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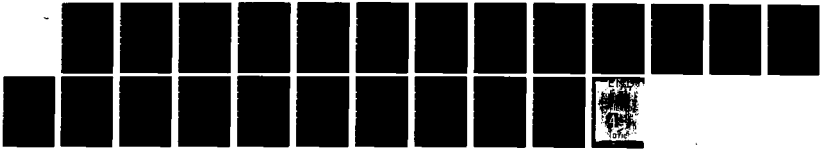

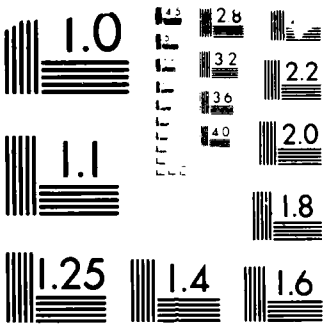

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Report Number 3

POST TRAUMATIC CHANGES IN SERUM FATTY ACIDS AND THEIR RELATIONSHIP TO ORGAN FAILURE - LUNG AND LIVER

Final Report

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SUMMARY

Seriously injured patients are suseptible to poorly understood problems with major organ function, including post traumatic respiratory distress. The relationship of altered fat metabolism and pulmonary failure and liver dysfunction is not well defined, due in part to limitations in fatty acid analytical methods. The purpose of this study was to develop an accurate and rapid method to quantify plasma free fatty acids in trauma patients developing pulmonary insufficiency and to study the pathophysiology and molecular dysfunction produced by post traumatic alterations in plasma fatty acid levels both in vitro and in vivo model systems.

Under this contract, a highly reporducible gas chromatographic method was developed for the analysis of plasma free fatty acids. An animal injury model, the rat, was also developed and found highly reproducible. It is an excellent model for the study of trauma induced hepatic dysfunction. Several analytical methods were honed and/or developed so that accurate and reliable measurements of hepatic drug metabolizing enzymes could be performed. In both injured animal models and human trauma patients, it was shown that injury produces a highly significant rise in plasma free fatty acids within 24 hours of injury. The free fatty acid levels in animals return to normal within 72 hours, but in most human trauma patients studied, free fatty acid levels more slowly return to normal. In animal models, the rise in plasma free fatty acids correlates with large decreases in hepatic drug metabolizing activities, specifically cytochrome P-450 and FAD-monooxygenase. The decrease in free fatty acid levels as the animal recovers is accompanied by a return of these enzyme activities to control levels. In the animal experiments, it was indicated that inflammation, not associated with polymorphonuclear neutrophiles, is linked to post traumatic alteration of drug metabolism. It is suggested that depression of drug metabolism may be caused by an acute phase reactant.

The assessment of hepatic dysfunction in trauma patients could not be made directly by measuring hepatic drug metabolizing enzyme activities. Instead, a drug, antipyrine, was used to assess liver function. This compound is metabolized via the cytochrome P-450 system. A selective and necessarily limited study in multisystem trauma patients revealed that plasma half life of the antipyrine was significantly longer in trauma patients 24-48 hours post injury than in healthy volunteers. In several cases, antipyrine plasma half life in the trauma patient was within normal limits 5-10 days post injury.

Relationships between plasma free fatty acids in trauma patients and clinical parameters proved statistically insignificant. There was definitely an increased free fatty acid level at the time of injury which steadily, not always linearly, decreased over a period of several days. The high level of free fatty acids was significantly reduced initially if albumin was administered within 36 hours of trauma, however, the course of the decline was not affected and albumin administration did not affect free fatty acid levels after day two following trauma.

FOREWARD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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PRELIMINARY OBJECTIVES

The study of major organ dysfunction in trauma required an appropriate small animal trauma model as, at the time of the study, the few described in the literature were not reproducible. Furthermore, in order to understand trauma induced biochemical alterations in fatty acid metabolism, a reproducible and accurate measure of plasma free fatty acids was required, since the methods in use were conspicuously nonselective, relatively slow, and often not reproducible. Initial objectives were therefore to develop a rapid and accurate method of determining free fatty acids and to select a small animal model on which trauma studies could be performed. As work progressed, it also became necessary to develop and refine some of the assay procedures.

Method for Free Fatty Acid Determination in Plasma. The details of the development of the analytical method for determination of free fatty acids are discussed at length in the 1978-1979 Progress Report. Briefly, we investigated many aspects of fatty acid extraction, derivatization, and quantitation, finally choosing analysis by gas chromatography. High performance liquid chromatography methods were assessed, but derivatization required a significant amount of time. The methyl esters used in the gas chromatographic method were rapidly and quantitatively formed. For this method, solvents, internal standards and column packings were evaluated. The method, as presently used, provides quantitative data on individual fatty acids extracted from plasma, including the sixteen and eighteen-carbon series as well as arachidonic acid (20 carbon atoms). Confirmation of the fatty acid structures was performed by gas chromatography-mass spectrometry. Samples are automatically assayed with an autosampler interfaced with a microprocessor. The microprocesor determines peak areas and prints out data for each sample. Thus the gas chromatography analysis of plasma free fatty acids provides total and individual free fatty acids in plasma determined as the methyl esters. Table I indicates the total number and type of plasma analyzed over the past three years. Although we have quantitated the commonly occurring fatty acids, the method readily detects the higher unsaturated fatty acids such as those with twenty and twenty-two carbon chains and 4 or 5 double bonds.

