Loyola University Chicago
Loyola eCommons

Dissertations Theses and Dissertations

1989

Tumor Necrosis Factor and the Pathophysiology of Septic Shock

Mae J. Ciancio
Loyola University Chicago

Follow this and additional works at: https://ecommons.luc.edu/luc_diss

Part of the Medicine and Health Sciences Commons

Recommended Citation

Ciancio, Mae J., "Tumor Necrosis Factor and the Pathophysiology of Septic Shock" (1989). *Dissertations*. 2607.
https://ecommons.luc.edu/luc_diss/2607

This Dissertation is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Dissertations by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License.
Copyright © 1989 Mae J. Ciancio

LOYOLA UNIVERSITY MEDICAL CENTER LIBRARY

TUMOR NECROSIS FACTOR AND THE PATHOPHYSIOLOGY OF SEPTIC SHOCK

by

Mae J. Ciancio

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

April

1989

Copyright (c) 1989 by Mae J. Ciancio

ACKNOWLEDGEMENTS

I am truly grateful for the guidance and patience offered by my adviser, Dr. James P. Filkins, concerning both graduate studies and laboratory research. His continual interest and dedication to research, service, and teaching provided an excellent example. I also appreciate the time and efforts provided by my committee members: Dr. Mohammed M. Sayeed and Dr. Stephen B. Jones of the Physiology Department; Dr. Herbert L. Mathews of the Microbiology Department; and Dr. Anthony Cerami of Rockefeller University.

Sincere thanks go to all the faculty members and staff of the Physiology Department for their assistance throughout my training. The excellent technical help provided by Ms. Julia Hunt made completing this work easier and more enjoyable. I appreciate the help from Dr. Stephen B. Jones in establishing the cannulated rat model and determining plasma catecholamine concentrations. I appreciate Dr. Yelich's interest and the insulin assays run by his laboratory. The helpfulness of Dr. Linda Janusek in obtaining plasma corticosterone measurements is appreciated. Thanks go to Dr. John X. Thomas for providing the computer programs and help which made completing this dissertation a reasonable process.

Most importantly, I would like to thank my husband Don, my family, and my in-laws for their never ending emotional support, patience, and love. Without all that, this never would have been possible.

VITA

Mae J. Ciancio, the daughter of Joseph and Lucille Fulco, was born on September 29, 1959 in Chicago, Illinois. She attended elementary and secondary schools in Downers Grove, Illinois and graduated from Downers Grove North High School in June, 1977.

In September of 1977, Mae entered Northwestern University in Evanston, Illinois. While an undergraduate at Northwestern, she worked with Dr. Walter E. Bollenbacher on the regulation of ecdysone synthesis by the prothoracic glands in Manduca sexta. She received a Bachelor of Arts degree in Chemistry in June, 1981. She continued her research interests with Dr. Andrew R. LaBarbera in the Center for Endocrinology, Metabolism, and Nutrition at Northwestern University Medical School during June, 1981-1983.

Mae began her graduate studies in the Department of Physiology at Loyola University Stritch School of Medicine in July, 1983. Her dissertation work was completed under the direction of Dr. James P. Filkins. Mae was the recipient of a Loyola University Dissertation Fellowship in 1987. She also received a first place award in the Seventeenth Annual Sigma Xi Graduate Research Forum competition in 1987.

In February, Mae will begin post-doctoral studies under the direction of Dr. Eugene B. Chang in the Department of Medicine at the University of Chicago.

PUBLICATIONS

Journal Articles

1. Ciancio, M.J. and A.R. LaBarbera. Insulin stimulates granulosa cells: increased progesterone and cAMP production *in vitro*. Am. J. Physiol. 247: E468-E474, 1984.

2. Watson, R.D., M.J. Ciancio, W.P. Gunnar, L.I. Gilbert and W.E. Bollenbacher. Regulation of insect prothoracic glands: existence of a haemolymph stimulatory factor in *Manduca sexta*. J. Insect Physiol. 31: 487-494, 1985.

3. Ciancio, M.J., R.D. Watson and W.E. Bollenbacher. Competency of *Manduca sexta* prothoracic glands to synthesize ecdysone during development. Mol. Cell. Endocrinol. 44: 171-178, 1986.

4. Ciancio, M.J. and J.P. Filkins. *In vivo* effects of tumor necrosis factor and endotoxin. In preparation.

5. Ciancio, M.J. and J.P. Filkins. Tumor necrosis factor and hepatic glucoregulation. In preparation.

Abstracts

1. LaBarbera, A.R. and M.J. Ciancio. Insulin affects granulosa cells: increased progesterone and cAMP production *in vitro*. Fed. Proc. 42(3): 315, 1983.

2. Ciancio, M.J. and J.P. Filkins. Effect of interleukin-1 on hepatic glucoregulation. Circ. Shock. 18(4): 371, 1986.

3. Ciancio, M.J., S.B. Jones, M.R. Yelich and J.P. Filkins. Glucoregulatory and sympathoadrenal responses to TNF in conscious rats. Fed. Proc. 46(3): 561, 1987.

4. Ciancio, M.J., S.B. Jones, N.A. Sacco and J.P. Filkins. Metabolic and histologic alterations in TNF-induced shock. Circ. Shock. 21(4): 355, 1987.

5. Ciancio, M.J. and J.P. Filkins. Tumor necrosis factor (TNF) and hepatic glucoregulation. Circ. Shock. 24(4): 296, 1988.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS ii

VITA iii

LIST OF TABLES ix

LIST OF FIGURES x

Chapter

I. INTRODUCTION 1

II. REVIEW OF RELATED LITERATURE 4

A. HISTORY OF TUMOR NECROSIS FACTOR 4

B. BIOCHEMISTRY OF TUMOR NECROSIS FACTOR. 6

1. Isolation and structure 6
2. Source 8
3. Production and regulation 10
4. Receptor 12

C. EFFECTS OF TUMOR NECROSIS FACTOR AT THE CELLULAR LEVEL 15

1. Hematocrit and white blood cell counts 15
2. Polymorphonuclear neutrophils 17
3. Fibroblasts 19
4. Endothelial cells 22
5. T and B lymphocytes 26

D. EFFECTS OF TUMOR NECROSIS FACTOR AT THE ORGAN AND TISSUE LEVEL 28

1. Liver 28
2. Muscle 31
3. Adipose tissue and adipocytes 33
4. Organ pathology 36
5. Alterations in body weight 38

E. FEVER INDUCING PROPERTIES OF TUMOR NECROSIS FACTOR . 39

F. HEMODYNAMIC CHANGES INDUCED BY TUMOR NECROSIS FACTOR . 41

G. METABOLIC CHANGES INDUCED BY TUMOR NECROSIS FACTOR . 42

Page

1. Plasma glucose 42
2. Plasma lactate 43

H. NEURO-ENDOCRINE CHANGES INDUCED BY TUMOR NECROSIS FACTOR 44

1. Insulin and glucagon 44
2. Plasma catecholamines 47
3. Plasma cortisol 48

I. MORTALITY AND MORBIDITY INDUCED WITH TUMOR NECROSIS FACTOR 49

J. RATIONALE. 50

III. MATERIALS AND METHODS 52

A. ANIMALS 52

B. AGENTS 52

1. Tumor necrosis factor (TNF). 52
2. Heat-inactivated tumor necrosis factor . . . 53
3. Endotoxin (ETX). 53

C. IN VIVO EXPERIMENTS 53

1. Cannulation procedure and feeding regimen . . 53
2. Experimental procedure for cannulated rats . . 54
3. Glucan treated animals 55

D. ISOLATED PERFUSED RAT LIVER EXPERIMENTS 56

1. Liver perfusion apparatus 56
2. Liver removal 57
3. Liver perfusion 57

E. ISOLATED LIVER PARENCHYMAL CELL EXPERIMENTS 59

1. Isolation procedure 59
2. Incubation conditions 60
3. Glucose utilization by isolated hepatocytes . 61

F. GLUCOSE AND LACTATE MEASUREMENTS 61

1 Glucose determination 61
2. Lactate determination 62

G. INSULIN MEASUREMENTS 63

Page

H. NOREPINEPHRINE AND EPINEPHRINE MEASUREMENTS 63

I. CORTICOSTERONE MEASUREMENTS 63

J. PROTEIN DETERMINATION 63

K. STATISTICAL ANALYSIS 64

IV. RESULTS 65

A. *IN VIVO* EFFECTS OF TUMOR NECROSIS FACTOR 65

1. Experimental groups 65
2. Mortality and morbidity induced with TNF, ETX, and TNF plus ETX co-treatment 65
3. TNF- and ETX-induced mortality after glucan pretreatment 69
4. Hemodynamic effects of ETX, TNF, and ETX plus TNF co-treatment: mean blood pressure 71
5. Hemodynamic effects of ETX, TNF, and ETX plus TNF co-treatment: pulse rate 79
6. Metabolic effects of TNF, ETX, and TNF plus ETX co-treatment: plasma glucose 87
7. Metabolic effects of TNF, ETX, and TNF plus ETX co-treatment: plasma lactate 96
8. Endocrine effects of TNF, ETX, and TNF plus ETX co-treatment: plasma insulin 105
9. Neuro-endocrine effects of TNF: plasma catecholamines 113
10. Endocrine effects of TNF: plasma corticosterone 118

B. *IN VITRO* EFFECTS OF TNF: ISOLATED PERFUSED RAT LIVERS 121

C. *IN VITRO* EFFECTS OF TNF: ISOLATED LIVER PARENCHYMAL CELLS 127

V. DISCUSSION 137

A. *IN VIVO* EVALUATION OF TNF AS A MEDIATOR OF ENDOTOXIC SHOCK 137

1. Mortality and morbidity comparisons. 138
2. Hemodynamic comparisons: mean blood pressure and pulse rate 140
3. Metabolic comparisons: plasma glucose and lactate 141
4. Neuro-endocrine comparisons: alterations in insulin, catecholamines, and corticosterone. . 143
5. Additional pathophysiological effects . . . 146
6. Other mediators 147

Page

B. *IN VIVO* EFFECTS OF TNF PLUS ETX CO-TREATMENT . . . 149

  1. Mortality and morbidity. 149
  2. Hemodynamic, metabolic and endocrine effects . 151

C. *IN VITRO* EFFECTS OF TNF ON HEPATIC GLUCOREGULATION . 153

VI. SUMMARY AND CONCLUSIONS. 160

REFERENCES 165

LIST OF TABLES

Table Page

1. TUMOR NECROSIS FACTOR RECEPTORS 13

2. TIME AND DOSE DEPENDENT EFFECTS OF TNF- AND ETX-INDUCED MORTALITY IN FASTED CANNULATED RATS 67

3. LETHAL EFFECTS OF HIGH DOSE TNF IN FED CANNULATED RATS . . 68

4. EFFECTS OF GLUCAN PRETREATMENT ON TNF- OR ETX-INDUCED MORTALITY 70

5. EFFECTS OF TNF ON GLUCOSE PRODUCTION RATES BY ISOLATED PERFUSED RAT LIVERS 123

6. EFFECTS OF TNF ON LACTATE PRODUCTION RATES BY ISOLATED PERFUSED RAT LIVERS 125

7. EFFECTS OF TNF ON OXYGEN CONSUMPTION RATES BY ISOLATED PERFUSED RAT LIVERS 126

8. EFFECTS OF TNF ON BASAL AND GLUCAGON-STIMULATED GLUCOSE PRODUCTION RATES BY ISOLATED HEPATOCYTES 129

9. EFFECTS OF TNF ON BASAL AND VASOPRESSIN-STIMULATED GLUCOSE PRODUCTION RATES BY ISOLATED HEPATOCYTES . . . 130

10. EFFECTS OF TNF ON LACTATE PRODUCTION RATES BY ISOLATED HEPATOCYTES 132

11. EFFECTS OF TNF ON LACTATE PRODUCTION RATES BY ISOLATED HEPATOCYTES IN THE ABSENCE OR PRESENCE OF VASOPRESSIN . . 133

12. EFFECTS OF TNF ON GLUCOSE UTILIZATION BY ISOLATED HEPATOCYTES 134

LIST OF FIGURES

Figure Page

1. EFFECTS OF HIGH DOSE TNF AND ETX ON MEAN BLOOD PRESSURE IN FASTED CANNULATED RATS 74

2. EFFECTS OF HIGH DOSE TNF ON MEAN BLOOD PRESSURE IN FED CANNULATED RATS 76

3. EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON MEAN BLOOD PRESSURE IN FASTED CANNULATED RATS 78

4. EFFECTS OF HIGH DOSE TNF AND ETX ON PULSE RATE IN FASTED CANNULATED RATS 82

5. EFFECTS OF HIGH DOSE TNF ON PULSE RATE IN FED CANNULATED RATS 84

6. EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PULSE RATE IN FASTED CANNULATED RATS. . 86

7. EFFECTS OF HIGH DOSE TNF AND ETX ON PLASMA GLUCOSE IN FASTED CANNULATED RATS 91

8. EFFECTS OF HIGH DOSE TNF ON PLASMA GLUCOSE IN FED CANNULATED RATS 93

9. EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PLASMA GLUCOSE IN FASTED CANNULATED RATS. 95

10. EFFECTS OF HIGH DOSE TNF AND ETX ON PLASMA LACTATE IN FASTED CANNULATED RATS 100

11. EFFECTS OF HIGH DOSE TNF ON PLASMA LACTATE IN FED CANNULATED RATS 102

12. EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PLASMA LACTATE IN FASTED CANNULATED RATS. 104

13. EFFECTS OF HIGH DOSE TNF AND ETX ON PLASMA INSULIN IN FASTED CANNULATED RATS 108

14. EFFECTS OF HIGH DOSE TNF ON PLASMA INSULIN IN FED CANNULATED RATS 110

15. EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PLASMA INSULIN IN FASTED CANNULATED RATS. 112

Page

16. EFFECTS OF HIGH DOSE TNF ON PLASMA EPINEPHRINE IN FASTED CANNULATED RATS 115

17. EFFECTS OF HIGH DOSE TNF ON PLASMA NOREPINEPHRINE IN FASTED CANNULATED RATS 117

18. EFFECTS OF HIGH DOSE TNF ON PLASMA CORTICOSTERONE IN FED CANNULATED RATS 120

19. TUMOR NECROSIS FACTOR AND THE PATHOPHYSIOLOGY OF SEPTIC SHOCK 164

CHAPTER I

INTRODUCTION

Septic shock induced by invading gram negative bacteria (GNB) is a significant concern of modern day health professionals (68,169,172,286). The prevalent use of antibiotics, invasive life support systems, catheterizations, immunosuppressive therapy, and organ transplants in medical centers have caused the number of GNB-induced cases of septic shock to escalate (9,70,80,105,146,270,281,286,288). Indeed, septic shock with concomitant hemodynamic, metabolic and neuro-endocrine alterations has been called the ultimate disease of medical progress (70). Prior to the 1920's, less than 100 cases of GNB-induced shock were reported (72). In the U.S. today, approximately 1% of hospital admissions develop GNB-induced sepsis, with more than 100,000 resultant deaths yearly (68,170).

