or following severe burns or shock of any type induces a precipitous fall in the plasma ascorbic acid and the amount excreted in the urine with or without load tolerance test (Lund and Grandon, 1941; Lund et al., 1947). As early as 1946, Levenson et al., reported on the use of ascorbic acid together with vitamin B₁, B₂ and nicotinic acid in cases of severe injury, hemorrhage and infections in humans. More recently the surgical requirements of vitamin C have quite logically demanded even further revaluation in view of the fact that stress accentuates the need for the vitamin and that the seriously injured human behaves physiologically in a manner similar to what is observed in case of scurvy (Levenson et al., 1957).
Ascorbic acid is necessary for wound healing. Orandon et al., (1961) noted that wound dehisence rate in the ascorbic acid deficient group of surgical patients was about eight times to that observed in the group showing adequate level of blood ascorbic acid. The danger of wound dehisence appeared to be very high when the buffy coat ascorbic acid concentration falls to very low levels and this tragedy could be prevented by intake of 100—300 mg of ascorbic acid daily.

Ascorbic acid and dehydroascorbic acid are members of reversible oxidation reduction system. The redox potential depends on the relative amounts of each component in the system. For healthy tissue processes there should be very low level of dehydroascorbic acid in comparison with free ascorbic acid, the level of which is required to be predominantly high so that the redox potential is kept low. Thus, the system is spared of the toxic effect of dehydroascorbic acid which is diabetogenic (Patterson, 1950) and neurotoxic (Patterson and Mastin, 1951; Sjostrand, 1970). But in stress conditions the picture is reverse, that is predominantly high amount of dehydroascorbic acid accumulates while ascorbic acid level is markedly diminished. We have investigated different types of stress conditions experienced by human subjects, namely, severe head injury, burn cases, crush injury/lacerated injury or trauma of any type. Pregnancy during labour and cases of mental derangements. The results are illustrated in Tables 1.2 through Table 1.9.
and summarised in Table 1.10. It would appear that there is accumulation of significantly high amount of DHA in the blood while the ascorbic acid content diminishes alarmingly. In certain types of stresses like severe head injury, burn cases, it is present only in trace amounts.

Results presented in Section B of Chapter-I indicates clearly that there is also a sharp fall in plasma ascorbic acid under the influence of variety of stressful conditions such as severe head injury, burns, lacerated injury/crush injury or trauma of any type. The mean value of plasma ascorbic acid in different stress conditions was $0.08 \pm 0.14$ S.D. mg/dl (normal $0.56 \pm 0.17$ S.D. mg/dl).

Cancerous growth is uncontrolled tissue development and expansion because of the fact that the tissue loses the normal restraints on cell division and growth. The cancer grows in a wild manner at the cost of surrounding normal tissues. Cancer and its present day therapy are intense biochemical stresses that is expected to deplete the bodies of the cancer victims of their ascorbic acid. Leukemia is a cancerous disease of the blood forming tissues in which there is a over-production of the white blood cells. Different types of leukemia results from different varieties of leukocytes involved in the disease process. Hence the patients suffering from
leukemia are by any means under the constant and direct influence of a severe biochemical stress.

In our present investigation new untreated cases of carcinoma and new untreated cases of leukemia were studied. The blood AA level decreased appreciably, the AA values were $0.28 \pm 0.27 \text{ S.D. mg/dl}$ and $0.16 \pm 0.22 \text{ S.D. mg/dl}$ respectively, while the DHA levels were $0.47 \pm 0.38 \text{ S.D. mg/dl}$ and $0.44 \pm 0.29 \text{ S.D. mg/dl}$ respectively. The results are elaborated in Table 1.6 and 1.7.

Thus, it would appear that significantly high amount of DHA accumulates in blood under the influence of various stress conditions while blood or plasma AA content is very low or even trace in certain cases. The results obtained from the study on a group of surgical patients revealed that DHA accumulated in the blood of such patients as a results of surgical stress, apparently disappears with removal of the stress and AA reappears in blood or plasma, thus resembling the values of DHA and AA obtained from a group of patients prior to surgical operations. The results are illustrated in Tables 1.13 through Table 1.16 of Chapter-I. In the aforesaid study it has also been observed that a group of surgical patients receiving i.v. injection of vitamin C after surgery does not show any improvement of the blood or plasma vitamin C.
status, indicating thereby increased utilization of ascorbic acid under the influence of surgical stress. The results are illustrated in Table 1.13 and Table 1.14 (Chapter-I).