TABLE I

Analysis for total and individual free fatty acids in plasma

Year	Patients	Animals	Collaborative Projects	Healthy Subjects
1980-81	185	44	107	58
1979-80	337	531	167	--
1978-79	138	160	--	--

The Animal Injury Model. Development of the animal injury model was difficult because previously reported models were either unsuccessful or not reproducible. A background of the literature pertaining to these models is listed and discussed in the 1978-79 Proposal and Progress Report. Initially, in developing a small animal model, we used outbred rats. These animals exhibited tremendous individual variation in trauma induced hepatic enzyme alterations. In further studies, we showed that inbred rats provided significant and reproducible results on relatively small groups (n=6 or 8). See Table II. Briefly, the injury in the rat is produced by infrarenal aorta ligation. The effect of this injury is to dramatically decrease the activity of cytochrome P-450 and FAD-monooxygenese, two of the most important drug metabolizing enzymes. Recovery of the animals from the trauma occurs 24-48 hours post injury during which period the activity of the two enzymes slowly recovers. Associated with the injury and with the change in enzyme activity is a significant rise in plasma free fatty acid levels. See Figure 1.

Development of Enzyme and Drug Assays. During the course of this research project, it became necessary to develop several research procedures and techniques. In addition to the two initial objectives already described, fatty acid determination and an in vivo animal trauma model, new assays were required for some of the hepatic enzymes and for the isolation and determination of drugs and metabolites from plasma and urine.

Cytochrome P-450 analysis was found to be marginally reliable using the commonly performed techniques which relied on multielectron substrate oxidation. A new technique was developed which uses a stable nitroxide radical as a substrate for the cytochrome P-450 one electron reduction of this nitroxide to its electron paramagnetic resonance (epr) invisible hydroxylamine. This assay is accurate and to date is the most reliable technique available for determination of cytochrome P-450 activity, as the procedure relies on only one electron transfer from cytochrome P-450.

Drug metabolism studies must depend on reliable analysis of the drug and its metabolites. Fortunately, the drugs we have studied, antipyrine and diazepam, in animals and in humans are well characterized as to pharmacokinetics and metabolite identification and distribution. There were, however, some problems in that a high performance liquid chromatographic assay for these drugs and their metabolites was not available that was reasonably rapid and accurate. Considerable effort was made to find a relatively rapid analysis, particularly for antipyrine and metabolites in urine which tends to contain many interfering substances. The result of this effort, led by our collaborator, Dr. Christian Tschanz, was a reproducible and accurate assay of antipyrine, 3-hydroxyantipyrine, 4-hydroxyantipyrine, and N-desmethylantipyrine. A method was also found to determine diazepam, temazepam, oxazapam, and N-desmethyldiazepam using reverse phase columns and high performance liquid chronatography. Thus using serial blood samples and periodic urine collections, drug metabolism data can be collected and plasma half lives, clearances and metabolite ratios determined.

Assay of one of the major drug metabolizing enzymes, FAD-monooxygenase, was modified to provide a more accurate estimation of enzyme levels. Extensive kinetics of enzyme reactions were determined. It was found that endogeneous substrates existed in the microsomes and these compounds complicated the assay procedure. Although base rates cannot be determined, reliable assays can be performed using excess additional substrate and/or by extensive washing of the microsomes. A more detailed description is given by Cavagnaro et al. (1981).

MAIN OBJECTIVES

The role of free fatty acids in major organ dysfunction associated with trauma was a main focus of this study. During the course of the research, a relation between plasma free fatty acids and alterations in hepatic drug metabolizing enzymes was defined in the animal trauma model. In the human trauma patient, drug metabolizing dysfunction was assessed as an indicator of depressed hepatic drug metabolizing activity, and a relation between plasma free fatty acids and respiratory distress was indicated in a small number of patients. The use of albumin as a "sponge" for elevated, and presumed toxic levels of fatty acids was evaluated with respect to several clinical parameters. In brief, several significant findings concerning the relation of free fatty acids and drug metabolizing enzyme alterations with respect to trauma induced hepatic dysfunction were found.

Studies on Human Patients: Traumatic Injury and Altered Drug Metabolism. As indicated in studies by Meguid et al. (1979), plasma free fatty acids, lactate, and glycerol rose significantly, as did plasma cortisol, in injured patients. It is no surprise that in multisystem injury nearly every major organ in the body can show effects of the trauma, so that organ failure, despite no direct injury to that organ, is often observed.

Our studies using injured animal models (Rauckman et al. 1980) clearly demonstrate that traumatic injury in the rat induces a rapid decrease in activity of several drug metabolizing enzymes, including cytochrome P-450 and FAD-monooxygenase activities. Other work has correlated the highly significant decreases in drug metabolizing enzyme activities with rises in plasma free fatty acids. We have observed significant increases in plasma free fatty acid concentrations in human trauma patients (Moylan, unpublished) as have Nixon and Brock-Unte (1978). These observations suggested that humans, as well as rats, might have drug metabolizing enzyme activities affected by injury. This appears to be the case, as indicated by our first study of antipyrine metabolism in severely injured patients.