The chain of events responsible for the lethal demise of the septic host is complex and as yet unresolved. One long-standing, major pathogenetic theory emphasizes that endotoxin (ETX), a lipopolysaccharide complex isolated from the cell wall of GNB, is the direct causative agent for the morbidity and mortality of GNB induced septic shock(107,286,288). Indeed, ETX treated animals as well as human subjects demonstrate the septic symptoms such as fever, cation shifts, increased white blood cell counts, muscle wasting, acute phase protein synthesis, decreased lipogenesis, hyperinsulinemia, and glucose dyshomeostasis (73,106,107

243,283) which are associated with septic shock, multiple systems organ failure and eventual mortality.

In recent years an alternate, novel theory of septic shock pathogenesis proposes that ETX elicits all its pathophysiological effects by stimulating cells of the immune system to release septic mediators. In particular, macrophages activated either by GNB or their constituent ETX release a number of mediators which are now recognized as key components in the pathophysiology of septic shock, such as prostaglandins, leukotrienes, platelet-activating factor, and the monokines tumor necrosis factor (TNF) and interleukin 1 (IL-1) (75,180,220).

Of all the pathophysiological changes which occur during septic shock, altered substrate metabolism may be the eventual critical component in the demise of the septic host (17,177). During infection and tissue injury cells tend to rely heavily on glucose for the energy required to function and survive. Because the liver is the major site for systemic glucose production, alterations in hepatic glucoregulation may produce the changes in circulating plasma glucose concentrations, which in turn impact detrimentally on cell function. In particular, monokines released from the liver macrophages, i.e. Kupffer cells, in response to invading GNB and ETX (64) may be critical in the glucose alterations of septic shock.

Therefore, the overall purpose of this dissertation is to contribute fundamental new knowledge regarding the role of the mononuclear phagocyte system in the pathogenesis of gram negative septic shock. The major focus is on the role of TNF in the pathophysiology of lethal endotoxicosis in conscious rats. The specific aims include: 1) an *in vivo* evaluation of TNF as a mediator of the endotoxic syndrome including mortality, hemodynamic, metabolic, and neuro-endocrine aspects; 2) an *in vivo* examination of the pathophysiological changes induced by co-treatment with TNF and ETX; and 3) an *in vitro* evaluation of the direct glucoregulatory effects of TNF on hepatic carbohydrate metabolism using isolated perfused rat livers and isolated liver parenchymal cells.

CHAPTER II

REVIEW OF RELATED LITERATURE

The related literature published up to June 1, 1988 was reviewed in this dissertation. Because of the extensive interest in the biological activities of TNF, the author realizes that numerous relevant papers have been published more recently. When considered to be of the utmost importance to the interpretation of the data, a few of the most recent papers have been included in this dissertation.

A. HISTORY OF TUMOR NECROSIS FACTOR

For years, physicians noted that some cancer patients who became infected subsequently demonstrated significant tumor regression. Intrigued by these findings, Coley devoted his career to the treatment of cancer patients with bacterial products. After one of his cancer patients recovered from a neck sarcoma after 2 attacks of erysipelas (49), Coley induced erysipelas in his cancer patients as a means of treatment (50). However, Coley quickly realized that infecting patients already compromised by the cancerous insult frequently led to a fatal outcome. Therefore, Coley implemented a filter sterilized preparation of the soluble toxins produced by Streptococcus erysipelas and Bacillus prodigiosus as a means of cancer treatment rather than live bacteria. In 1894, Coley reported the successful use of "Coley's Mixed Toxins" in the treatment of a patient with inoperable abdominal wall sarcoma (51). An extensive report of Coley's works and mixed toxin preparations was later published by his daughter in 1953 (184).

In 1942, Zahl, et al. (287), suggested that the tumor necrotizing agent produced by gram negative bacteria might be identical with a polypeptide component of the endotoxin antigen (287). Shear, et al. (233), had isolated the necrotizing agent from Serratia marcescens cultures and determined it to be a polysaccharide-rich fraction; sub-microgram quantities produced significant tumor necrosis within a few hours (232).

In 1962, O'Malley, et al. (189), demonstrated that serum from normal mice injected with Serratia marcescens polysaccharide had dose and time dependent tumor necrotizing activity when injected into recipient tumor bearing mice. In 1975, Carswell, et al. (40), further examined the hypothesis that a serum factor was involved in the bacteria-evoked tumor necrotizing activity. Mice were pretreated with the macrophage stimulant bacillus Calmette-Guerin (BCG) 14 days prior to challenge with lipopolysaccharide (LPS). Within a few hours after LPS challenge, donor mice demonstrated significant tumor regressing activity. Carswell, et al., suggested that lipopolysaccharide acted indirectly to cause tumor regression by stimulating the release of a necrotizing substance from the macrophages which they called "tumor necrosis factor."

Concurrently, a group of investigators at Rockefeller University was examining the mechanism responsible for the severe cachexia associated with chronic infection. In 1980, Rouzer and Cerami (215) reported that rabbits infected with the parasite Trypanosoma brucei became cachectic in spite of elevated circulating levels of triglycerides. In order to examine this further, Kawakami and Cerami utilized an ETX model of infection rather than a parasitic model (119). They demonstrated a dramatic decrease in lipoprotein lipase (LPL) activity and a concomitant increase in serum triglyceride levels 16 hours after ETX challenge. A similar response was produced in endotoxin-resistant mice administered serum from endotoxin-sensitive mice 2 hours after an ETX challenge. Thus, a serum factor present in the donor blood was associated with the hyperlipemia produced during bacterial or parasitic infection. They determined that peritoneal exudate cells produced a LPL-suppressing factor after stimulation with ETX.

Kawakami, _et al_. (121), continued to study the LPL-suppressing factor using the well-defined pre-adipocyte cell line, 3T3-L1 (121). Media isolated from peritoneal exudate cells stimulated _in vitro_ with ETX markedly suppressed LPL activity in 3T3-L1 cells. Beutler, _et al_. (25), purified this cachexia-inducing factor and named it "cachectin". Beutler, _et al_. (23), subsequently determined that the macrophage-released mediators, "cachectin" and "tumor necrosis factor," were one in the same.

B. BIOCHEMISTRY OF TUMOR NECROSIS FACTOR

1. Isolation and structure

Studies reported between 1976 and 1981 used serum from mice and rabbits treated in the manner described by Carswell, _et al_. (40), as a source for TNF. TNF isolated from mouse and rabbit sera was a protein with a molecular weight of 67,000-150,000 daltons, as determined by polyacrylamide gel electrophoresis (94,167,216) or 39,000-225,000 daltons when determined by gel filtration (132,157,167,168,216). TNF dissociated to a smaller molecular weight form under denaturing conditions, thus indicating that TNF may exist as an oligomer under natural conditions (94,157). Mouse TNF was a glycoprotein (94,132) with a pI=4.8 (157), whereas rabbit TNF was a protein with little or no carbohydrate moieties and a pI=5.1 (167,168,216). Both mouse and rabbit TNF were labile at temperatures ≥ 70°C (94,132,167,168,216)

Fransen, et al. (85) and Pennica, et al. (197) constructed the cDNA for mouse TNF by isolating the mRNA from the monocytic cell line PU5.1.8. The amino acid sequence predicted from the cDNA indicated that mouse TNF is synthesized as a pre-TNF protein, with a 79 amino acid pre-sequence attached to the 156 amino acid protein. When produced *in vivo*, TNF is glycosylated at the specific amino acid site predicted by the cDNA (197). The amino acid sequence for rabbit TNF also was determined from its corresponding cDNA. Like mouse TNF, rabbit TNF is synthesized as a pre-TNF protein, with an 80 amino acid pre-sequence followed by a 154 amino acid protein (112).

Human TNF, isolated from human peripheral blood monocytes and monocytic cell lines, was determined initially to be a protein with a molecular weight of 40,000-70,000 daltons by gel filtration. Similar to mouse and rabbit TNF, human TNF was pH and temperature sensitive (7,188,280). Aggarwal, et al. (7) suggested that TNF existed as a dimer or trimer under natural conditions which dissociated into a monomer of 17,000 daltons under denaturing conditions. Pennica, et al. (198) and Wang, et al. (273) described the cDNA cloning for human TNF and its expression in *E. coli* to produce recombinant TNF. TNF is synthesized as a larger precursor molecule, with a leader sequence of 76 amino acids attached to the 157 amino acid protein containing 2 cysteine residues, no glycosylation sites, and a pI≈5.3 (7,198,273).

TNF is a highly conserved protein, as illustrated by the homology between mouse, rabbit, and human TNF. Mouse and human TNF have approximately 80% homology in amino acid sequence (160,197). Similarly, Ito, et al. (112) reported an 81.9% homology in amino acid sequence between human and rabbit TNF (112). A 33 nucleotide sequence is totally conserved in the 3'-untranslated region of mouse and human TNF mRNA (39). This 33 nucleotide sequence is composed entirely of A and T residues in a repeating octamer of TTATTTAT. The human gene encoding for TNF was identified (234) and determined (239) to be encoded within the major histocompatibility complex on chromosome 6 and to contain 3 introns (111,160).

Whether the active form of TNF is a monomer or trimer is still under investigation. Smith, et al. (237) reported a 5.5 fold lower binding activity of the TNF monomer than that of the TNF trimer. The dissociation constants also differed substantially: the K_D=90 pM for the trimer and K_D=70 nM for the monomer. The molecular form of TNF in solution was dependent on its concentration: as the concentration of TNF in solution decreased, TNF dissociated into either a monomer or dimer dependent upon the pH in the solution (183). The structure of TNF, as determined by Davies, et al. (60), is a non-helical β-sheet rich structure which may be a noncovalently associated oligomer.

2. Source

Since Carswell's suggestion that macrophages were the cellular source of TNF (40), in vivo studies by Green, et al. (95) and Mannel, et al. (159) indirectly supported that theory. Green observed that the concentration of TNF in the serum of BCG-primed, endotoxin challenged mice correlated with a measurable hepatosplenomegaly and suggested that the macrophage-rich tissues were the source of serum TNF. Mannel, et al. (159) determined that the host's ability to necrotize an invading tumor after an endotoxin challenge was dependent on the ETX responsiveness of bone marrow cells.

In vitro studies with isolated macrophages demonstrated directly that macrophages were the primary site for synthesis and release of TNF. TNF production by mouse, rat, rabbit, and human macrophages was stimulated by ETX, BCG, C. parvum, zymosan, and poke weed mitogen (55,81,155,158,165,188). Stimulated macrophages synthesized and released more TNF than unstimulated, plastic adhered macrophages (162,164). TNF is not stored intracellularly; it is produced in response to macrophage activation (163). Cycloheximide and actinomycin D treatment inhibited TNF production in vitro (81,163,164,165), therefore substantiating that TNF is not stored intracellularly but is produced in response to macrophage activation. Studies with rabbit antiserum specific for serum TNF established that TNF produced in vitro by isolated macrophages was the same as TNF produced in vivo (155,158). Macrophage cell lines also produce TNF in vitro (25,133) after activation.

Although macrophages are the primary cellular source of TNF, Peters, et al. (201) and Cuturi, et al. (56) demonstrated detectable TNF production by natural killer cells. Christmas, et al. (47), however, reported little or no TNF production by natural killer cells, but significant production by T lymphocytes after stimulation with phytohaemagglutinin (PHA). T lymphocytes stimulated with phorbol-12,13-dibutyrate, A23187, PHA, anti-T3 + IL-2, or PMA produced significant quantities of TNF (56,241,246). The Ca^{++} -ionophore A23187 potentiated TNF production by T cells stimulated with phorbol-12,13-dibutyrate (56) and anti-CD3 monoclonal antibody (246). Additionally, epithelial tumor cell lines provoked with cycloheximide produced TNF (240).

3. Production and regulation

As briefly discussed in section 2, TNF is produced primarily by activated macrophages. Agents that enhanced the reticuloendothelial system, such as bacillus Calmette-Guerin (40,108,222), *Corynebacterium granulosum* (40), *Corynebacterium parvum* (40,90,95,108,223), zymosan (40), *Propionibacterium acnes* (100), *Listeria monocytogenes* (101), and thioglycollate (90,91) significantly increased TNF production after an endotoxin challenge (83,90,101). However, endotoxin alone stimulated significant TNF production both *in vivo* (83,90) and *in vitro* (25,90,131). The lipid A moiety of endotoxin and not the polysaccharide-rich fraction was responsible for the stimulatory effect (100).

TNF also is produced *in vitro* by monocytic cell lines such as HL-60 and U937 (6,102,198). Stimulation with either PMA, interferon-gamma (IFN-γ), interferon-alpha (IFN-α), lymphotoxin (TNF β) or TNF produced a transient increase in TNF mRNA which is inhibited with the protein kinase C inhibitor, H7 (102). As quickly as 20 minutes after activation, measurable levels of TNF mRNA were produced (221). The stimulus for TNF production must be continuously present during the incubation in order for TNF production to continue: for example, removal of ETX at any time during the incubation period immediately halted TNF production (91).

TNF production is enhanced by IFN-γ and inhibited by prostaglandin E_2 and dexamethasone. IFN-γ 'primes' the macrophages so that a greater quantitiy of TNF is produced after ETX stimulation (27,36,91,92). IFN-γ increased TNF production by acting at both the transcriptional (27,36,52) and post-transcriptional (27,36) levels. Incubation with cycloheximide increased the transcription of TNF mRNA 12 fold in response to stimulation with phorbol ester (221), whereas transcription of TNF mRNA was increased 50 fold in peritoneal macrophages incubated with cycloheximide plus IFN-γ (52).

Prostaglandin E_2 and dexamethasone act in a similar manner to decrease TNF production. Kunkel, et al. (135,136) reported a dose dependent decrease in endotoxin-induced TNF production by prostaglandin E_2: prostaglandin E_2 at concentrations greater than 10 nM decreased TNF production by 80%. Prostaglandin E_2 acted primarily to decrease the transcription of TNF mRNA (135). Pretreatment with dexamethasone significantly inhibits TNF production: TNF production is inhibited 50-90% after pretreatment with dexamethasone (266,267). Just as IFN-γ and prostaglandin E_2, dexamethasone acted at both the transcriptional and post-transcriptional levels to decrease TNF production (24).