It was considered that the decrease in the blood or plasma AA level and increase in blood DHA level in various stress conditions might be due to (i) increased turnover of AA to DHA or (ii) decreased rate of reduction of DHA to AA, or both.

DHA reduction in erythrocytes is not enzymic but chemical. As shown in Chapter-II, Section A, the reduction is dependent on the GSH content of the hemolysate and the rate of reduction is markedly enhanced if instead of GSH, a GSH regenerating system is used. Data presented in Chapter-II, Section C demonstrate that the rate of reduction of DHA in the erythrocyte from trauma patients is similar to that from normal subjects. This indicates that accumulation of DHA in blood of trauma patients is not due to lack of reduction of DHA to AA. This is further supported by the observation that the erythrocyte GSH level and the G6PD activity (EC 1.1.1.49) which regulate the DHA reduction as shown in the scheme presented here (Scheme 1), are similar in normal and traumatic blood.
Scheme-1

G6P → G6PD → 6PG → NADP → NADPH → GR → GSSG → GSH → AA → DHA → G6P
The data presented in Chapter-II, Section B show that DHA reduction in guinea pig tissues is also not enzymic but chemical was carried out by DTNB (5,5-dithiobis-(2-nitrobenzoic acid)) reacting thiol compounds, particular GSH, and in this case also the reduction is markedly enhanced if instead of GSH, a GSH regenerating system is used.

The erythrocyte GR activity (EC 1.6.4.2) is, however, significantly higher (P < 0.001) in trauma than that of normal patients. The reason for this, however, is not clear.

It would, therefore, appear that accumulation of DHA in traumatic blood is probably a result of increased turnover of AA to DHA. Data presented in Chapter-III, Section A supports increased turnover of AA to DHA in trauma. The trauma patients were supplemented with a single dose of 500 mg vitamin C (i.v.) and the results obtained were compared with the values obtained from normal volunteers under similar conditions. The blood and plasma, AA and DHA levels twenty four hours after AA administration to trauma patients were in mg/dl: blood, AA 0.16 ± 0.06 S.D.; DHA 0.89 ± 0.24 S.D.; plasma, AA 0.15 ± 0.07 S.D.; DHA 0.17 ± 0.04 S.D. The corresponding values from normal volunteers were, blood, AA 1.26 ± 0.23 S.D.; DHA 0.12 ± 0.07 S.D.; plasma, AA 1.07 ± 0.20 S.D.; DHA 0.06 ± 0.01 S.D.
In case of a high turnover of AA to DHA, one would expect a high DHA level in the plasma of traumatic patients. The data presented in Chapter-I, Section B shows that plasma DHA level in trauma is significantly higher (P < 0.001) than that of normal but the mean value is relatively low (0.17 ± 0.06 S.D.). This can be explained by the fact that the erythrocytes have no permeability barrier to DHA. The data presented in Chapter-III, Section B demonstrates that the uptake of erythrocytes is very rapid in normal or traumatic subjects. This would explain that even when blood DHA level in stress condition was quite high plasma DHA level would remain low.

Apparently, stress in any form results in increased release of epinephrine and nor-epinephrine into the general circulation with consequent alteration in several metabolic processes, including catecholamine induced changes in energy substrates and regulatory hormones.

Catecholamines have profound effect on carbohydrate and lipid metabolism. In humans, catecholamines have profound lipolytic effect (Steinberg, 1966). The lipolytic activity may be the major determinant of the elevated concentration of circulating FFA, a major characteristic of stress. In addition, in vitro experiments have demonstrated that catecholamines possess glycogenolytic (Sherline et al., 1972) and ketogenic
(Exton and Park, 1972) properties. Thus, the secretion of catecholamines during stress provides the organism with an increased availability of the energy yielding substrates such as glucose, ketone bodies and free fatty acids. Wilmore et al., (1974) have directly attributed the hypermetabolic response observed following thermal injury to stress resulting in increased catecholamine secretion.