Severely traumatized patients have a series of pathophysiological disturbances that may alter their drug eliminating ability. Trauma may cause severe hemolysis, hypoxemic tissue dysfunction, hormonal disturbances, thrombosis or necrosis so that organ perfusion is decreased and drug elimination by hepatic mechanisms is impaired. It is difficult to assess drug metabolizing capacity under different pathophysiological conditions, partly due to the complex nature of the reactions involved. There are two major groups of reactions participating in drug elimmination in the liver (e.g. hydroxylation or demethylation) and conjugation reactions in which the metabolite is subsequently conjugated with glucuronic acid, sulfate, glutathione, or other amino acids. How these hepatic drug elimination pathways are influenced by trauma is not well understood at the present time. The investigation of the metabolic profile of antipyrine was chosen because the drug is widely used, safe, and can be administered shortly post trauma to provide an indication of any significant alteration in hepatic drug metabolizing activities.

Antipyrine was administered orally to 14 severely injured multisystem trauma patients and to 4 healthy volunteers. No attempt was made to group the trauma patients, as the idea was to detect gross diferences in drug metabolism between healthy and traumatized individuals. Normal ranges may vary, as genetic background, diet, age, personal habits such as drinking and smoking, as well as other parameters not easily defined, may have a profound effect on individual ability to metabolize drugs. Ideally, because so called normal ranges of metabolites and metabolic rates may vary, a normal metabolism for an individual should be established compared with that in his traumatized condition.

There are a number of reasons drug half life in trauma patients might be prolonged, including alterations in hepatic enzyme drug metabolizing activities. Changes in enzyme

TABLE II

Table 1. Free fatty acid and hepatic enzyme drug-metabolizing activities in rats following infrarenal aortic ligation

Rat strain	N*	FAD-monooxygenase (nmoles·mg^{-1}·min^{-1})	Cytochrome P-450 (nmoles/mg)	Cytochrome c reductase (nmoles·mg^{-1}·min^{-1})	Non-esterified fatty acids (μmoles/l)
Wistar-Furth					
Control	8	2.58 ± 0.38	0.407 ± 0.068	51.25 ± 2.40	62 ± 25
Day 1	8	2.25 ± 0.21†	0.283 ± 0.043†	50.45 ± 4.34§	169 ± 46‡
Day 3	8	2.18 ± 0.19†	0.349 ± 0.0268	54.14 ± 5.15§	96 ± 91§
Fischer 344					
Control	8	2.28 ± 0.14	0.521 ± 0.053	54.08 ± 2.99	129 ± 33
Day 1	7	1.71 ± 0.17‡	0.269 ± 0.018‡	45.08 ± 3.62‡	203 ± 52‡
Day 3	6	1.27 ± 0.12‡	0.187 ± 0.034‡	44.68 ± 2.44‡	136 ± 69§
Lewis					
Control	8	1.91 ± 0.21	0.410 ± 0.048	41.46 ± 3.55	123 ± 44
Day 1	8	1.55 ± 0.14‡	0.291 ± 0.037†	42.45 ± 2.61§	175 ± 35‡
Day 3	8	1.68 ± 0.13‡	0.307 ± 0.053†	42.02 ± 4.70§	79 ± 29†
Fischer 344					
Control	8	1.89 ± 0.12	0.426 ± 0.036	48.21 ± 3.65	84 ± 26
Day 1	8	1.37 ± 0.13‡	0.270 ± 0.032‡	41.92 ± 2.89‡	189 ± 55‡
Day 3	8	1.17 ± 0.11‡	0.187 ± 0.044‡	42.76 ± 3.93‡	145 ± 48*

* N equals the number of individual rats. Data are means ± S.D.
† $P < 0.05$, compared to control.
‡ $P < 0.01$, compared to control.
§ Not different.