As reported by Beutler, et al. (25), TNF is a major macrophage secretory product, comprising 1-5% of total protein synthesized by activated macrophages. Within 15 minutes of macrophage activation, measurable levels of TNF are produced (22,28,90). Peak serum TNF levels are reached approximately 90 minutes after endotoxin injection in humans (103,174,175), mice (90,95), rats (266), rabbits (28,161), and baboons (103). Similar results are observed for TNF production by activated macrophages in vitro (102). The half-life for TNF ranges from 0.5 to 27 minutes (28,31,82,161,266), and the kidneys are important in TNF breakdown and elimination (200).

4. Receptor

As documented in table 1, receptors for TNF are present on a number of different cell types. The number of receptor sites per cell ranges from 230-11,000, with K_D values of 2.6×10^{-13} - 0.8×10^{-9} M. TNF binding is saturable and dependent on experimental conditions, such as dose, temperature, and time. After binding to its receptor, TNF is internalized and degraded intracellularly (14,110,258). The presence of TNF receptors, alone, is insufficient to predict whether the cell will respond to TNF (134,244,258). Beutler, et al. (25) determined that TNF binding to as few as 5% of the receptors will produce a significant cellular response.

In vivo binding sites for TNF were determined by Beutler, et al. (28) after an i.v. bolus injection of ^{125}I-TNF. Thirty-one percent of the radiolabeled TNF was recovered in the liver, approximately 30% in the skin, 9 and 8% in the G.I. tract and kidneys, respectively. Only 2% was found in the lungs and 1% was found in the spleen. When expressed as a percentage of the average counts found per gram of tissue wet weight, the liver and kidneys possessed the greatest degree of radioactivity, followed by the lungs, spleen, and skin.

The structure of the TNF receptor was examined using radiolabeled TNF covalently cross-linked to its receptor prior to isolation. Tsujimoto, et al. (256) and Scheurich, et al. (226) determined that the TNF-receptor complex had a molecular weight of 100 kda and 76 kda,

TABLE 1

TUMOR NECROSIS FACTOR RECEPTORS

CELL TYPE	RECEPTOR NUMBER (sites/cell)	K_D(M)	REFERENCE
3T3-L1 ADIPOCYTES	10,000	0.33×10^{-9}	Beutler et.al. (25)
C2 MUSCLE CELLS	10,000	0.33×10^{-9}	
L-M	1100	3×10^{-12}	Kull et.al. (134)
L929	770	3×10^{-12}	
3T3A31	1050	2.5×10^{-12}	
J774.1	1100	3×10^{-12}	
CPAE	1500	3×10^{-12}	
HeLa	6000	2×10^{-10}	Baglioni et.al. (14)
JURKATT	1100	2.5×10^{-10}	
L929	2200	6.1×10^{-10}	Tsujimoto et. al., (258)
FS-4	7500	3.2×10^{-10}	
(HUMAN PERIPHERAL BLOOD MONOCYTES)	230	2.6×10^{-13}	Imamura et.al. (110)
	3800	1.5×10^{-10}	
ME-180	2250	0.31×10^{-9}	Sugarman et.al. (244)
W1-38	2172	0.22×10^{-9}	
T24	1512	0.25×10^{-9}	
PMN	6000	1.37×10^{-9}	Shalaby et.al. (231)
MCF-7	2500	0.13×10^{-9}	Creasey et.al. (54)
	4000	0.8×10^{-9}	

respectively. Kull, et al. (134) observed 2 bands of autoradiographic activity; a prominent band at 95 kda and a second band at 75kda. Four bands of activity were isolated by Creasey, et al. (54) with molecular weights of 138 kda, 90 kda, 75 kda, and 54 kda. Three of the four components, 90 kda, 75 kda, and 54 kda, were present on cells whether they did or did not respond to the cytotoxic, proliferative, or stimulatory effects of TNF. The 138 kda component was determined to exist only on cell lines that were sensitive to the cytotoxic effects of TNF. The 350 kda globular protein demonstrated by Smith, et al. (238) corresponds to the molecular weight of the TNF receptor when all four subunits are present.

TNF receptor binding is significantly influenced by agents such as interferon-γ, conconavalin A (Con A), and protein kinase C activators. Interferon-γ and Con A significantly enhance TNF receptor binding. Aggarwal, et al. (5), Tsujimoto, et al. (257,259) and Ruggiero, et al. (217) demonstrated a transient 30-300% increase in TNF receptor binding with no change in receptor affinity after treatment with IFN-γ. Interferon-γ increased TNF receptor binding by increasing receptor synthesis, since treatment with actinomycin D blocked the effect (217). The stimulatory effect of IFN-γ was dose, temperature, and time dependent, and for some cell lines, was influenced by type I interferon (257,259). Con A treatment, on the other hand, significantly increased receptor binding without increasing TNF receptor synthesis or affinity (8). Con A significantly increased receptor binding by decreasing receptor internalization and degradation.

TNF receptor binding is significantly reduced by treatment with protein kinase C activators. Agents such as 4β-phorbol 12-myristate 13-acetate (PMA), 1-oleoyl-2-acetyl glycerol (OAG), and Ca^{++} ionophore A23187 significantly reduced TNF receptor binding (4,109,115,262). Receptor binding decreased from 50-95% (4,262) due to either a decrease in receptor affinity (115,262) or a redistribution of TNF receptors (4,109). Pretreatment with the protein kinase C inhibitors, H7 or staurosporine (115,262), significantly reduced the inhibitory effect of PMA on TNF receptor binding. Furthermore, interleukin 1 also decreased TNF receptor binding (109).

C. EFFECTS OF TUMOR NECROSIS FACTOR AT THE CELLULAR LEVEL

1. Hematocrit and white blood cell counts

Depending on the model, a single injection of TNF can elicit either no change (253) or an increase in hematocrit (16,210,249). Tracey, et al. (253) reported no significant change in hematocrit for as long as 3 hours after infusion of non-lethal (10 μg/kg) or lethal (100 μg/ml) doses of TNF, as demonstrated using anesthetized beagle dogs. However, a significant hemoconcentration was observed in mice (16,210) and rats (249) after a single injection of TNF. Mice injected with 5 and 10 μg of TNF had a significant hemoconcentration response by 2 hours: hematocrit increased significantly from a control value of 38.6 to 46.6 and 48.8%, respectively (210). After injection of a larger dose of TNF, 3 mg/kg, an increase in hematocrit of 150% relative to control mice was produced by 6 hours (16). Similar observations were reported by Tracey, et al. (249) after a lethal injection of TNF in rats (249).

After repeated doses of TNF, a significant hemodilution response is observed in both humans and rats. Cancer patients administered TNF twice weekly for 4 weeks demonstrated a significant drop in hemoglobin content from 12 to 9.3 g/dl (31). Tracey, et al. (254) reported a similar observation in rats given TNF twice daily for 8 days: hematocrit dropped from a control value of 45% to approximately 28% by the eighth day. Accounting for the drop in hematocrit was the significant depression in red blood cell mass and hemoglobin content (254).

The effect of TNF on white blood cell (WBC) counts was time and model dependent. Remick, et al. (211) observed in mice a transient initial lymphocytosis 15-30 minutes after TNF injection which was followed by a rapid drop in lymphocyte number. By 2 hours, a marked lymphopenia was observed in mice given TNF, with the relative as well as the absolute number of lymphocytes decreasing from 77 to 21% and from 294 x 10^4 to 62 x 10^4 cell/ml, respectively (210). In anesthetized beagle dogs, however, Tracey, et al. (253) reported a significant drop in white blood cell count within 15 minutes after lethal infusion of TNF; white blood cell count dropped from a control value of 6.0 to 1.6 cells/mm^3 in TNF treated dogs. In human cancer patients, a 50% decrease in lymphocyte concentration was observed 4 hours after TNF injection (31). When TNF was given repeatedly to rats, a marked leukocytosis was observed. Tracey reported that the number of leukocytes increased from a pre-injection value [illegible] 5.8 x 10^{-3} cells/mm^3 to 14.7 x 10^{-3} cells/mm^3 (254).

Concomitant with the decrease in lymphocytes was a marked increase in neutrophils. Ulich, et al. (261) and Remick, et al. (211) reported a transient initial drop in neutrophil number within the first 30 minutes after TNF injection which was followed by a significant increase between 1 and 2 hours post-injection. Remick, et al. (211) reported both a relative and absolute increase in neutrophils: the percentage increased from 21 to 79%, while the absolute number of neutrophils increased from 75×10^4 to 237×10^4 cells/ml (210). Blick reported a significant increase in granulocyte number 4 hours after the administration of TNF: granulocytes increased from a control value of 4.8×10^3 cells/mm^3 to 7.03×10^3 cells/mm^3. However, the neutrophilia was a transient response: neutrophil counts returned back to control values by 24 hours (31,261). The neutrophilia induced by TNF was caused by the demargination of mature PMNs and the influx of immature cells from the bone marrow (210,211).

These studies demonstrated that the hematocrit and white blood cell count changes induced by TNF were dependent on the model used and the time of measurement.

2. Polymorphonuclear neutrophils

PMNs' function in cell-mediated immunity is vital in host defense. TNF increases the bactericidal activity of PMNs through: 1) enhancement of phagocytic activity; 2) increased superoxide anion production; and 3) increased chemotaxis to the site of infection.

One way by which TNF increased the bactericidal activity of PMNs was by augmenting their phagocytic activity. Blanchard, et al. (30) and Shalaby, et al. (230) examined the influence of TNF on the phagocytic activity of PMNs. TNF dose dependently (10-1000 U/ml) enhanced the bactericidal activity of PMNs for *Legionella pneumophilia in vitro* (30). Similarly, TNF pre-treatment, 10,000-50,000 U/rat for 2-4 hours, increased the removal of L. pneumophilia bacteria from infected lungs and subsequently protected 50% of the animals from the lethal effects of L. pneumophilia (30).

A second means by which TNF enhances bactericidal function of PMNs is by augmenting superoxide anion production. Larrick, et al. (140) and Tsujimoto, et al. (260) demonstrated the direct stimulatory effect of TNF on PMN superoxide anion production. Larrick, et al., reported a 2 to 3 fold increase in superoxide production by PMNs after incubation with 0.01-1 μg/ml TNF for 15 minutes. Tsujimoto, et al., reported maximal stimulation of superoxide production after incubation for 90 minutes with 10^{-9} M TNF. Inhibition of intracellular Ca^{++} mobilization significantly suppressed superoxide anion production in response to TNF (260). Others have reported TNF to be either a weak direct stimulus (129) or to have no direct effect (10,20) on superoxide anion production.

In the presence of PMN activators such as zymosan or f-methionyl-leucyl-phenylalanine (FMLP), PMN superoxide production is greatly enhanced by TNF. Klebanoff, et al. (129) observed a 10 fold increase in superoxide production stimulated by unopsonized zymosan plus 50 U of TNF over the amount produced after stimulation with TNF alone (129). Pretreatment with TNF for only 5-10 minutes increased, 0.5 to 18 fold, superoxide anion production stimulated by FMLP (10,20). Atkinson reported that TNF enhanced superoxide anion production in response to FMLP stimulation by changing all the FMLP binding sites to high affinity sites: the total number of receptors remained unchanged but the binding affinity increased (10).

Thirdly, TNF stimulates PMN migration to the sites of inflammation. Ming, et al. (176) demonstrated in vitro the PMN migratory response to a TNF concentration gradient. In the presence of a positive TNF concentration gradient of 10-10^3 U/ml, PMN migration across a polycarbonate filter was significant. Cybulsky, et al. (58) demonstrated a similar response in vivo: intradermal injections of 10^{-11} -10^{-10} moles of TNF/injection site induced significant PMN migration to the injection site within 1-1.5 hours. TNF also increased the adherence properties of PMNs as a means of enhancing their localization to the site of infection. Seow, et al. (229) demonstrated a significant increase in PMN adherence as early as 5 minutes after treatment with 500 U/ml TNF. Gamble, et al. (87) demonstrated enhanced PMN adherence to human umbilical vein endothelial cells (HUVE) after treatment with TNF for as little as 5 minutes. PMNs' rapid response to TNF stimulation is important for the quick mobilization of PMNs to a site of infection.

3. Fibroblasts

TNF significantly influences fibroblast function in a number of ways. For instance, TNF significantly enhances fibroblast proliferation in a dose dependent manner. After screening 39 human and mouse cell lines, Sugarman, et al. (244) demonstrated a dose dependent mitogenic effect of TNF on the human lung fibroblast cell line, WI-38: TNF at doses of 5- 5000 U/ml significantly increased cell number after 48-72 hours of incubation. Vilcek, et al. (265) reported the growth enhancing effect of 10^{-10}-10^{-13} M TNF on the human diploid fibroblast cell line, FS-4. TNF stimulated cell proliferation even in contact inhibited FS-4 cells when 5% fetal calf serum was present in the incubation medium.

Thus, in the presence of additional serum-derived growth factors, TNF's mitogenic activity overrides growth inhibitory conditions.

The mitogenic response resulted from either growth factor or c-fos and c-myc mRNA production by fibroblasts in response to TNF stimulation. For instance, when protein synthesis was inhibited with 0.1 mg/ml cycloheximide, TNF at a dose of 10 ng/ml was cytotoxic for human diploid fibroblasts (128). However, pretreatment for 4 hours with TNF completely protected fibroblasts from the cytotoxicity observed after TNF plus cycloheximide co-treatment. This protective effect may be attributed to two proteins of 36,000 and 42,000 molecular weights that are synthesized in response to TNF (128), although as many as nine proteins were determined after incubation with 1000 U/ml TNF for 24 hours (19). The induction of c-fos and c-myc oncogene expression may be more important in the mitogenic response to TNF stimulation than protein production. Lin and Vilcek (149) reported that incubation with 30 ng/ml TNF for 20 minute induced a transient increase in c-fos and c-myc mRNA expression, with peak concentrations by 30 minutes followed by a rapid decline to control levels by 60 minutes.

TNF also stimulates the synthesis and release of prostaglandin E_2 and collagenase by cultured fibroblasts. Human synovial cells and dermal fibroblasts incubated for 3 days with 3.0 nM TNF maximally produced prostaglandin E_2 and collagenase, as described by Dayer, et al. (61). Normal lung fibroblasts stimulated for 3 days with 20 ng/ml TNF demonstrated an increased synthesis of glycosaminoglycan (69): Elias et al. reported an 159% increase in ^{3}H-glucosamine incorporation into glycosaminoglycan.