Catecholamines, principally epinephrine and nor-epinephrine, are derived from sympathetic nervous systems including adrenal medulla (Axelord & Weinshilbaum, 1972). They have numerous physiological effects in human, ranging from positive ionotropic cardiac activity to inhibition of gut motility. Increased sympathetic activity has been reported during major trauma (Frankson et al., 1954), diabetic keto-acidosis (Christensen, 1974), infection (Grooves et al., 1973), severe anxiety (Taggart et al., 1973), exposure to cold (Hsich & Carson, 1957) and hypoxia (Porte and Robertson, 1973). In fact the symptomatology that characterizes counter regulatory hormone response has classically been attributed to elevation of catecholamines. The symptom complex of tachycardia, diaphoresis, anxiety and peripheral-vasoconstriction may be absent in patients treated with catecholamine blocking agents such as propanolol (Bray & De Quattro, 1972).
Participation of catecholamines in the counter-regulatory response is well documented, particularly in response to hypoglycemia (Callingham, 1975; Garber et al., 1976). In addition, catecholamines serve an important role as modulators of other hormones. The classical study of Porte and coworkers have convincingly demonstrated that catecholamines directly inhibit the secretion of endogenous insulin (Porte et al., 1966). The direct suppression of insulin at the beta cell is counteracted at the same time by catecholamine induced hyperglycemia. The net result of these two events is insulin secretion at a reduced rate (Samols et al., 1966). Thus, in severely burned patients with elevated catecholamine secretion, plasma insulin is suppressed relative to the characteristic hyperglycemia (Batstone et al., 1976). Finally, catecholamines also stimulate the secretion of other counter-regulatory hormones, particularly glucagon (Gerich et al., 1972; Schade and Eaton, 1976) and growth hormone (Blackard and Heidengsfelder, 1968). This activity is important in producing additional stimulation of lipolysis, ketogenesis and gluconeogenesis of the stressed organism. In summary catecholamines are major stress hormones and abnormalities in their secretion and regulation may be expected to precipitate in disease states.

As has already been mentioned there is an increased adrenal function in stress conditions and adrenal stimulation
in non-specific stress conditions has been ascribed to the net result of an increased output of pituitary ACTH (Grant and Eversole, 1949). Stressful conditions such as fever, infection and surgery are also characteristically accompanied by increased output of gluco and mineralocorticoids thereby indicating sufficient increase in the activity of adrenal cortex (Liddle, 1974). Tissue contents of both gluco and mineralocorticoids were increased in traumatic shocks in rats (El’skii and Samsoneko, 1977). These data suggest that increased ACTH secretion result in an increased plasma corticosteroid level. Increased secretion of ACTH under the influence of stress is again under the feedback control of the circulating corticosteroids. This control is exerted via the structures residing within medial basal hypothalamus (Abe and Critchlow, 1977). Further experimental results also support the importance of hypothalamic control of adrenal activity. Jones et al., showed (1976) that corticosteroids inhibit the release of corticotrophic releasing factor (CRF) from the hypothalamus in rats. Although it was reported that corticosteroids inhibit ACTH release from the anterior pituitary gland (Protaseeva and Sayers, 1973; Buckingham and Hodges, 1976; Tang and Phillips, 1977), available evidences indicate that ACTH, enhanced secretion of which takes place in stress conditions, may be
Involved in the conversion of ascorbic acid to its oxidation product namely dehydroascorbic acid. It has been observed that administration of ACTH depletes adrenal cortical gland of its ascorbic acid content (Sayers et al., 1944; Sayers and Sayers, 1949). Lipscomb and Nelson (1960) showed that there was an immediate rise in adrenal vein ascorbate level after the administration of ACTH. Earlier Salmon (1957) reported that the ascorbic acid recovered from the adrenal vein was in the form of its oxidation product viz., dehydroascorbic acid and was not in its reduced form. The results presented in Section E, Chapter-I indicated that single administration of ACTH (5 units/animal S.C.), hydrocortisone (8 mg/animal, S.C.) or adrenaline (25 µg—100 µg/animal, S.C.) had no significant effect on the plasma AA levels of guinea pigs (n = 8) 0.63 ± 0.04 mg/dl and rabbits (n = 4) 0.58 ± 0.07 mg/dl. The results also indicated that ACTH injected at doses of 2 units b.d. per guinea pig for four days had no significant effect on plasma AA level. Similarly five repeated doses of adrenaline (25 µg/guinea pig at 30 min interval) or conjoint effect of δ-methasone (0.5 mg/rabbit, S.C.) and five repeated doses of adrenaline (50 µg/rabbit at 30 min interval) had no significant effect on the plasma AA level. Cyclic AMP was also found to be ineffective in stimulating the rate of oxidation of AA to DHA in the liver mitochondria of guinea pigs. This in vitro experiment
corroborated the observation that plasma AA level was not altered by administration of different stress hormones.