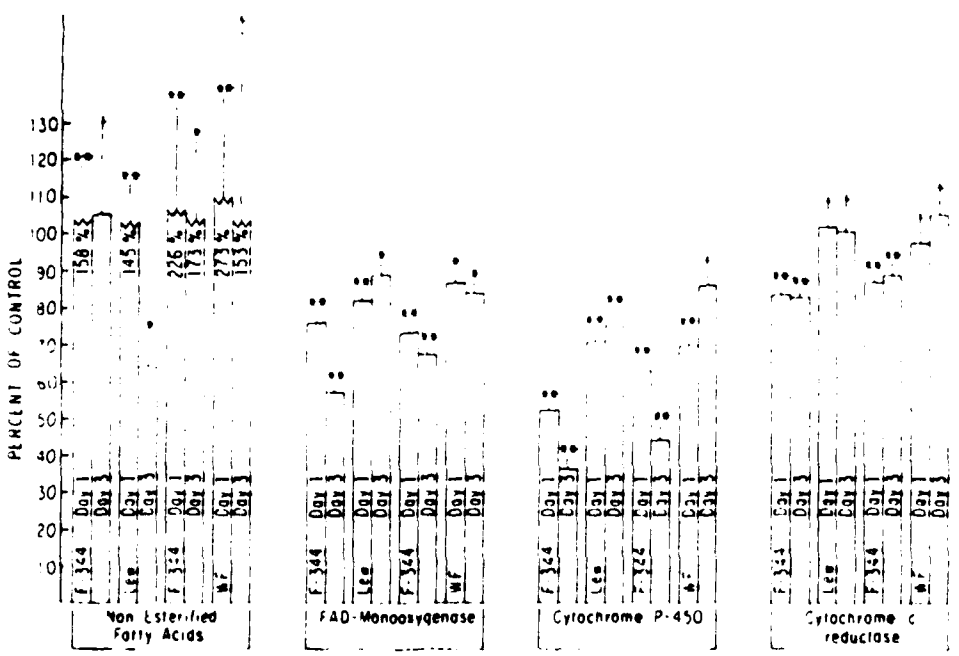

Fig. 1. Changes in plasma non-esterified fatty acids and hepatic enzymes relative to controls, days 1 and 3 post-infrarenal aortic ligation in inbred rats. Strains are Fischer 344 (F 344), Lewis (Lew), and Wistar–Furth (WF). Error bars represent the standard error. Levels of significance are (*) $P < 0.01$ (*) $P < 0.05$, (†) not significant. Data were analyzed by one way analysis of variance, followed by Dunnett's test [7].

activity could be indicated by changes in metabolite distribution, i.e., study of the pattern of urinary metabolites, or changes in plasma half life. We reasoned that if the trauma patients did in fact undergo an alteration in hepatic drug metabolizing activity, and if it were a relatively transient change, recovery to normal levels should occur reasonably rapidly during the recovery process. This would be reflected in drug plasma half life, provided the patient was not administered drugs in the initial stages post trauma that might alter drug metabolism. The majority of the severely injured trauma patients in this initial study usually received antibiotics, pain medication, and cymetidine. The latter has been found to alter antipyrine metabolism in healthy volunteers. Its effect is to prolong antipyrine half life by approximately 25%. Thus normal half life for antipyrine is 10-12 hours, whereas if preceded by cymetidine, antipyrine half life increases to 12.5-15 hours. Thus one would expect an increased antipyrine half life in those patients who received cymetidine.

The results shown in Table III were taken from an initial study of 12 severely injured patients to whom 600 mg. of antipyrine was administered orally within 24 hours of injury. Mean plasma half life of the antipyrine was 21.8 + 8.1 hours for these patients. Normal half life in healthy adults is 10-12 hours (Forrest et al., 1977) which we also confirmed in healthy volunteers (10.8 + 2.1 hours, n=3). The antipyrine plasma half life in the volunteers corresponded well with the published half life determined by other researchers and increased confidence in the high performance liquid chromatographic method and quantitative values. Clearly, antipyrine plasma half life is significantly increased in the severely injured. It is increased well beyond that expected from the inhibiting effect on antipyrine metabolism expected from cymetidine.

TABLE III

Plasma Half Life of Antipyrine (hours)

Patient	Half life (day one)	Half life (day as indicated)
1	30.1	
2	10.8	
3	26.5	
4	13.9	
5	34.7	
6	21.7	
7	22	
8	9.8	
9	31.5	
10	18.6	13 (9)
11	17.2	
12	24.9	8.2 (8)
13	--	7.3 (5)
14	--	13.5 (6)
Healthy Adults		
1	11.6	
2	8.4	
3	12.3	

Ideally, each patient should be used as his own control and should be evaluated several weeks or even months post recovery. This was not feasible owing to the distance of most patients from the Medical Center. However, it had been shown in the animal model that hepatic drug metabolizing activities recovered fairly rapidly post trauma, i.e. in approximately 48-72 hours. On this basis, one might expect to observe recovery in the human trauma patients by perhaps 5-7 days barring complications. In four patients investigated at 5-9 days post trauma, the antipyrine half life was within the normal range and in the two patients used as their own control (day 1 versus day 8 or 9), the decrease in plasma half life was significant. Continued study is necessary, but the first results indicate that there is a longer than normal half life for antipyrine immediately post injury despite cymetidine effects and that several days later the half life is in the normal range, suggesting recovery of the drug metabolizing system.

Studies in progress are investigating trauma patients who have not been on cymetidine. Preliminary results suggest the half life of antipyrine in these severely injured patients is significantly prolonged.

Effect of albumin administration on plasma free fatty acids. Post traumatic respiratory failure (RDS) is a common occurence in patients suffering from multisystem trauma. It is generally believed that fatty acids are an important, if not crucial factor. Fatty acids themselves have been shown to be toxic (Kreis et. al., 1976; Cahill et. al, 1974) to lung tissue and it has been documented that high levels of plasma free fatty acids are associated with multisystem injury (Nixon and Brock-Utne, 1978; Stoner et. al., 1979).