TNF not only stimulates production of specific growth factors, but also stimulates production of specific growth factor binding sites. Confluent fibroblasts stimulated with 50-500 ng of TNF for 24 hours produced specific growth factors for colony formation by human bone marrow granulocytes-macrophages (CFU-GM), erythroid (BFU-E), and multipotential progenitor cells (CFU-GEMM) (289). Furthermore, TNF stimulated the production of epidermal growth factor (EGF) binding sites. Palombella, et al. (193) reported a 67% increase in ^{125}I-EGF binding by human diploid fibroblasts after 24 hour incubation with 1 ng/ml TNF (193). TNF treatment increased both the synthesis and maturation of EGF receptor, thereby eliciting a 76% increase in the number of specific EGF binding sites from 25,000 to 40,000 sites/cell (193).

Fibroblasts treated with TNF also are protected from the pathological effects of encephalomyocarditis virus (EMC). Kohase, et al. (130) reported that pretreatment for 24 hours with TNF at doses greater than or equal to 3 ng/ml completely protected FS-4 fibroblasts from the pathological effects of EMC virus. This protective effect, as determined by Kohase, resulted from IFN-β_2 production by TNF stimulated fibroblasts. However, closer examination by Reis, et al. (209) determined that the protective effect resulted from IFN-β rather than IFN-β_2 production by TNF treated fibroblasts.

Lastly, TNF has an immunomodulatory effect on fibroblasts. Collins, et al. (53) demonstrated that human dermal fibroblasts treated with TNF had an enhanced expression of class I major histocompatibility (MHC) antigens. Class I MHC antigen expression increased approximately 9 fold after treatment with 20 U/ml of TNF for 4 days as a result of increased mRNA synthesis. An additional immunoregulatory effect includes the inability of TNF treated fibroblasts to perform an accessory role in the production of IFN-γ by PHA stimulated T cells. Le and Vilcek (141) demonstrated that FS-4 cells pretreated with 1-100 ng/ml TNF for 24 hours failed to perform their accessory role in IFN-γ production by PHA stimulated T cells due, perhaps, to the release of a T cell inhibitor by the TNF-activated fibroblasts.

4. Endothelial cells

One of the functions of TNF is to enhance endothelial cell adhesiveness, thereby increasing the propensity for PMN adherence to endothelial cell surfaces. Gamble, et al. (87) and Pohlman, et al. (205) examined the stimulatory influence of TNF on endothelial cell adhesiveness. Incubation with 100 U/ml TNF for as little as 5 minutes increased PMN adherence to the endothelial cell surface (87). A maximal 25% increase in PMN adherence was achieved after endothelial cells were incubated for 4 hours with 100 U/ml of TNF. TNF enhanced adherence of PMNs to endothelial cells may be by a protein synthesis dependent process (87,205). Pohlman, et al. (205) determined that TNF treatment induced the expression of an endothelial cell surface factor which augmented neutrophil attraction. The function of the TNF induced endothelial cell surface factor was to interact with the neutrophil membrane antigen complex CDW18, thereby increasing the endothelial cell surface adherence for PMNs. This hypothesis prevailed after the observation that PMN adherence to TNF treated endothelial cells decreased 65% after treatment with the monoclonal antibody specfic for the membrane antigen complex CDW18. A similar mechanism is involved in the TNF enhanced leukocyte adherence to endothelial cell surfaces observed by Pober, et al. (203,204). Endothelial cells incubated with 200 U/ml of TNF for 4.5 hours demonstrated a significant increase in antibody H4/18 binding, indicative of the expression of an endothelial cell surface protein which is involved in endothelial cell-leukocyte adherence (203,204).

TNF also increases endothelial cell surface adhesiveness for T and B lymphocytes. Cavender, et al. (43) observed a significant increase in T and B lymphocyte binding to endothelial cells treated with 10 U/ml TNF for 4 hours (43). Experiments with specific monoclonal antibodies suggested that TNF induced endothelial cell-lymphocyte binding is mediated by an endothelial cell surface receptor (43). Pober, et al. (204) established that TNF treatment induced a rapid elevation of the endothelial cell intercellular adhesion molecule, ICAM-1. ICAM-1 expression increased progressively during 24 hour treatment with 100 U/ml TNF and may be the mechanism involved in T lymphocyte binding to endothelial cells (204).

TNF not only influences the adhesiveness of the endothelial cell surface for PMNs and lymphocytes, but TNF also modulates the growth of endothelial cells both in vivo and in vitro. In vivo, TNF had a significant angiogenic effect; doses of 0.5 to 10 μg of TNF induced a significant increase in the vascularization of the rabbit cornea (86). In vitro, however, TNF had a growth inhibitory effect. Endothelial cells incubated with 1 ng/ml TNF for 7 days had a 50% inhibition in cell proliferation (86). Normal cell growth resumed after TNFs' removal from the incubation medium (86). Stolpen, et al. (242) evaluated the morphological changes that accompanied the growth inhibitory effect of

TNF observed *in vitro*. Human umbilical vein endothelial cells treated with 20 to 100 U/ml of TNF for 72 to 96 hours became very elongated and plump. The cells were arranged in whorls and the uniform cell-cell contacts were disrupted, which resulted in areas of overlapping cells. Cytoskeletal changes included parallel arrays of actin filaments rather than dense parallel bands and proteolytic degradation of fibronectin.

TNF is a potent inducer of blood procoagulant activity. Bevilacqua, *et al*. (29) and Nawroth and Stern (186) evaluated the procoagulant activity induced with TNF treatment of cultured endothelial cells. An increase in endothelial cell procoagulant activity was demonstrated as early as 15 minutes after incubation with 100 U/ml TNF, with a 4 to 15 fold rise evident after 4 hours (29). This effect was blocked by treatment with cycloheximide or actinomycin D, which suggested that it was a protein synthesis dependent process (29). Nawroth and Stern attributed the procoagulant activity observed after TNF treatment of endothelial cells to: 1) induction of tissue factor expression on the endothelial cell surface; and 2) depressed thrombomodulin-dependent protein C activated anticoagulant activity.

Growth factor production by endothelial cells is stimulated with TNF. Endothelial cells incubated with 100 U/ml of TNF for 8-24 hours produced the hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor, GM-CSF (32,33). This effect resulted from TNF stimulated GM-CSF mRNA production. Endothelial cells cultured with TNF were demonstrated by Hajjar, *et al*. (98) to produce a smooth muscle cell mitogenic factor. Conditioned medium from endothelial cells cultured for 18 hours with 10 ng/ml of TNF induced a 90% increase in smooth muscle cell proliferation, whereas TNF alone stimulated only a 17% increase in smooth muscle cell mitogensis. Antibodies to platlet-derived growth factor completely abolished the mitogenic activity, suggesting that TNF-stimulated endothelial cells produced a platlet-derived growth factor-like molecule that was responsible for the smooth muscle cell proliferation.

Endothelial cell surface MHC class I antigen expression is induced by TNF. Collins, *et al*. (53) and Wedgewood, *et al*. (278) demonstrated that TNF increased endothelial cell surface MHC class I antigen but not class II antigen expression. Incubation with TNF for 96 hours enhanced HLA-A,B antigens and β_2-microglobulin expression 2 to 9 fold by increasing 100 fold the steady state level of the 1500 base pair transcript for class I MHC heavy chains (53,278). This was a protein synthesis dependent process, for incubation with cycloheximide prevented the increase in MHC class I antigen expression (53). Leeuwenberg, *et al*. (143) postulated that TNF increased endothelial cell MHC class I antigen expression by stimulating IFN-β_1 production. They demonstrated, with antibodies directed against IFN-β_1, that IFN-β_1 was important in stimulating MHC class I expression by endothelial cells. However, Lapierre, *et al*. (139) determined that TNF induced MHC class I antigen expression most likely was not mediated through the production of either IFN-β, IFN-α, or IFN-β_2.

Prostacyclin synthesis by vascular endothelial cells is increased by treatment with TNF, as reported by Kawakami, *et al*. (120). Endothelial cells incubated with 3.4×10^{-9} M TNF for 24 hours produced more than twice the amount of prostacyclin than did unstimulated cells. The maximal prostacyclin secretory rate was observed between 3 and 12 hours after stimulation with 3.4 x 10^{-8} M TNF.

Lastly, TNF stimulates endothelial cells to synthesize and release IL-1. Maximal IL-1 activity is produced by confluent endothelial cells after incubation with 200 pM TNF for 20 hours (185). TNF acted directly on the endothelial cells to stimulate the production and release of IL-1, since the addition of cycloheximide to the incubation medium prevented the response.

5. T and B lymphocytes

TNF has important immunomodulatory effects on both T and B lymphocytes. TNF does not effect resting T lymphocytes (225) but does influence T cells activated with agents such as PHA or specific T cell monoclonal antibodies (97,208,225,285). Scheurich, et al. (225) demonstrated that activated T cells incubated with 100 ng/ml of TNF for 48 hours had an increase Of 136% in HLA-DR expression. A 50% increase in IL-2 receptors on OKT3 activated T cells was observed after 24 hour incubation with 10 ng/ml TNF, thereby enhancing the sensitivity to IL-2 (225).

TNF also enhances the proliferation of activated T cells (97,208, 225,285). Yokota, et al. (285) and Hackett, et al. (97) reported a 100-600% increase in ^{3}H-thymidine incorporation by activated T cells stimulated with TNF for 72-96 hours. TNF increased the number of mitogen activated T cells entering the cell cycle (97). Ranges, et al. (208) reported that the thymocyte proliferatory response to TNF was species specific: recombinant murine TNF, not recombinant human TNF, at concentrations of 78 to 2500 U/ml stimulated murine thymocyte proliferation in response to PHA. These TNF induced responses on activated T cells are related to the significant increase in TNF receptor number, from 300 to 5000 sites/cell, subsequent to T cell activation (225). Additionally, PHA activation of T cells stimulated the production of TNF (208). Moreover, the production of TNF by activated T cells is enhanced by IL-1, IL-2, and IL-4, suggesting that TNF production by activated thymocytes might be involved in the thymocyte proliferation stimulated by IL-1, IL-2, and IL-4 (208).

TNF is also important in cytotoxic T lymphocyte development. Ranges, *et al*. (207) reported that 10^2 U/ml of TNF restored cytotoxic T lymphocyte (CTL) generation in mixed lymphocyte cultures suppressed by transforming growth factor β (TGF-β). Part of the suppressive effect of TGF-β on CTL generation was related to the complete inhibition of TNF generation by mixed lymphocyte cultures incubated with 1 ng/ml TGF-β (207). From the observations of Ranges, *et al*. (207) and Jongeneel, *et al*. (118), TNF was important in CTL development but was not essential.

Not only does TNF significantly influence activated T cells, but TNF also significantly influences activated B cells. Similar to activated T cells, activated B cells were responsive to TNF stimulation as a consequence of the increased number of surface TNF receptors: activated B cells have 6000 TNF receptor sites/cell, compared to quiescent cells which lacked detectable TNF receptors (122). TNF significantly enhanced the B cell immune response to T cell dependent antigens. Jelinek, *et al*. (114) demonstrated that TNF enhanced the generation of immunoglobulin secreting B cells stimulated by *Staphylococcus aureus* in the presence of IL-2. Ghiara, *et al*. (89) reported a similar finding: TNF enhanced the in vivo B cell response to the T cell dependent antigen, sheep red blood cells, but did not enhance the B cell response to the T cell independent antigen, type III pneumo- coccal polysaccharide (89).

TNF also stimulates ^{3}H-thymidine incorporation by activated B cells. In the presence of B cell activators, such as the polyclonal B cell activator and Staphylococcus aureus, TNF enhanced ^{3}H-thymidine incorporation by B cells 4 to 6 fold (114,122). The incorporation of ^{3}H-thymidine by activated B cells was further augmented by co-treatment with IL-2 plus TNF (114,122). Janssen and Kabelitz (113) reported a similar stimulatory effect of TNF on Epstein-Barr virus (EBV) stimulated B cells. TNF induced the rapid proliferation and differentation of a macrophage-free B cell preparation stimulated with EBV.

D. EFFECTS OF TUMOR NECROSIS FACTOR AT THE ORGAN AND TISSUE LEVEL

1. Liver

The liver performs vital roles in protein, glucose, and lipid metabolism. This section will review the literature concerning the effects of TNF on hepatic: 1) amino acid uptake and acute phase protein synthesis; 2) glucose production; and 3) lipid production.

In vivo and in vitro effects of TNF on hepatic amino acid uptake was assessed by Warren, et al. (275) using male rats. TNF at a dose of 1 mg/kg significantly increased hepatic α-amino [1-^{14}C]isobutyric acid (AIB) uptake in vivo: the AIB distribution ratio was elevated 5 fold within 4 hours. However, TNF failed to directly enhance basal AIB uptake in vitro by isolated hepatocytes incubated for 20 hours with TNF at doses of 0.03 to 30 pM. Under stimulatory conditions with 5 nM glucagon, 0.3

pM TNF significantly enhanced glucagon-stimulated AIB uptake from 200% above basal to greater than 300% above basal.

TNF has a direct stimulatory effect on hepatic acute phase protein synthesis both in vivo and in vitro. Six hours after an i.p. injection of 10-10^4 ng TNF/mouse, Sipe et al. (236) observed a dose dependent increase in serum amyloid A (SAA) concentration. Serum amyloid A concentrations increased from a basal value of 2 μg/ml to 80 and 130 μg/ml by 6 and 20 hours, respectively, after the administration of 10^4 ng of TNF. Mortensen, et al. (181) reported a significant increase in serum amyloid P (SAP) concentrations 24 hours after an i.p. injection of TNF: SAP concentrations increased 4 fold after an i.p. injection of 5 x 10^4 U of TNF/mouse (approximately 1 μg). When 7.5 μg of TNF was given to each mouse, a measurable increase in 2 other acute phase proteins was observed 24 hours post-injection; serum C3 and fibrinogen (181). Serum C3 concentration was elevated to 809 μg/ml compared to 747 μg/ml for saline treated controls, and fibrinogen concentrations doubled 24 hours after TNF injection. A significant increase in a fifth acute phase protein, C-reactive protein, was demonstrated 24 hours after the subcutaneous injection of 100-175 μg/m^2 of TNF to cancer patients (276). C-reactive protein concentrations more than doubled, increasing from a pre-injection concentration of 4.4 to 11.6 mg/dl within 24 hours after TNF administration.

Moldawer, et al. (178) examined the effect of chronic TNF administration on hepatic acute phase protein synthesis in vivo. Total liver protein synthesis was significantly elevated to 167 mg/day in mice given daily injections of 0.02 nmoles of TNF for 5 days compared to 136 mg/day produced by freely fed control mice. Chronic TNF treatment significantly increased SAP concentrations approximately 7 fold to 46 μg/ml compared to the SAP concentrations in the control mice. On the contrary, chronic TNF treatment significantly decreased serum albumin concentration to 34 mg/ml compared to 44 mg/ml reported for freely fed control animals.