In these times when every incident, real or imaginary is weighed in the balance of medicolegal litigation, the problem of whether trauma or stressful conditions of any type may cause or increase the possibility of diabetes is very logistic. The experimental results presented in Chapter-IV show that there is a persistently high level of blood DHA irrespective of age, sex, family history and duration of diabetes mellitus. The data presented in Chapter-IV further indicate that the rate of erythrocyte DHA reduction was similar in diabetic patients and normal subjects. The high blood DHA and low plasma AA levels in diabetes was not due to lack of reduction of DHA to AA. It was also observed that there is no difference in erythrocyte GSH level and G6PD activity between diabetic and normal subjects. However, the GR activity was higher ($P < 0.001$) in diabetic subjects. Eppes et al., (1964) have also observed that erythrocyte G6PD activity is not altered in diabetes. This is in contrary to the observations of Channugham et al., (1964) who reported decreased G6PD activity in diabetes mellitus. Contradictory reports regarding the blood GSH level of diabetic patients are also found in the literature. Seltzer (1957) reported a decreased blood GSH level in diabetes while some investigators observed
that GSH level in blood was decreased only in diabetes with ketosis (Illing et al., 1951; Lai, 1967).

It is interesting that erythrocyte GR activities are significantly higher ($P < 0.001$) in diabetics than that of normal subjects (Table 4.2, Chapter IV). The reason is not clear. High GR activity in diabetes was also reported by some authors (Long, 1961; Heath et al., 1963).

The low plasma AA level is apparently due to high turnover of AA in the body (Table 4.3, Chapter IV). The high turnover is possibly due to increased oxidation of AA to DHA, because the rate of oxidation of AA to DHA is greatly increased in tissue mitochondrial fractions of diabetic rats. In case of increased oxidation of AA to DHA in the body, one would expect a high level of DHA in the plasma of diabetic subjects. Though the mean plasma DHA level of diabetic patients is significantly ($P < 0.001$) higher than that of normal individuals, the value is relatively low. This may be explained by the fact that erythrocytes have no permeability barrier to DHA (Section B, Chapter-III). So even in case of increased oxidation of AA to DHA, plasma DHA level would remain low.

In stress, as has already been discussed, there is temporary marked increase in blood DHA which disappears
rapidly with the removal of the stress. It is unlikely that a temporary increase in blood DHA under stress condition would lead to diabetes. Nevertheless, the modern concept suggests that if a person has latent diabetes, the sum of stresses over a period of time may bring about overt stage of the disease which might otherwise never have occurred (Williams, 1970; Marks et al., 1971). The reasoning then continues that a massive stress or traumatic blow can accelerate the process, although it has been indicated that stress per se did not produce diabetes.