It has been our clinical protocol to administer albumin after 24 hours to trauma patients as required to maintain serum albumin levels above 3 gms. percent. The rationale of this was to bind as much unbound free fatty acid as possible in order to lower plasma free fatty acid levels (Moylan, 1976). Therefore, we undertook a random clinical study intended to evaluate the use of albumin for this purpose.

Over a period of three years, approximately 90 patients were evaluated. Of these, approximately two thirds were eliminated for various reasons including injury type (e.g. head injury or burn) and age (under 21 or over 65). The patients were classed into two groups: those receiving no albumin and those who received albumin on different days post trauma. Within the first few hours of trauma few patients were given albumin so that the most useful comparisons are made on patients who received albumin 12-36 hours post injury. In this time frame, compared to the 13 patients who did not receive albumin, 14 patients who received albumin twice during this time period had significantly lower total plasma free fatty acids ($p < 0.05$) with levels at 340 + 32 S.E.) as compared with levels of 486 + 60 uM/L without treatment. For 22 patients who received one treatment of albumin within the first 36 hours, plasma free fatty acid levels were 340 + 31 uM/L, virtually identical with those who had received only one treatment. After 48 hours post trauma, there appeared to be no difference in total plasma free fatty acid levels in patients receiving albumin versus those not receiving albumin. It did not matter whether the patient received albumin every day post injury or just one time. Administration made a difference, i.e. lowered total free fatty acid levels, only in relation to levels in untreated patients within the first two days post injury. It is to be noted that we have no cases in which albumin administration was initiated later than 48 hours post injury, so we do not know if on day 3 or 4 for example, the albumin receivers would have lower fatty acid levels than the "controls". However, it seems unlikely that later initiation of albumin therapy would change these results. Table IV summarizes the results of the study of the effect of albumin administration on plasma free fatty acid levels in multisystem trauma patients.

TABLE IV

Plasma Free Fatty Acids in Trauma Patients:
Albumin versus No Albumin Post Injury

No Albumin			Albumin	
Day	N	FFA (uM/L)	N	FFA (uM/L)
1-2	13	486±60 (SEM)	14	340±30[1]
			22	340±31[2]
3	15	327±44	11	381±55[1]
			25	363±32[2]
			16	337±45[3]
4	16	298±36	7	287±76[1]
			19	314±41[4]
			24	300±34[3]
5	14	246±22	17	222±39[5]
			20	231±35[3]
6	10	206±40	10	237±35[6]
			18	253±34[3]
7	8	238±35	1	210
			8	232±36[7]
			6	242±48[8]
			4	281±51[9]
			8	232±36[3]

1Albumin administered on all days studied
2Albumin administered on day 1 or day 2
3Albumin administered on 2 out of 3 days
4Albumin administered on 3 out of 4 days
5Albumin administered 3 out of 5 days
6Albumin administered 4 out of 6 days
7Albumin administered 3 out of 7 days
8Albumin administered 4 out of 7 days
9Albumin administered 5 out of 7 days

The data show that both groups of patients have elevated plasma free fatty acid levels post trauma and that these levels decline to relatively constant lower levels by day five. Without albumin, initial levels of plasma free fatty acids are higher, but decline more rapidly in the first two days to levels no different from the group that received albumin within the first day and a half of trauma. We note that free fatty acid levels do not remain significantly elevated beyond 4-5 days post trauma, however, we do not know the real basal values of these patients. It is possible that their normal average daily values are somewhat lower than the 232 uM/L average obtained here and that over a several period the values we have recorded would slowly decrease as body metabolism recovered to normal function.

ANIMAL STUDIES

The development of a reproducible animal injury model in the rat paved the way for several studies of trauma-induced hepatic dysfunction relevant to the understanding of the alterations in this organ during trauma and suggestive of differential treatment regimens, particularly in regard to drug therapy for humans.

Effects of Endotoxin on Hepatic Enzymes. Our work has shown that experimental traumatic injury in rats by abdominal aorta ligation results in rapid decreases in hepatic cytochrome P-450 reductase content, FAD-monooxygenase and cytochrome c reductase. Another type of insult to the animal, systemic endotoxin shock, also causes decreases in hepatic microsomal enzymes. As shown by Gorodischey et. al. (1976) and Renton and Mannering (1976), cytochrome P-450, UDP-glucuronyl transferase, aniline hydroxylase, benzene hydroxylase, ethyl morphine N-demethylase, and cytochrome b were depressed in endotoxin treated rats. We undertook to confirm the effect on some of these enzymes, as well as to examine the effect of endotoxin on cytosolic glutathione peroxidase and glutathione reductase.

Adult CDF rats were used for this study. Each was injected with lypolysaccharide from E. Coli and sacrificed 24 or 72 hours post treatment. Livers were removed and hepatic microsomes prepared and assayed for cytochrome P-450, FAD-monooxygenase, cytochrome c reductase, UDP-glucuronyl transferase, and cytosol for glutathione peroxidase and glutathione reductase. Table V summarizes the results.