The in vitro effect of TNF on hepatic acute phase protein synthesis was examined using isolated hepatocytes and hepatoma cell lines. Mortensen, et al. (181) evaluated the direct in vitro effect of TNF on hepatic SAP synthesis using isolated hepatocytes. A three fold increase in SAP synthesis was produced after 24 hour incubation with 10^2-10^4 U/ml TNF. Perlmutter, et al. (199), Darlington, et al. (59) and Mackiewicz, et al. (151) evaluated the effects of TNF on acute phase protein synthesis using human hepatoma cell lines. TNF, incubated with isolated cells for 18 to 48 hours, significantly enhanced the synthesis of the following acute phase proteins; factor B (199), factor C3 (59,199), and ceruloplasmin (151). Perlmutter (199) and Mackiewicz (151) reported a direct inhibition of hepatic albumin synthesis with 100 and 250 ng/ml TNF, respectively, whereas Darlington (59) reported no change in albumin synthesis after treatment with 20-10,000 U/ml TNF. The TNF induced changes in hepatic acute phase protein synthesis reflected the changes in respective mRNA synthesis induced by TNF (199).

The effects of TNF on hepatic glucose metabolism has not been studied as extensively as the effects of TNF on acute phase protein synthesis. Rofe, et al. (212) examined the direct glucoregulatory effects of TNF using isolated hepatocytes. The glycogenolytic capacity of hepatocytes isolated from fed rats was unaltered by treatment with 1.2 nM TNF for 60 minutes. Furthermore, the gluconeogenic ability of hepatocytes isolated from 24 hour fasted rats also was not effected by TNF treatment: incubation with 1.2 nM TNF treatment for 30 minutes did not alter the gluconeogenic response to lactate, alanine, or glycerol.

Thirdly, the effect of TNF on hepatic lipid metabolism was tested. Feingold, et al. (71) observed a stimulatory effect of TNF on hepatic lipogenesis in vivo. Within 16-17 hours after the administration of 0.125 mg/kg TNF, rats demonstrated a significant increase in liver ^3H_2O incorporation into fatty acids (67 fold), cholesterol (2.9 fold), and total non-saponifiable lipids (2.6 fold). Liver slices obtained from rats 16 hours after the administration of 0.125 mg/kg TNF demonstrated similar effects in vitro: acetate incorporation into fatty acids, cholesterol, and total non-saponifiable lipids was increased 97%, 95%, and 74%, respectively (71). Closer examination revealed that TNF enhanced lipogenesis by increasing HMG CoA reductase synthesis (71). TNF had no effect, however, on hepatic fatty acid utilization, triglyceride synthesis, or ketone body production (212).

2. Muscle

TNF significantly influenced 3 aspects of muscle physiology: muscle fiber membrane potential; glucose metabolism; and protein metabolism. TNF triggered muscle membrane depolarization both in vivo and in vitro. Tracey, et al., demonstrated the muscle membrane depolarization activity of TNF both in rats (251) and in dogs (253). Intravenous infusion of 0.6 and 1.8 mg/kg TNF produced a measurable depolarization of hindlimb adductor muscle fibers 1 hour later; membrane potentials decreased from pre-injection values of -95.0 and -93.4 mV to -85.5 and -75.6 mV potentials 1 hour after infusions of 0.6 and 1.8 mg/kg TNF, respectively (251). Infusion of TNF directly into the hindlimb muscle demonstrated significant depolarization within 5-10 minutes, the magnitude and duration of which were dose dependent (253). *In vitro* studies conducted by Tracey, *et al*. (251,252) using rat extensor digitorum longus and soleus muscles supported the hypothesis that TNF acted directly on the muscle fibers *in vivo* to elicit an observable membrane depolarization. In agreement with the *in vivo* studies that infused TNF directly into the hindlimb muscle, TNF dose dependently induced significant muscle fiber membrane depolarization *in vitro* within 5 minutes. The largest change in membrane potential was observed with 10 nM TNF: membrane potential decreased from a pre-injection value of -71.6 mV to -58.6 mV after TNF treatment.

A second area influenced by TNF treatment is muscle glucose metabolism. A significant increase in glucose transport by L6 myotubes was observed after 19 hours of incubation with 0.1-10 μg/ml TNF (142). A 100% increase in ^{3}H-2-deoxy-D-glucose uptake over control values was demonstrated by myotubes incubated with 10 μg/ml TNF. Concomitant with the rise in glucose uptake was a significant increase in lactate production by the TNF treated muscles. On the contrary, Rofe, *et al*. (212) observed no significant increase in lactate production by rat hemidiaphragms treated with 1.2 nM TNF for 2 hours. However, Rofe did demonstrate a 31% increase in glucose oxidation by the TNF treated rat hemidiaphragms.

A less defined, third effect of TNF on muscle physiology concerns muscle protein metabolism. Kettelhut, et al. (126) reported no significant effects on in vitro muscle protein degradation or synthesis using soleus and extensor digitorum longus muscles isolated from TNF pretreated rats. Regardless of the route of administration, intravenous or subcutaneous, pretreatment with 4 mg/kg TNF in vivo did not influence muscle metabolism in vitro 24 hours later. Daily administration of TNF for 5 days also failed to cause any measurable difference in muscle protein metabolism in vitro by muscles isolated 6 hours after the fifth injection (126). Furthermore, TNF at doses of 10^4 to 10^5 U/ml incubated directly with soleus and extensor digitorum longus muscles for 2 hours in vitro also failed to significantly influence muscle protein metabolism (126). A comparable study performed by Rofe, et al. (212) measuring alanine release as an index of rat hemidiaphragm protein degradation, further supports the findings that TNF does not directly influence muscle protein metabolism.

Contrary findings concerning muscle protein metabolism were reported by Warren, et al. (276). Cancer patients given an i.v. injection of 10-100 $\mu g/m^2$ TNF demonstrated a significant net efflux of amino acids from the forearm by 6 hours: total amino acid efflux nearly doubled. More specifically, net alanine and glutamine efflux increased from control values of 105 and 144 nmoles/min/100ml to 278 and 214 nmoles/min/100ml, respectively, 6 hours after TNF injection.

3. Adipose tissue and adipocytes

As discussed earlier in this dissertation, TNF mediates the hypertriglyceridemia observed during chronic infection. Chronic administration of exogenous TNF in vivo induced the same response. Cancer patients administered TNF twice weekly for 4 weeks developed significant hypertriglyceridemia (31). Plasma triglyceride concentrations increased significantly from a pretreatment value of 119 to 125 mg/dl after 4 weeks of treatment. However, the hyperlipemic effects of TNF were also demonstrated much earlier (195). As early as 3.5 hours after an i.v. injection of 250 μg/kg TNF, rats developed a significant hypertriglyceridemia, with triglyceride concentrations elevated to 210 mg/dl from a pre-injection value of 67 mg/dl (195). This was not a transient response since triglyceride concentrations remained elevated for 7 hours (195).

There are several mechanisms responsible for the hyperlipemic effect of TNF. One mechanism is the lipoprotein lipase inhibiting effect of TNF. Semb, et al. (228) evaluated the effects of TNF on lipoprotein lipase (LPL) activity using mice, rats, and guinea pigs. TNF at doses of 10, 20, and 30 μg significantly depressed adipose tissue LPL activity in mice, rats, and guinea pigs at 4, 6, and 15 hours post-injection, respectively. However, TNF significantly increased liver and plasma LPL activity in all 3 animal models (228). The hypothesis that TNF directly suppressed adipose tissue LPL activity in vivo is supported by the in vitro studies of Patton, et al. (196). Using the murine adipocyte cell line, 3T3-L1, a dose dependent inhibition of heparin-releasable LPL activity was exhibited after incubation with 0.01-100 nM TNF fo. 16 hours. Approximately 90% inhibition was demonstrated with 1 nM TNF.

Secondly, TNF induces hyperlipemia through the inhibition of lipid synthesis. Using 3T3-L1 cells, the influence of 0.0001-10nM TNF on ^{3}H-acetate uptake was investigated (196). TNF inhibited ^{3}H-acetate uptake in a dose dependent manner: 80% inhibition of ^{3}H-acetate uptake was induced by treatment with 1 nM TNF for 24 hours. TNF inhibited ^{3}H-acetate incorporation into all classes of lipids, but especially the triglycerides.

Direct inhibition of key lipogenic enzymes is the mechanism responsible for TNF-induced inhibition of lipid synthesis. Pape, et al. (194) determined that TNF directly inhibited the synthesis of an important lipogenic enzyme using the pre-adipocyte cell line, 30A-5. Incubation with 200 U/ml TNF for 8 days directly inhibited acetyl-CoA carboxylase mRNA accumulation by 80% (194). Treatment with TNF also prevented pre-adipocyte differentiation and cellular lipid accumulation as a result of inhibiting lipogenic enzyme synthesis (194,248). Failure to maintain lipid synthesis was observed after incubating mature adipocytes with TNF (248). After 6 days of culture with macrophage conditioned medium containing TNF, only 10% of the mature adipocytes had identifiable lipid droplets; the rest of the cells had lost their stored triglycerides. The resultant loss in lipid droplets was caused by a rapid decrease in the synthesis of key lipogenic enzymes: within 12 to 24 hours after TNF addition, there was a 90% drop in lipogenic enzyme mRNA levels (248).

Thirdly, TNF directly stimulates lipolysis, thereby increasing serum triglyceride levels. Using 3T3-L1 adipocytes, Patton, et al. (196) demonstrated the lipolytic effect of TNF in vitro. TNF at a dose of 1.5 nM directly increased free fatty acid efflux from 3T3-L1 adipocytes within 8 to 24 hours. TNF, however, failed to influence fatty acid efflux from epidydimal fat pads removed from rats pre-treated with 4 mg/kg TNF for 4 hours (126). Furthermore, both epidydimal fat pads (126) and adipocytes (212) isolated from control rats failed to demonstrate a direct effect of TNF on in vitro lipolysis. Neither epidydimal fat pads nor isolated adipocytes demonstrated an increase in glycerol release as a result of incubation with 4 μg/ml or 1.2 nM TNF, respectively (126,212).

4. Organ pathology

Close examination of major organs after in vivo treatment with TNF revealed significant pathophysiological changes. The gastro-intestinal tract was very sensitive to TNF treatment. For instance, Patton (195) observed a 2.5 to 4.5 fold increase in the stomach weights of mice and rats 3.5 hours after the i.v. injection of 0.25 mg/kg TNF. Incomplete gastric emptying, accompanied by swelling and gas retention, was responsible for the increase in stomach weights. Areas of infarction on the cecum were rapidly observed even after sub-lethal doses of TNF (195,249). The small intestines exhibited areas of segmental ischemia, inflammation, and edema after TNF treatment (195,210,249). Microscopic examination demonstrated mononuclear cell and neutrophil accummulation in the lamina propria of villi and intestinal glands (195). Electron microscopic examination demonstrated necrosis of intestinal epithelial cells with significant vacuolization of the cytoplasm (210). The blood vessels were lined by damaged endothelial cells, thus accounting for the increased adherence of mononuclear cells and neutrophils to the endothelial cell surface (195), edema formation, and the accumulation of PMNs and RBCs in the interstitium (210). In some animals, TNF administration resulted in intussusception of the ileocecal valve into the cecum (210).

Assessment of TNF-induced liver damage revealed a significant increase in liver weight compared to controls (88). Histological examination of TNF treated livers demonstrated hepatocyte swelling, narrowing of liver canaliculi, and increased Kupffer cell number (88). After chronic administration of TNF for up to 10 days, Tracey, et al. (254) reported areas of focal parenchymal cell necrosis, extramedullary hematopoiesis, and proliferation of small bile ducts in the portal tracts.

Discrete areas of hemorrhage and scattered hyperemia were observed in the lungs after TNF treatment (249,253). Histological examination of these areas revealed large arteries occluded by thrombi composed primarily of PMNs (249). In rats, significant pulmonary edema occurred, as reflected in the significant increase in lung wet weights from 696 to 941 mg after TNF treatment (88). Tracey (253) reported significant thickening of the alveolar membranes with peribronchiolar pneumonitis and PMN infiltration.

Chronic exposure to TNF resulted in inflammatory cell infiltration into the subendocardium of the heart (254). This was associated with significant hypertrophy and hyperplasia of intimal endothelial cells and subintimal fibroblasts. Focal areas of myocardial cell hypereosinophilia and necrosis were also reported (254).

Changes also were observed in the kidneys, pancreas, and adrenal glands as a result of TNF administration. Kidneys became enlarged and hyperemic, with discrete areas of tubular necrosis and damage to the nephrons (249,253). Focal areas of hemorrhage and inflammation were present in the pancreas (249). Medullary hemorrhagic necrosis as well as prominent inflammatory changes were observed in the adrenal glands after TNF treatment (249,253).

5. Alterations in body weight

Since TNF is responsible for mediating the severe cachexia associated with chronic infection, then repeated administration of TNF should result in severe weight loss. Oliff, _et al_. (191) used an interesting technique to replicate the chronic release of TNF _in vivo_ without administering repeated injections of TNF. Oliff constructed a rodent tumor cell line, CHO/TNF-20, which continuously secreted TNF and inoculated nude mice with the TNF-secreting tumor cells. During the first 14 days, they reported an initial weight gain, after which the mice began to lose weight. By 32 days post-inoculation with the TNF secreting tumor cells, animals had lost an average of 3.4g, or 17.6% of total body weight. Moreover, the TNF treated animals lost all body fat: autopsy revealed no prominent intrascapular, perirenal, or subcutaneous fat. The severe wasting associated with the TNF secreting tumor was associated with a significant drop in food intake.

The suppressed food consumption associated with TNF secretion from the TNF-secreting tumors was substantiated in young and adult rats given repeated injections of TNF (126,254). Within 24 hours after the first TNF injection, a 33% and 60% drop in food consumption were reported for young and adult rats, respectively (126,254). Even though repeated TNF administration induced a 20-30% decrease in food intake, the rate of growth relative to food consumption was similar for both the control and TNF treated rats (126). The weight loss observed in the TNF treated rats was similar to the weight loss observed in the pair-fed controls (254).

There was a significant difference, however, in protein and fat content between the pair-fed controls and the TNF treated rats. Pair-fed control animals demonstrated a drop in percent body fat from 11.9% to 6.0% and no change in protein content. TNF treatment, however, resulted in little fat mobilization, observed as a slight drop in percent body fat from 11.9% to 8.3%, and a significant mobilization of protein, reflected by a decrease in percent protein from 22.7 to 19.8%. Therefore, TNF induced cachexia resulted from suppressed food intake and altered fuel mobilization.