The difficulty in evaluating mental or emotional stress is obvious since living in modern times is itself a stressful adventure. Danowski (1963) discusses emotional stress as a possible cause of diabetes. He cites Hinkle et al., (1950) who reported non-diabetic ketosis becomes manifest in response to certain types of stress and this ketosis resembles that of uncontrolled diabetes although the former is decidedly more benign. Danowski further points out that diabetes is a spectrum which extends from 0 to 100% with those clearly non-diabetic at 0 and clearly diabetic at 100. Thus, if a person has enough etiological influence such as heredity or obesity to become an 85% possibility for overt diabetes, sufficient and sustained stress might well precipitate the disease. Increased utilisation of ascorbic acid under stressful conditions would advocate increased intake of the vitamin to
combat stress. But the danger involved in connection of the intake of megadoses of ascorbic acid must be given due consideration. Many reports of hyperglycemia occurring after large doses of ascorbic acid ascribe diabetic symptoms to ascorbate (Barness, 1975). Moreover, in diabetic patients administration of any extra amount of ascorbic acid may results in a further increase of the toxic dehydroascorbic acid level. One of the feared consequences of using a urinary acidifying agent like AA is the possible development of renal stones or nephrocalcinosis (Goldsmith, 1971). While stone formation may be accelerated with the administration of large doses of ascorbate, this is difficult to quantitate. A rise in cholesterol was reported in artherosclerotic patients receiving large doses of ascorbic acid, this may aggravate artherosclerosis (Morin, 1972). It has recently been noted that when ascorbic acid is ingested with food, substantial amounts of vitamin $B_{12}$ are destroyed and it was suggested that there should be a regular evaluation of vitamin $B_{12}$ status in anyone taking more than 500 mg of vitamin C daily (Herbert & Jacob, 1974). Vitamin C overdosage would also seem to be contraindicated in patients suffering from disease accompanied by actual or potential hypoxia (Schrauzer et al., 1975). A suggestion has been made that a systemic conditioning can occur after prolonged intake of 2 to 3 gms of extradietory ascorbic acid.
Case histories have been reported concerning several adults who may have developed scurvy following the cessation of intake of the extra amount of the vitamin (Schrauzer and Rhead, 1973).

There are some evidences concerning the effects of ascorbic acid on G6PD deficient erythrocytes. Brewer et al. (1967) mention that 1500 mg of ascorbic acid has produced mild hemolysis in G6PD deficient subjects. Beutler (1959) noted earlier a fall in erythrocyte glutathione levels when G6PD-deficient cells are incubated in vitro with ascorbic acid. In this connection it would perhaps be of interest to cite the example of a case which represents the first reported death in which megadoses of ascorbic acid were a contributing factor (Campbell et al., 1975). The patient was a 60 years old black man admitted for treatment of acute renal failure. Six days prior to admission he suffered second degree of burns of the hand. He received 40 g of ascorbic acid intravenously on each of two consecutive days. Before treatment urine analysis and hemoglobin concentrations were normal but on the third day the patient became oliguric. The urine was dark in colour and the serum was red. The erythrocyte glucose-6-phosphate dehydrogenase (G6PD) was only 1.76 I.U. per g hemoglobin (normal values for Western people, 8.4 ± 1.3 I.U.). Electrophoresis revealed the isoenzyme GDA which is relevant since individual of African descent with G6PD deficiency show this isoenzyme.
The patient was dialyzed with little change in clinical status. The neurological status and the general condition deteriorated steadily and finally the patient died on the 22nd day. However, the cause of hemolysis by megadoses of AA was not clear. Megadoses of AA would give rise to a high concentration of DHA in blood and tissues. According to the Scheme (Chapter-II, Section A) presented before, DHA in blood and tissues is reduced back to AA by GSH and this reduction is dependent on G6PD activity. In G6PD deficiency, which is the most common hereditary enzyme deficiency of the erythrocytes (Beutler, 1971), there would be lack of GSH and NADPH. Consequently, DHA reduction would be greatly inhibited. As a result, in G6PD deficient persons administration of megadoses of AA would result in accumulation of large amounts of DHA in blood and tissues. DHA is highly neurotoxic (Patterson and Mastin, 1951; Sjostvand, 1970). This might be a cause of death. Therefore, due caution should be taken while administering megadoses of ascorbic acid to a patient. It is desirable that G6PD activity of patients should be checked prior to administration of large doses of ascorbic acid.