In agreement with previous reports, there was a marked decrease in hepatic cytochrome P-450 content both 24 and 72 hours post treatment. FAD-monooxygenase activity decreased in 2 of 3 experiments. Since both cytochrome P-450 and FAD-monooxygenase are responsible for the oxidative detoxification of a wide variety of substances, the impairment of this mechanism during endotoxemia has potential implications for drug and nutritional therapy in humans.

TABLE V

Expt.	Treatment	Mortality	P-450 content (% of control)	FADM (% of control)	Cytochrome c Reductase (% of control)	Glutathione Peroxidase (% of control)	Glutathione Reductase (% of control)	Glucuronyl Transferase (% of control)
1	.9 mg. LPS 24 hrs.	44	65.0	84.3	86.8	76.7	86.4	135.7
	.9 mg. LPS 72 hrs.	11	73.3	75.8	70.4	87.2	90.9	104.1*
2	1 mg. LPS 24 hrs.	0	61.0	96.1*	91.5*	94.9*	92.4*	166.3
	1 mg. LPS 72 hrs.	0	55.4	76.6	65.1	97.4*	94.9	95.4*
3	1 mg. LPS 24 hrs.	0	53.8	78.5	85.0	83.0	90.6	145.6
	1.5 mg. LPS 72 hrs.	60	54.9	59.8	74.1	82.5	83.4	106.3*

Table V. Effect on LPS on rat hepatic enzymes. Values are the mean + S.E. of the percentages of the individual saline control values. Mean saline control values for a typical experiment (#2) were (in nmol/mg-min): P-450 content, 0.464 + 0.028; FADM 2.56 + 0.12; cytochrome c reductase, 90.46 + 8.07; glutathione peroxidase, 51.18 + 4.54; glutathione reductase, 121.6 + 28.8; and glucuronyl transferase, 0.086 + 0.011. Mortality of controls was 0%. Except where noted, all experimental values are significantly different from controls ($p < .05$) according to a two-tail Student's t-test.

*Not significant

Cytochrome c reductase, glutathione reductase, and glutathione peroxidase activities were all observed to decrease significantly except in experiment 2. It is noteworthy that in experiment 2 no mortality was observed for the endotoxin treated animals, suggesting that the overall effect of endotoxin in this experiment was not so profound as in the other experiments.

UPD-glucuronyl transferase was observed to increase in activity 24 hours after endotoxin treatment in all experiments; this is in contrast to the results of Gorodischer et al. (1976) who reported a decrease. We postulate that endotoxin treatment results in damage to the microsomal membrane containing UDP-glucuronyl transferase such that the accessibility of either hydrophobic substrates or UDPGA to the enzyme is increased. This hypothesis is supported by the observation that protease or detergent treatment of mocrosomal in vitro results in increased levels of UDP-glucuronyl transferase activity (Winsnes, 1969). In all experiments, UDP-glucuronyl transferase activity returned to control levels by 72 hours after endotoxin administration.

The slight changes observed in glutathione peroxidase and glutathione reductase suggest that this protective system of hepatocytes is relatively well preserved during endotoxemia. Endotoxin can injure cells indirectly by bringing about hemodynamic alterations resulting in tissue anoxia, by activating the complement and clotting cascades and by causing the extracellular release of lysosomal contents from polymorphonuclear leukocytes (Bradley, 1979). It also is directly toxic to some cells (Ho, 1964). Which effects(s) of endotoxin leads to the altered hepatic enzyme levels in endotoxemic animals is not known.

It is clear that alterations in hepatic drug metabolizing enzymes are not limited to those disease states affecting the liver primarily, but that disorders distant from the liver, such as regional ischemia (Rauckman et al., 1980) or endotoxemia may play an important role in altering drug metabolism.

Influence of Age on Post Traumatic Alterations of Hepatic Drug Metabolizing Enzymes. The metabolism of drugs, either by oxidation, conjugation or a combination of both is a major factor in controlling the circulating level of therapeutic agents. Environmental factors which influence the rate of drug metabolism alter the therapeutic efficacey of agents which generally require a certain minimal level for effectiveness and are limited to a maximal level to prevent toxicity. The effect of traumatic injury on drug metabolism is poorly defined from both a theoretical and practical view point. The purpose of these studies is to define the effect of model injury on hepatic drug metabolism. This is particularly germane since most severe trauma victims require multiple therapeutic agents.

The effect of infrarenal aorta ligation on hepatic cytochrome P-450 levels, FAD-containing monooxygenase activities and UDP-glucuronyl transferase activities is shown in Table VI for old (300 day) and young (42-46 day) Sprague-Dawley) rats. Cytochrome P-450 loss in old rats was maximal 3 days post trauma and returned to control levels by 6 days post trauma. Young animals, however, demonstrated a maximal reduction in cytochrome P-450 levels 2 days post trauma followed by a slight recovery phase and a second peak of cytochrome P-450 loss at 8 days post trauma. At 10 days post trauma the animals still had a 20% lower cytochrome P-450 level than their untreated age matched cohorts.