E. FEVER INDUCING PROPERTIES OF TUMOR NECROSIS FACTOR

TNF has thermoregulatory effects in rabbits, rats, mice, and humans. Dinarello, et al. (66) were first to demonstrate the dose-dependent fever-inducing properties of TNF. Using rabbits, a monophasic fever response was elicited after an i.v. injection of 1 μg/kg TNF: peak temperature elevation occurred within 48-54 minutes post-injection and returned back to basal levels by 180 minutes. When the dose of TNF administered was increased 10 fold to 10 μg/kg, a biphasic fever profile was demonstrated (66). The initial peak in temperature, which corresponded to the monophasic peak observed after the 1 μg/kg dose of TNF, was followed by a second peak at 3.5 hours post-injection. Pretreatment with ibuprofen (10 mg/kg) blocked the initial rise in temperature, indicating that it resulted from TNF-induced prostaglandin release. The second fever peak, however, was due to TNF-induced IL-1 release (66).

Thermoregulatory changes induced by TNF administration were dependent not only on the dose but also on the route of administration.

Young rats (60g) given an i.v. injection of 4 mg/kg TNF had a measurable drop in rectal temperature from 36.8 to 34.3°C by 1 hour (125). Just prior to death, i.e. 3-4 hours after injection, rectal temperature had dropped further to 29.5-32°C. However, i.p. or subcutaneous injections of 4 mg/kg TNF resulted in the opposite response (126). A significant hyperthermia was measured in these rats 3 and 4 hours after i.p. and subcutaneous injections, respectively. Three hours after the peak hyperthermic response, temperatures began to fall and returned to control levels within 24 hours.

Contaminating endotoxin in the recombinant TNF preparation was not responsible for the thermoregulatory activity of TNF. Mannel evaluated the thermoregulatory activity of TNF using both ETX-sensitive (C3H/HeN) and ETX-resistant mice (C3H/HeJ) (154,156). Within 5 hours after i.v. injections of 3-4 mg/kg TNF to C3H/HeN and C3H/HeJ there was a significant decrease in body temperatures from 38 to 30°C in both groups of mice. If the hypothermic effect of TNF had resulted from contaminating ETX in the TNF preparation, then the ETX-resistant mice would have failed to demonstrate a significant thermoregulatory effect.

Finally, there is substantial evidence which demonstrated significant febrile responses shortly after TNF administration to humans. Blick, _et al_. (31) and Warren, _et al_. (276) examined the _in vivo_ effects of TNF administered to cancer patients as an anti-neoplastic drug twice weekly for 4 weeks. Regardless of the route of administration, intravenous, intramuscular, or subcutaneous, a significant febrile response was determined. The time to peak temperature elevation, however, varied according to the route of administration: patients that received 10-100

$\mu g/m^2$ TNF i.v. demonstrated peak elevation in body temperature within 1-2 hours whereas 4-8 hours were required after the subcutaneous injection of 100-175 $\mu g/m^2$ TNF (276). Associated with the febrile response were numerous clinical flu-like symptoms such as headache, fatigue, chills, and rigor (31,276).

F. HEMODYNAMIC CHANGES INDUCED BY TUMOR NECROSIS FACTOR

The hemodynamic effects of TNF were examined using beagle dogs (253), rats (13), and cancer patients (31,276). Anesthetized beagle dogs, infused intra-arterially with a non-lethal dose of 10 μg/kg TNF, had a measurable drop in mean arterial blood pressure (MBP) from a pre-injection control value of 145 mmHg to 113 mmHg 1 hour post-infusion (253). In spite of the fall in MBP, there was no measurable change in heart rate. When a non-lethal dose of TNF was infused into conscious rats, a slightly different hemodynamic profile was observed. Bagby, _et al_. (13) reported no measurable changes in MBP and heart rate either during the infusion of 0.15 mg/kg TNF over a period of 3 hours or for 2 hours after completion of the infusion. Similar findings were reported in the clinical trials conducted by Blick, _et al_. (31) and Warren, _et al_. (276): conscious cancer patients administered non-lethal doses of TNF demonstrated no measurable change in mean arterial pressure.

More dramatic hemodynamic changes were observed after highly lethal doses of TNF were administered to beagle dogs (253) and rats (249). Anesthetized beagle dogs infused over 40 minutes with 100 μg/kg TNF demonstrated a rapid drop in mean arterial pressure from 141 to 45 mmHg by 3 hours after completion of the infusion (253). Heart rate, however, was unaltered and remained at an average rate of 166 beats per minute inspite of the significant hypotensive response. Similarly, anesthetized Sprague-Dawley rats infused with a highly lethal dose of 1.8 mg/kg TNF demonstrated a significant 40 mmHg drop in systolic pressure approximately 75 minutes after onset of the infusion (249). This was a transient hypotensive response, for systolic pressure returned to near control values by 120 minutes. Unlike the anesthetized beagle dogs, anesthetized rats demonstrated a significant elevation in heart rate from 450 to 550 beats per minute within the first 60 minutes after onset of TNF infusion.

G. METABOLIC CHANGES INDUCED BY TUMOR NECROSIS FACTOR

1. Plasma glucose

Non-lethal doses of TNF have no effect on plasma glucose concentrations in both human cancer patients and experimental rats. Plasma glucose concentrations remained at control values for as long as 6 hours after the subcutaneous injection of 100-175 $\mu g/m^2$ TNF to human cancer patients (276). Although there was no net change in plasma glucose concentrations for conscious rats infused with a sub-lethal dose of TNF (13), there were measurable changes in the rates of glucose appearance and utilization. Bagby, et al. (13) demonstrated a 25% increase in the rate of plasma glucose appearance during the 3 hour infusion of 0.15 mg/kg TNF. Concomitant with the increased rate of glucose appearance was a 28% increase in glucose utilization (173). Meszaros, et al. (173) determined that the macrophage-rich tissues, such as spleen, liver, and kidneys, were largely responsible for the increased glucose utilization measured after TNF administration (173). Hence, even though there were no measurable changes in plasma glucose concentrations, TNF did increase both glucose production and glucose utilization.

Significant changes in plasma glucose concentrations were demonstrated after lethal doses of TNF. A significant hyperglycemic effect of TNF was determined in 60 g rats as well as adult rats. Kettelhut, et al. (125), using 60 g rats, evaluated the glucoregulatory effects of TNF by changes in venous plasma glucose concentrations from samples taken by jugular vein puncture. TNF significantly increased venous glucose concentrations from 130 mg/dl to 200 mg/dl within 1 hour after the i.v. administration of 4 mg/kg TNF. Tracey, et al. (249), using anesthetized adult rats infused with 0.2-1.8 mg/kg TNF, reported elevated plasma glucose concentrations betweem 15 and 90 minutes after TNF administration. At the later time points after lethal injections of TNF, significant hypoglycemia was observed. Two hours after TNF injection, Kettelhut (125), using 60 g rats, reported a significant drop in venous plasma glucose concentrations to approximately 30 mg/dl. Bauss, et al. (16) reported a similar hypoglycemic response in C3H/HeN mice 6 hours after injection with 3.0 mg/kg TNF. Plasma glucose concentrations, determined for samples taken under ether anesthesia, dropped to approximately 60 % of control values.

2. Plasma lactate

Dose dependent changes in plasma lactate were observed after TNF administration. Non-lethal doses of TNF induced no significant changes in plasma lactate concentrations for as long as 3-5 hours after administration to adult rats (13) or anesthetized beagle dogs (253). In contrast, significant hyperlactacidemia was observed after the administration of lethal doses of TNF. Tracey, et al. (253) reported a 4 fold increase in plasma lactate 3 hours after the infusion of a lethal dose of TNF, 100 μg/kg, to anesthetized beagle dogs: plasma lactate concentrations increased from 0.66 to 2.55 mM by 3 hours. When Tracey, et al. (249) examined the hyperlactacidemia-inducing property of TNF in anesthetized rats, a similar response was observed. Anesthetized rats given a lethal infusion of 1.8 mg/kg TNF demonstrated a 3 to 5 fold increase in plasma lactate concentrations compared to pre-infusion values. A smaller increase was reported for mice injected with a lethal dose of TNF: only a 160% increase in plasma lactate concentrations was determined for samples taken under ether anesthesia within 6 hours after the injection of 3.0 mg/kg TNF (16).

H. NEURO-ENDOCRINE CHANGES INDUCED BY TUMOR NECROSIS FACTOR

1. Insulin and glucagon

The effects induced by TNF on the pancreatic hormones, insulin and glucagon, were examined *in vivo* as well as *in vitro*. The *in vivo* changes in plasma insulin and glucagon concentrations induced by TNF were dose dependent and model dependent. When tested in cancer patients, non-lethal doses of TNF (100-175 $\mu g/m^2$) injected subcutaneously elicited no significant changes in either plasma insulin or glucagon concentrations (276). Plasma insulin and glucagon concentrations were 65 μU/ml and 354 pg/ml, respectively, prior to injection and were not significantly different 6 hours after TNF injection at 43 μU/ml and 429 pg/ml, respectively. Significant changes in plasma insulin as well as glucagon concentrations, however, were reported for studies performed in animal models. Bagby, et al. (13) demonstrated a transient 3 fold increase in plasma insulin concentrations within 1 hour after the onset of a non-lethal infusion of TNF in conscious rats. Plasma insulin concentrations were 30 and 9 μU/ml in TNF and saline treated rats, respectively. Although the plasma insulin concentrations were 3 fold higher in the TNF treated rats compared to the saline controls, the pre-injection insulin concentrations were not determined for either group of rats. Since the real control value for the TNF treated rats is the pre-injection insulin concentration, it is difficult to definitely conclude that TNF induced a hyperinsulinemic response.

Significant dose dependent changes in plasma glucagon concentrations also were induced after TNF administration. Tracey, et al. (253) reported no significant change in plasma glucagon concentrations 3 hours after the infusion of a non-lethal dose of TNF (10 μg/kg) in anesthetized beagle dogs. Although there was a slight elevation in plasma glucagon concentration 3 hours after TNF infusion, the difference was not statistically significant, ie. 179 and 98 pg/ml, respectively. However, a significant rise in plasma glucagon concentration was induced after the administration of a lethal dose of TNF (100 μg/kg) to anesthetized beagle dogs (253). Plasma glucagon concentrations increased from a control value of 64 to 558 pg/ml within 3 hours after a lethal dose of TNF. Similarly, a significant elevation in plasma glucagon concentration was observed in conscious rats infused with a non-lethal dose of TNF. Bagby, et al. (13) reported significantly elevated plasma glucagon concentrations for the TNF infused rats compared to the saline treated rats at 1 and 3 hours after start of the TNF infusion: plasma glucagon concentrations were 530 and 607 pg/ml, respectively, for the TNF treated rats compared to 276 and 287 pg/ml, respectively, for the saline treated rats. The limitation of this study is that Bagby failed to report the pre-infusion glucagon concentrations for both groups of rats, thereby making it difficult to definitely conclude that TNF induced significant hyperglucagonemia.

Direct in vitro effects of TNF on pancreatic hormone secretion were examined using isolated pancreatic Islets of Langerhans. TNF reduced insulin release from isolated rat islets by 40% (18). As reported by Bendtzen, et al. (18), islets incubated with TNF at doses greater than 1 μg/ml for 6 days exhibited a 40% reduction in insulin release. TNF suppressed insulin release without damaging islet integrity (153) or changing insulin content (18).

Profound modulation of pancreatic hormone release was observed after treatment with TNF in combination with IL-1 (152) and IFN-γ (206). TNF, at doses as low as 25 ng/ml, potentiated 5 to 10 fold the inhibitory effect of IL-1 on glucose-stimulated insulin release by cultured islets (152). Moreover, cultured islets pretreated with 2 ng/ml IL-1 prior to incubation with 2.5 ng/ml TNF released 36% less insulin than islets pre-treated with TNF prior to incubation with IL-1 (152). TNF also enhanced the cytotoxic effects of IFN-γ. Pukel, et al. (206) determined that rat islet cell monolayers incubated for 4 days with 25 ng/ml TNF plus 1000 U/ml IFN-γ released more intracellular ^{51}Cr than did cells incubated with IFN-γ alone; 36.8% versus 7.6%, respectively. Even greater islet cell cytotoxicity was demonstrated after treatment with all three mediators; TNF, IL-1 and IFN-γ. Co-treatment with 1000 U/ml IFN-γ plus 25 ng/ml TNF plus 0.39 ng/ml IL-1 resulted in the release of 47% of intracellular ^{51}Cr.

TNF mediates β cell destruction in vivo not only by enhancing the cytotoxic activity of IFN-γ and IL-1, but also by increasing the expression of MHC class I antigens on β cells (37). Campbell, et al. (37) reported that incubation for 6 days with 20 ng/ml TNF increased the expression of MHC class I proteins on human and mouse β cells. An increase in class II MHC antigen expression was observed after co-treatment with IFN-γ (500 U/ml) plus TNF (10 ng/ml).

2. Plasma catecholamines

Changes in plasma epinephrine and norepinephrine concentrations induced by TNF administration were dose dependent. Non-lethal doses of TNF induced either no change (253) or an increase (13) in plasma catecholamines. Tracey, et al. (253) reported no significant changes in plasma epinephrine or norepinephrine concentrations measured in anesthetized beagle dogs after infusion of a non-lethal dose of TNF (10 μg/kg). Bagby, et al. (13), however, demonstrated a significant increase in plasma epinephrine and norepinephrine concentrations after the infusion of a non-lethal dose of TNF (150 μg/kg) in conscious rats. Plasma epinephrine concentration was significantly elevated 1 hour after onset of the TNF infusion relative to the concentration reported for the saline treated rats; 447 vs. 170 pg/ml, respectively. By 3 hours, both plasma epinephrine and norepinephrine concentrations were significantly elevated relative to the saline treated rats: plasma epinephrine and norepinephrine concentrations for the TNF treated rats, 583 and 460 pg/ml, respectively, were significantly greater than the corresponding values determined for the saline treated rats, 167 and 182 pg/ml.

The influence of a highly lethal dose of TNF on plasma catecholamine concentrations was examined by Tracey, et al. (253). Anesthetized beagle dogs infused with 100 μg/kg TNF demonstrated significant elevations in plasma epinephrine and norepinephrine concentrations. Plasma epinephrine increased from a pre-injection value of 58 pg/ml to 1148 pg/ml within 15 minutes after the infusion of TNF. By 3 hours, plasma epinephrine was significantly elevated at 1623 pg/ml relative to the baseline control. Plasma norepinephrine concentrations increased significantly from a baseline value of 282 pg/ml to 1400 and 1982 pg/ml by 15 and 180 minutes, respectively, after TNF infusion.