FAD-containing monooxygenase activity was found to drop to a minimum at 2-3 days post trauma in old animals and to recover to control levels by the sixth day post trauma. Young animals, on the other hand, showed an initial peak of FAD-containing

monooxygenase activity loss at 3 days post trauma, a slight recovery of activity at 4 days and a second peak of activity loss at 8 days post trauma. At 10 days post trauma, FAD-containing monooxygenase activity still remained 20% below control level.

UDP-glucuronyl transferase activity was found to undergo an initial increase in specific activity at 1 day post trauma in both young and old animals, however, by day 3 both showed a significant loss in hepatic microsomal UDP-glucuronyl transferase activity. In old animals the peak loss of activity occurred at day 3 and recovery to control levels occurred on day 6 post trauma. Young animals demonstrated maximal loss of UDP-glucuronyl transferase activity at 5 to 6 days post trauma and even by day 10 little recovery, as compared to age matched controls, had occurred.

The sensitivity of young and old animals to model trauma, as measured by alterations to hepatic drug metabolizing enzymes, is not significantly different, however, the recovery phase is dissimilar. The measured hepatic drug metabolizing enzymes of old rats return to control, or near control, levels within 6 days post trauma. These same drug hepatic drug metabolizing enzymes in young rats do not return to control levels even after 10 days. It was not determined if this loss of particular hepatic drug metabolizing enzymes is permanent. Since the growth of these animals is also disrupted by the model injury, it is felt that hormonal imbalances as a result of the injury may affect the hepatic drug metabolizing enzymes in the developing animal.

The effect of model trauma on UDP-glucuronyl transferase, the major hepatic drug conjugating enzyme in humans, differs from the effect on cytochrome P-450 and FAD-containing monooxygenase. An initial increase in activity is observed at day 1 post trauma followed by a decrease in activity by day 3. The activity of hepatic UDP-glucuronyl transferase is known to be increased by certain alterations of membrane structure (Winsnes, 1969). One model for activation of this enzyme suggests that an opening of the membrane lipid structure allows more ready access to the enzyme by either substrate (p-nitrophenol) or cofactor UDPGA (Vessey and Zakim, 1978). The observed post traumatic increases in in vitro microsomal UDP-glucuronyl transferase activity therefore suggests that the ischemic injury to the hind limbs causes an alteration of the hepatic cellular endoplasmic reticular membrane resulting in initially increased UDP-glucuronyl transferase activity. The latter decrease in activity could be caused by further membrane damage, perhaps to the enzyme itself. This corresponds well with the known effects of proteolytic enzymes on microsomal membranes in vitro (Graham et al., 1979).

The influence of age on post traumatic drug metabolism was investigated using model traumatic injury on Sprague-Dawley rats. Young (42-46 days old) and old (300 day) animals were submitted to infrarenal abdominal aorta ligation and were sacrificed between one and ten days later. Cytochrome P-450 and FAD-containing monooxygenase were found to undergo a rapid decrease in activity with the old animals recovering more rapidly than the young animals. UDP-glucuronyl transferase was found to undergo an initial increase in activity post trauma, followed by a decrease in activity. Old animals were also observed to recover more rapidly than young animals. This effect of trauma may have important applications in treating humans who have undergone severe trauma.

Role of Inflammation in Post Trauma Hepatic Dysfunction. The role of inflammation in the etiology of post traumatic disposition of hepatic drug metabolizing enzymes was investigated due to protection afforded these enzyme systems by dexamethazone. Limited previous studies (Bect and Whitehouse, 1974) have indicated that adjuvant arthritis produces diminished ability to demethylate aminopyrine and prolong hexabarbital sleeping time. Intraperitoneal injection of acid washed celite was chosen as a means of stimulating an acute inflammatory response. Because of the inertness of the particulate, direct effects, such as toxicity and enzyme induction, can be ignored. A dose-response curve for the effect of celite (i.p.) on UDP-glucuronyl transferase (UDPGT), cytochrome P-450, and FAD-containing monooxygenase (FADM) was generated and is shown in Table VII below.

TABLE VII

Effect of celite on hepatic enzymes: celite/175 g rat

Enzyme (% of control)	20	100	200
UDPGT	+25	+42	+13
P-450	-29	-40	-43
FADM	-29	-37	-38

On the basis of this experiment, 500 mg/kg was chosen as an optimal dose of celite. Dexamethazone (DMS) was found to protect against the effects of celite on hepatic cytochrome P-450 as shown in Table VIII below.

TABLE VIII

Enzyme (% of control)	Celite	DMS	DMS + Celite	IDM	IDM + Celite
UDPGT	+35	-26	N.S.	+280	+240
P-450	-38	-9	-9	-12	-36
FADM	-31	N.S.	N.S.	N.S.	N.S.

Indomethicin, however, was found to protect FAD-containing monooxygenase and no conclusion could be made concerning UDPGT and indomethecin. This experiment established the probability of an inflammatory response being responsible for celite induced loss of hepatic drug metabolizing enzymes. The quantitation similarities between celite and aortic ligation and their similar responses to dexamethazone also suggested a major role for inflammation in hepatic response to model trauma.

Irradiated rats (800 rads,-radiation 96 hours prior to experiments) which were shown to have a 98% reduction in polymorphonuclear neutrophile (PMN) were submitted to celite injection or abdominal aorta ligation. Irradiation did not greatly alter the hepatic response to either celite injection or abdominal aorta ligation indicating that polymorphonuclear neutrophiles are not responsible for trauma induced loss of hepatic

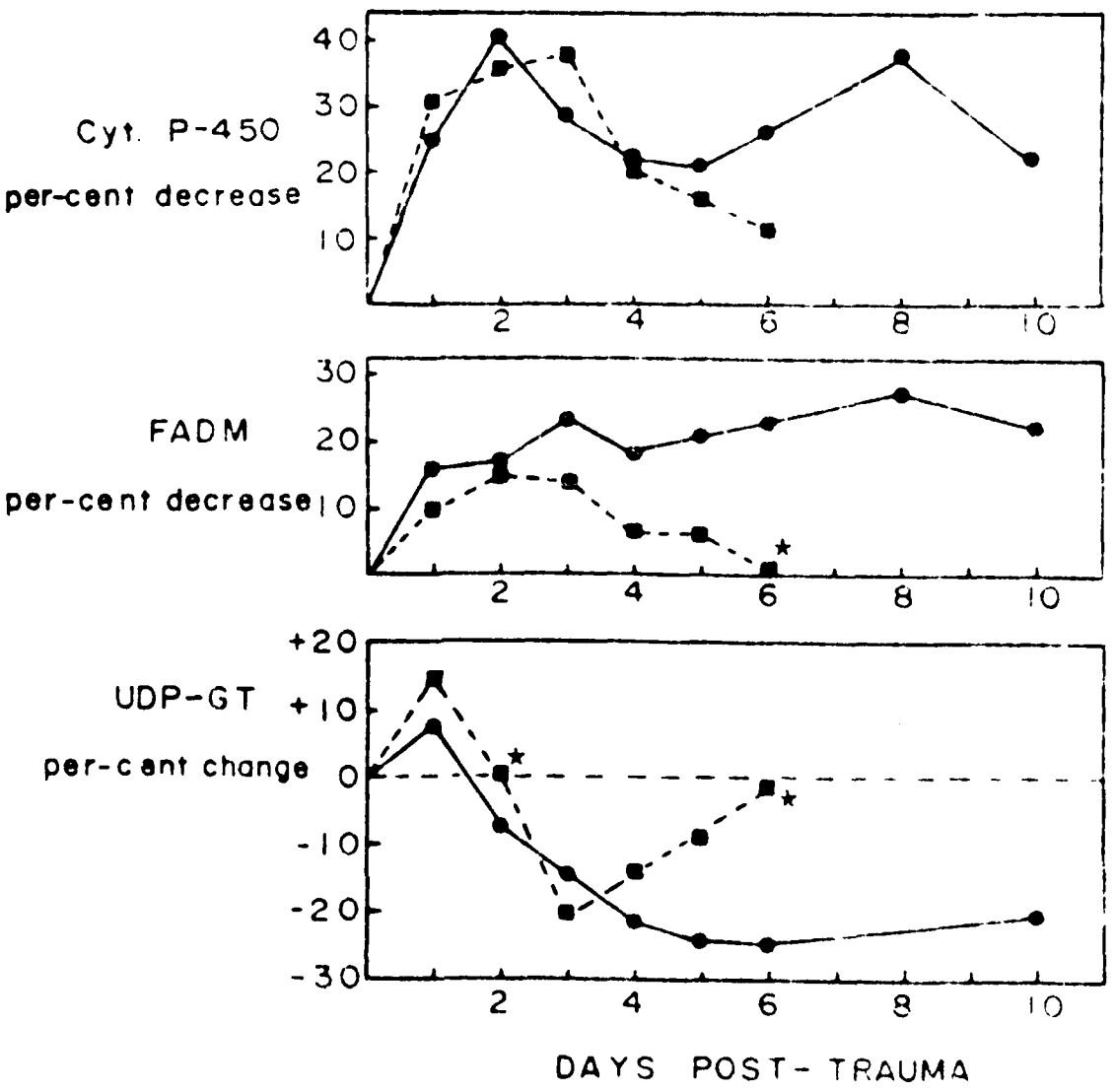

Figure 1: Change in hepatic enzymes at various time intervals after model trauma for young(●) and old (■) male Sprague-Dawley rats. Cyt-P-450, cytochrome P-450;FADM, FAD-containing monooxygenase; UDP-GT, UDP-glucuronyl transferase. Sixteen control and sixteen traumatized animals were used to obtain each point. Unless indicated (*), points are significantly ($p<0.05$, Students t-test) different from control.

drug metabolizing enzymes. Confirmation for this was an experiment in which rats were injected with shellfish glycogen, a nonspecific inflammatory agent, which stimulates PMN. Shellfish glycogen did not cause alteration of hepatic drug metabolizing enzyme levels.

These experiments suggest, then, that inflammation, not associated with PMN's, is associated with post traumatic alteration of drug metabolism. We propose that the responsible agent for post traumatic depression of drug metabolism is macrophage derived and is likely an acute reactant released by phagocytizing macrophages.

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