3. Plasma cortisol

The influence of TNF on a stress and glucoregulatory hormone, cortisol, also was evaluated. Plasma cortisol concentrations are elevated even by non-lethal doses of TNF. As reported by Warren, et al. (276), cancer patients injected subcutaneously with a non-lethal dose of TNF, 100-175 $\mu g/m^2$, had significantly elevated serum cortisol concentrations 6 hours after TNF administration: plasma cortisol increased from 18 to 30 μg/ml. Tracey, et al. (253), however, reported a significant increase in plasma cortisol only after a lethal infusion of TNF in anesthetized beagle dogs. Plasma cortisol increased greater than 3 fold, from 42 to 146 ng/ml, as early as 15 minutes after . lethal infusion of TNF and remained elevated for as long as 3 hours after the infusion.

I. MORTALITY AND MORBIDITY INDUCED WITH TUMOR NECROSIS FACTOR

Lethal, shock-like effects were induced by TNF in mice, rats and dogs. Mice were the most resistant, compared to rats and dogs, to the lethal effects of TNF. The LD_{50} dose for TNF administered as an i.p. bolus injection to adult mice was 300 µg/mouse (approximately 12 mg/kg) for a 48 hour observation period (15). Approximately 10% and 80% mortality rates were observed after subcutaneous and i.p. injections of 8 and 20 mg/kg TNF, respectively, in adult mice (145,214). However, when injected intravenously, mice were more sensitive to the lethal effects of TNF: 67% of the mice died by 24 hours after an i.v. bolus injection of 4.3-4.5 mg/kg TNF (16). Rats, on the other hand, were more sensitive than mice to the lethal effects of TNF. The LD_{50} dose, determined for a 12 hour observation period, was calculated to be 0.7 mg/kg for adult rats given an i.v. bolus injection of TNF (249). Although similar mortality rates of 64% were observed after the i.v. injections of 0.6 and 1.8 mg/kg TNF, the mean survival time was less for the 1.8 mg/kg treated rats (249). One hundred percent of both young and adult rats died after the i.v. injections of 4.0 and 3.6 mg/kg TNF, respectively (125,249). When TNF was administered as an i.v. continuous infusion, no deaths were observed after the 3 hour infusion of 0.15 mg/kg TNF (13). More chronic administration of TNF, as reflected in the 5 day infusion of 0.1 mg/kg TNF/day, produced a mortality rate of 20% during the 5 day study (88). When a comparable dose of TNF was infused into anesthetized beagle dogs, ie. 0.1 mg/kg, all of the dogs died within 3 hours (253), indicating that dogs were more sensitive than rats and mice to the lethal effects of TNF.

Additionally, lethal as well as non-lethal doses of TNF induce clinical signs commonly associated with infections in both animal models and human cancer patients. Clinical signs of morbidity, such as diarrhea, ruffled fur, piloerection, tachypnea, and ocular exudates were observed after TNF administration to mice and rats (16,88,125,145,214, 249). Similarly, clinical flu-like symptoms, such as headache, fatigue, chills, nausea, and rigor, were reported by human cancer patients injected with non-lethal doses of TNF (31,175,276).

J. RATIONALE

Information concerning the role of TNF as a mediator of the pathophysiological changes associated with septic shock has escalated since the demonstration by Beutler, et al. (26) that passive immunization against TNF protected mice from the lethal effects of ETX. Although numerous studies have evaluated and compared the alterations induced by TNF to those associated with endotoxicosis and sepsis (13,16,24,103,125, 126,142,145,156,161,173,174,175,249,250,251,253) a clear dose- and time-related study evaluating the hemodynamic, metabolic, and neuro-endocrine alterations induced by comparably lethal doses of TNF and ETX has not been examined in conscious, unrestrained, cannulated, adult rats. Additionally, the interactions of these two agents to elicit a highly lethal response has not been evaluated with relation to the hemodynamic, metabolic, and endocrine alterations induced after co-treatment with low doses of TNF and ETX. Furthermore, the direct in vitro ability of TNF to act as a glucoregulatory agent has not been examined using the isolated perfused rat liver preparation. Although Rofe, et al. (212) briefly evaluated the direct effect of TNF on isolated hepatocytes, a dose-dependent study has not been performed. Therefore, this study was performed to address specific in vivo as well as in vitro effects of TNF.

CHAPTER III

MATERIALS AND METHODS

A. ANIMALS

Male Holtzman rats weighing approximately 350-400 grams were used for all experiments. Rats were obtained from either the Holtzman Co. (Madison, WI) or Sasco-King (St. Louis, MO). The rats were housed in stainless steel wire mesh cages while maintained in a temperature of 24°C for at least 5 days prior to experimentation on a 12 hour light (0700-1900)/dark cycle. Purina rat chow and tap water were allowed ad libitum throughout the acclimation period. All experiments were initiated between 1000 and 1400 hours.

B. AGENTS

1. Tumor necrosis factor

Recombinant human tumor necrosis factor-α (TNF) used for this study was either donated by Genentech Inc. (courtesy of H. Michael Shepard; South San Francisco, CA) or purchased from Amgen Biologicals (Thousand Oaks, CA). The Genentech TNF was lot #3056-55 with a specific activity of 5.02 x 10^7 units/mg protein, as assessed by lysis of actinomycin D treated L-M cells. This supply of TNF was received as a solution of 0.5 mg TNF/ml of buffer, containing monobasic and dibasic sodium phosphate and sodium chloride, pH=7.0. Endotoxin contamination was determined by Genentech to be less than or equal to 0.125 endotoxin units/ml, which is equivalent to 0.25 endotoxin units/mg of TNF (Limulus Amoebocyte Lysate Assay; 1 endotoxin unit ≈ 0.1 ng of ETX). TNF purchased from Amgen Biologicals was lot #902 with a specific activity of 8×10^6 units/mg protein, as assessed by lysis of mitomycin treated mouse L929 cells. Amgen Biologicals' TNF was received as a solution of 0.3 mg TNF/ml of 40 mM Tris/0.1 M NaCl, pH=8.5. Contaminating endotoxin content was assayed by Amgen and was found to be less than 2.5 endotoxin units/mg protein (Limulus Amoebocyte Lysate Assay).

2. Heat-inactivated tumor necrosis factor

For one set of in vivo experiments, heat-inactivated TNF (H.I.-TNF) was used as a control for the contaminating endotoxin inherent in a recombinant TNF preparation. Heating the TNF solution to 80°C for 60 minutes inactivates the monokine but not the small quantity of contaminating endotoxin. For this reason, changes induced in the H.I.-TNF treated animals were probably the result of the contaminating endotoxin.

3. Endotoxin

Lyophilized Salmonella enteritidis lipopolysaccharide Boivin, lot #733346, was purchased from Difco Laboratories (Detroit, MI). For use in these experiments, the lipopolysaccharide (endotoxin; ETX) was suspended in 0.9% sodium chloride.

C. IN VIVO EXPERIMENTS

1. Cannulation procedure and feeding regimen

For one set of experiments, rats underwent surgery at 1400 hours on the day prior to experimentation. Rats were anesthetized with either 5% Halothane (Halocarbon Laboratory Inc., Hackensack, N.J.) in 100% oxygen until the animal became unconscious, or an i.v. injection of sodium pentobarbital (46 mg/kg). A midline incision was made on the neck and the area was blunt dissected to expose the left carotid artery and the right jugular vein. Heparinized polyethylene tubing (PE-50) was inserted into each vessel and advanced approximately 3 cm to either the ascending aorta aorta for the arterial line or to the junction of the right atrium and superior vena cava for the venous line. Each cannula was tied in place, was tunneled under the skin, and was exteriorized on the back of the neck. Animals were placed in individual cages with water and observed for post-surgical complications. These animals were not provided with food after surgery and so are considered to be in a fasted state for the experiment conducted at 1000 hours the next morning.

For the second set of experiments, cannulae were implanted on the morning of experimentation. _Ad libitum_ fed animals were anesthetized with Halothane and surgery was performed in the same manner as described above. The rats were given 4 hours to recover from surgery before the experiment was begun. Because the surgery was performed on rats that had free access to food, they are considered to be fed animals at the time of experimentation.

2. Experimental procedure for cannulated rats

Prior to the onset of experimentation, each cannula was extended with an additional piece of PE-50 tubing. The arterial line was connected to a saline filled Statham pressure transducer (model number P23) which was used to record pulsatile as well as mean blood pressure, to determine pulse rate, and to obtain arterial blood samples. Extension of the venous line provided a convenient means for i.v. injection of saline, H.I.-TNF, TNF at 0.1, 0.5, or 1.0 mg/kg, ETX at 1.55 or 30 mg/kg, or co-treatment with TNF plus ETX at 0.1 and 1.55 mg/kg, respectively. Before beginning each experiment, rats were acclimated to their environment as determined by normal, steady blood pressures and quiet behavior.

Arterial blood samples were taken prior to and at 30, 60, 90, 120, 180, or 360 minutes after i.v. injection, depending upon which plasma constituents were to be measured in each blood sample. For experiments in which plasma glucose, lactate, insulin, corticosterone, norepinephrine, and epinephrine concentrations were determined, arterial blood samples (1.5 ml) were taken prior to and at 30, 90, 180, and 360 minutes post-injection. Experiments for which only plasma glucose, lactate, and insulin concentrations were measured, samples (0.8 ml) were taken prior to and at 30, 60, 90, and 120 minutes post-injection. Regardless of which sampling schedule was followed, plasma was removed from each blood sample and the red blood cells (RBCs) were resuspended in an equal volume of saline and returned intravenously to each animal. Blood volume depletion and the complications associated with that condition were minimized by handling the animals in this manner.

Upon completion of each experiment, the carotid artery and jugular vein cannulae were resealed and rats were allowed free access to water. For the next 24 hours, these animals were periodically checked to determine survival. Additionally, each animal was surgically checked either at death or after 24 hours to insure that their cannulae were placed in the appropriate location and for any gross organ pathology.

3. Glucan treated animals

Under light ether anesthesia, rats were injected i.v. with saline or 10 mg/kg particulate glucan (lot #1F5500; Fleischmann Laboratories; Stamford, CT) suspended in saline. Animals were returned to our animal research facility and allowed food and water ad libitum. Seventy-two hours later, these rats were anesthetized with ether and injected i.v. with either saline, TNF (0.1 mg/kg), or ETX (1.25 mg/kg) and were monitored to determine 24 hour survival.

D. ISOLATED PERFUSED RAT LIVER EXPERIMENTS

1. Liver perfusion apparatus

Livers were perfused in a liver Perfusion-Aeration Apparatus (Medical Research Apparatus; Clearwater, FL). Krebs bicarbonate buffer (KRB; 118 mM NaCl, 4.7mM KCl, 2.5mM $CaCl_2$-$2H_2O$, 1.2mM KH_2PO_4, 1.2mM $MgSO_4$-$7H_2O$, and 25mM $NaHCO_3$) containing 2 mg/ml bovine albumin (RIA grade; Sigma) was continuously recirculated through the system using a Masterflex peristaltic pump (Cole-Parmer Instrument Co.; Chicago, IL). Total volume of perfusion fluid continuously recirculated through the system was 150 ml. Temperature was maintained at 36-37°C with a heat lamp and heating wires. The perfusate was oxygenated continuously with 95% O_2 / 5% CO_2 by passing over a gas exchange lung. Two lucite filters lined with silk mesh were used to filter the perfusate free of any particulate or fibrous material which the liver may have produced during the perfusion.

Oxygen tension in the perfusion fluid going to and coming from the liver was measured with a YSI model 53 biological oxygen monitor (Yellow Springs Instrument Co., Inc.; Yellow Springs, OH). Glucose and lactate concentrations in the perfusate as a result of net liver production were measured continuously with YSI model 26 continuous glucose and lactate monitors, respectively. Both glucose concentration and oxygen saturation in the perfusate were recorded continuously on a Linear 1202 chart recorder (Linear Instrument Corporation; Reno, NV). A Gilmont spherical float flowmeter (size number 3; Thomas Scientific; Swedesboro, NJ) was used to determine flow rate to the liver. Livers were perfused with an average flow rate of 50 ml/min.

2. Liver removal

Fed rats were injected i.v. with sodium pentobarbital until there was only a slight response to a tail pinch. This insured that the animals were anesthetized adequately without significantly depressing liver blood flow. A midline abdominal incision was made and followed by 2 lateral incisions, one to the right and left side. This provided easy access to the portal area. The intestines were reflected gently to the animal's left side, thus exposing the portal vein and abdominal vena cava. Heparin (500 units/rat) was injected into the abdominal vena cava and was allowed to circulate in the animal for 5 minutes before proceeding with the rest of the surgery. The portal vein was cannulated with PE-260 tubing and flow to the liver established immediately with KRB. Quickly the chest was opened with a midline incision and outflow from the liver was established by cannulating the thoracic vena cava through the right atrium with PE-260 tubing. With KRB rapidly flushing the liver of all RBCs, the liver was excised gently from the animal and was placed in the perfusion chamber within 4 minutes from the time the portal vein was cannulated.

3. Liver perfusion

The excised liver was placed on a glass platform, inflow established, and outflow adjusted such that the liver was not distended but was perfused well. Temperature, O_2 tension, glucose (mg/dl) and lactate (mg/dl) were measured in the perfusate coming from the liver before it returned to the reservoir. The perfusion fluid was mixed in the reservoir prior to being pumped through a lucite filter to the gas exchange lung. After passing over the lung, the perfusate was filtered through a second filter, and was pumped to the liver, constantly measuring flow rate and oxygen tension. The difference between the standing level of perfusate in the glass lung and the level of inflow to the liver was used to determine the pressure head, which averaged 20 cm of H_2O for these experiments.

Oxygen consumption rate by the liver was calculated from the difference in O_2 tension in the perfusate going to and coming from the liver using the formula: μl O_2/min/gm liver = Oxygen amount in solution(μl/ml) x flow rate(ml/min) x difference in O_2 saturation(%)/100 x 1/gm liver wet weight. Net glucose production rate by the liver was calculated in the following manner: μmoles glucose/gm liver wet weight/hour = [Δmg glucose produced in 5 minutes/100 ml] x 150ml x 1000 μmoles/180 mg x 12/hour x 1/gm liver wet weight. Net liver lactate production rate was calculated as follows: μmoles lactate/gm liver wet weight/hour = [Δmg lactate produced in 5 minutes/100 ml] x 150 ml x 1000 μmoles/90 mg x 12/hour x 1/gm liver wet weight.

The initial 30 minutes of perfusion were used as a control period. During this time, net glucose production and oxygen consumption reached a steady state. Perfused livers had to meet the following criteria in order to be included in this study: 1) average basal net glucose production rate $\approx$ 94 μmoles glucose/gm liver wet weight/hour; 2) average basal oxygen consumption rate $\approx$ 57 μl of oxygen/gm liver wet weight/

minute; 3) average flow to the liver ≈ 50 ml/minute; 4) good liver color; and 5) well perfused liver lacking areas of inadequate perfusion or swelling. If any of these criteria were compromised, the liver perfusion was terminated and data not included in the study.

After the control period, either 1 ml of phosphate buffered saline (PBS) or 150 or 300 μg of TNF was added directly to the perfusion reservoir to achieve an initial TNF concentration of 1 and 2 μg/ml. Measurements of net glucose and lactate production, oxygen tension, and flow rate were taken every minute for the subsequent 15 minutes. This was followed by maximal stimulation of liver glucose production with the addition of glucagon (1 μM final concentration; Sigma). As described above, measurements were taken every minute for the next 15 minutes, resulting in a total liver perfusion time of 64 minutes. Upon completion of the perfusion, liver wet weight was determined for use in the calculation of glucose and lactate production rates and oxygen consumption rates.

E. ISOLATED LIVER PARENCHYMAL CELL EXPERIMENTS

1. Isolation procedure

Parenchymal cells were isolated by a modification of the Berry and Friend technique (21). Livers were excised from fed rats and were perfused in a liver Perfusion-Aeration Apparatus as previously described. For these studies, however, the perfusate was a calcium-glucose-free-Hanks buffer (CGFH; pH=7.4; 137mM NaCl, 5.37mM KCl, 0.34mM Na_2HPO_4, 0.44mM KH_2PO4, 4.17mM $NaHCO_3$, 0.41mM $MgSO_4$-$7H_2O$, 0.49mM $MgCl_2$-$6H_2O$) containing 0.05% collagenase (Type 1, Sigma) and 0.10% hyaluronidase

(Type 1, Sigma). Perfusate was oxygenated continuously with 100% O_2 and was recirculated with a minimum flow rate of 40 ml/min.

After approximately 20 minutes, before any obvious breaks in the liver capsule were evident, the liver was removed from the system and placed in an ice cold Nalgene beaker with 50 ml of perfusion fluid. After chilling for 7 minutes, the liver was cut into extremely small pieces, was mashed gently, and was filtered through gauze and silk into 2 ice cold 50 ml Nalgene centrifuge tubes. The resulting cell suspension was centrifuged (50g x 3 minutes) and was washed 3 times with ice cold CGFH. After the final wash, the liver parenchymal cells were resuspended to 40ml final volume in ice cold KRB containing 2 mg/ml bovine albumin. Cells were counted with a hemocytometer and dye exclusion of trypan blue was used as a measure of cell viability, which ranged from 80-95%.

2. Incubation conditions

After determining cell number with the hemocytometer, KRB containing 2 mg/ml albumin was added to the cell suspension to adjust the final cell concentration to 2×10^6 cells/ml. Cells were placed in 25ml siliconized (Sigmacote; Sigma) Erlenmeyer flasks, were gassed with 95% O_2/ 5% CO_2 for 1 minute, and then were incubated at 37°C for 60 minutes in a gently shaking water bath. Effect of TNF on basal glucose production rate was determined by incubating the cells (2.8 ml x 2×10^6 cells/ml) with added saline or added TNF (0.01 to 5 μg/ml final concentration); final incubation volume was 3 ml. In order to ascertain the effect of TNF on hormone stimulated glucose production, cells were incubated with added saline plus glucagon or saline plus vasopressin (1 μM final concentration), or with added TNF plus glucagon or TNF plus vasopressin. At the end of the indicated incubation time, 200 μl of sample was removed from each flask and was used for glucose and lactate measurements.

3. Glucose utilization by isolated hepatocytes

Isolated liver parenchymal cells from fed rats were isolated in the manner previously described and were resuspended in KRB containing 2 mg/ml albumin, 1 mg/ml D-glucose and 0.1 μCi/ml ^{14}C-glucose (Amersham). Cells (5 ml) were incubated for 2 hours at 37°C in siliconized metabolic flasks in the absence or presence of added TNF (0.01 to 5 μg/ml final concentration). After the 2 hour incubation and then the addition of 1 ml of 62.5% citric acid to the incubation media, expired $^{14}CO_2$ was collected on methylbenzethonium hydroxide (Sigma) saturated filter paper in scintillation vials for 60 minutes. After the 60 minute collection period, liquid scintillation fluor (15 ml; 1 liter toluene, 4 gm PPO, 0.1 gm POPOP) was added to each vial. Vials were counted in a liquid scintillation counter for 10 minutes, after a dark adaptation period of 30 minutes.

F. GLUCOSE AND LACTATE MEASUREMENTS

1. Glucose determination

A YSI model 23A glucose monitor (Yellow Springs Instrument Co.; Yellow Springs, OH) was used to determine plasma glucose concentrations and isolated hepatocyte glucose production. For isolated perfused liver experiments, glucose production was determined using a YSI model 26 continuous glucose monitor.

The same operating principles pertain for both machines. The tip of the glucose probe is covered with a 3-layer membrane. The outer layer is a polycarbonate material which is freely permeable to glucose, oxygen, hydrogen peroxide, water and salt. The middle layer contains a thin layer of glutaraldehyde resin in which glucose oxidase is immobilized. The inner layer consists of a cellulose acetate material which is freely permeable only to hydrogen peroxide, oxygen, water, and salt. Hence, glucose in the sample chamber readily passes through the outer membrane layer and reacts with the glucose oxidase to produce gluconic acid and hydrogen peroxide by the following reaction: D-glucose + O_2 ---> gluconic acid + H_2O_2. A portion of the hydrogen peroxide produced diffuses through the inner layer and then is oxidized at the platinum anode: H_2O_2 ---> $2H^+ + O_2 + 2e^-$. Thus, the current produced at the platinum anode is directly proportional to the amount of hydrogen peroxide that was generated and had diffused through the inner membrane.

2. Lactate determination

Lactate concentration (mM) for in vivo and isolated cell experiments were determined with YSI model 23A lactate analyzer. Online measurements of lactate produced during isolated perfused liver experiments were made with a YSI model 26 continuous lactate monitor.

The principles of operation for lactate measurements are similiar to those for glucose determinations, except that a different membrane system was used. In this case, the 3-layer membrane contained an immobilized L-lactate oxidase and flavin dinucleotide (FAD) between the outer and inner membranes. Lactate in the sample chamber readily diffuses through the outer layer and reacts with L-lactate oxidase in the presence of FAD to produce pyruvate and hydrogen peroxide in the following manner: lactate + O_2 ---> pyruvate + H_2O_2. Hydrogen peroxide generated in this reaction diffuses through the inner membrane and is oxidized at the platinum anode. Thus, the current generated is directly proportional to the amount of hydrogen peroxide that is generated and diffuses through the inner membrane.

G. INSULIN MEASUREMENTS

Plasma insulin for in vivo experiments was measured using a double antibody radioimmunoassay (Cambridge Medical Technology Corp.; Billerica, MA). This assay had a sensitivity level of 2.0 μU/ml and an interassay coefficient of variability less than 10%.

H. NOREPINEPHRINE AND EPINEPHRINE MEASUREMENTS

Plasma norepinephrine and epinephrine levels were determined using a radioenzymatic assay (Upjohn Diagnostics; Kalamazoo, MI). A dose of 20 μl of a solution containing EGTA (90 mg/ml) and glutathione (60 mg/ml) was added to each milliliter of whole blood collected. The arterial blood sample was centrifuged, the plasma was removed and then was stored at -40°C until analyzed. This assay had a sensitivity of 2 pg/ 50 μl and an interassay variation of approximately 10%.

I. CORTICOSTERONE MEASUREMENTS

Plasma corticosterone levels were determined using a double antibody radioimmunoassay (Radioassay Systems Lab; Carson City, CA). The sensitivity of this assay was 2.5 ng/tube and an interassay variation of 6.5%.

J. PROTEIN DETERMINATION

For each isolated liver parenchymal cell experiment, samples were taken for protein determination by the Bio-Rad protein assay technique (Bio-Rad; Richmond, CA). Protein samples were diluted 1 to 3 in water and 100 μl quantities were used to measure protein concentration. The color change of Coomassie Brillant Blue G-250 in response to various concentrations of protein was reflected in OD_{595} value changes. Bovine albumin (20-140 μg/tube) was used to generate the standard curve. By linear regression analysis of the bovine albumin standard curve, the protein content of each experimental sample was determined from its corresponding OD_{595} value.

K. STATISTICAL ANALYSIS

Results are reported as mean ± standard error of the mean (mean±SEM). A 2-way ANOVA (treatment, time) with repeated measures followed by post-hoc analysis (Student Newman-Keuls, Tukey's, or Least Significant Difference tests) were performed on the in vivo data. Exact probability tests were performed on the survival data. For the isolated perfused liver experiments, statistical significance was determined by Student's unpaired t-test. A 1-way ANOVA was performed on the isolated liver parenchymal cell data. In each case, significance was accepted at $p \leq 0.05$.

CHAPTER IV

RESULTS

A. IN VIVO EFFECTS OF TUMOR NECROSIS FACTOR

1. Experimental groups

Initial experiments were conducted to evaluate the effects of TNF and ETX in conscious, unrestrained, fasted cannulated rats. The fasted rats were healthy, without any sign of discomfort, before they were arbitrarily assigned to one of the following eight treatment groups: 1) saline; 2) H.I.-TNF; 3) 0.1 mg/kg TNF; 4) 0.5 mg/kg TNF; 5) 1.0 mg/kg TNF; 6) 1.55 mg/kg ETX; 7) 30 mg/kg ETX; or 8) co-treatment with 0.1 mg/kg TNF plus 1.55 mg/kg ETX. In addition, two groups of fed, cannulated, unrestrained conscious rats were treated with either saline or high dose TNF (1.0 mg/kg).

2. Mortality and morbidity induced with TNF, ETX, and TNF plus ETX co-treatment

The mortality rates observed at 4, 6, and 24 hours after an i.v. bolus injection of saline, H.I.-TNF, TNF (0.1, 0.5, or 1.0 mg/kg), ETX (1.55 or 30 mg/kg), or TNF plus ETX co-treatment (0.1 + 1.55 mg/kg, respectively) in fasted rats are presented in table 2. The fractions represent the number of animals that died out of the total number of rats injected for each group. As anticipated, none of the animals in the two control groups died as a consequence of saline or H.I.-TNF injection. They appeared healthy at the onset of the protocol and remained that way for the subsequent 24 hours.

The mortality and morbidity observed after TNF administration were dose dependent (table 2). A low dose of TNF, 0.1 or 0.5 mg/kg, resulted in the death of 1 animal in each group during a 24 hour period. There were no deaths in either group early on, even though some of the animals began to look ill. When fasted rats were given a higher dose of TNF, 1.0 mg/kg, 4 out of 6 rats died within 4 hours. An additional rat died by 6 hours, but no additional rats died in the subsequent 18 hours. When a 1 mg/kg dose of TNF was injected into fed cannulated rats (table 3) a lower mortality rate was observed within the first 6 hours as compared to that observed with fasted cannulated rats given this same dose (table 2): 1 death was observed at 4 hours, 2 deaths were observed by 6 hours. The death rate by 24 hours was 3 out of 5. Rats given TNF manifested ruffled fur, cyanotic extremities, and labored breathing.

Two groups of animals were treated with different doses of ETX in order to compare the mortality and morbidity induced with TNF to that induced with ETX. Animals given a low dose of ETX, 1.55 mg/kg, demonstrated a mortality rate at 24 hours that was identical to the mortality rate observed in rats given a low dose of TNF, 0.1 mg/kg; 1 out of 5 rats died. This death occurred within 3 hours after ETX injection, suggesting that this rat was somewhat more sensitive to ETX than the others, even though there were no observable signs which indicated such a difference. When a much higher dose of ETX was given, 30 mg/kg, 2 deaths occurred within the first 4 hours. By 6 hours, 2 more rats had died, and by 24 hours all the rats had died. These animals demonstrated clinical signs of illness similar to those observed after a 1 mg/kg dose of TNF: their fur was ruffled, extremities were cyanotic, and their breathing was

TABLE 2

TIME AND DOSE DEPENDENT EFFECTS OF TNF- AND ETX-INDUCED LETHALITY IN FASTED CANNULATED RATS

TREATMENT	DOSE(mg/kg)	4 hours	6 hours	24 hours
SALINE	--	0/11	0/11	0/11
H.I.-TNF	--	0/5	0/5	0/5
TNF	0.1	0/6	0/6	1/5
	0.5	0/6	0/6	1/5
	1.0	4/6	5/6	5/6
ETX	1.55	1/5	1/5	1/5
	30	2/6	4/6	6/6
(TNF+ETX)	(0.1+1.55)	6/6*+	-	-

Left carotid artery and right jugular vein cannulae were surgically implanted 16 hours prior to experimentation. Fasted rats were injected i.v. with saline (0.1-0.65 ml), heat-inactivated TNF (H.I.-TNF; 0.65 ml), TNF (0.1, 0.5, or 1 mg/kg), ETX (1.55 or 30 mg/kg), or co-treatment with TNF plus ETX (0.1 + 1.55 mg/kg, respectively). $*p \leq 0.05$, low dose TNF or ETX alone vs. co-treatment with TNF plus ETX; $+p \leq 0.05$, high dose ETX vs. low dose TNF plus ETX co-treatment.

TABLE 3

LETHAL EFFECTS OF HIGH DOSE TNF IN FED CANNULATED RATS

TREATMENT	DOSE(mg/kg)	4 hours	6 hours	24 hours
SALINE	--	0/5	0/5	0/5
TNF	1.0	1/5	2/5	3/5

Left carotid artery and right jugular vein cannulae were implanted in fed rats under 5% Halothane. Animals were allowed 4 hours to recover from surgery before they were given an i.v. bolus injection of saline (0.65ml) or TNF (1.0 mg/kg).

labored. These rats, however, also manifested 2 other clinical signs that were not observed with the TNF treated animals, i.e. porphyrin-tinged ocular discharge and diarrhea.

Additionally, rats were co-treated with low dose TNF plus low dose ETX to evaluate the lethal response. Fasted cannulated rats were given an i.v. bolus injection of 0.1 mg/kg TNF followed immediately with an i.v. bolus injection of 1.55 mg/kg ETX. As reported in table 2, co-treatment with TNF plus ETX resulted in a mortality rate of 100% within 4 hours. Co-treatment with TNF plus ETX was significantly more lethal by 4 hours than a bolus injection of a high dose of ETX (30 mg/kg). Compared to the mortality rate observed by 4 hours after a high dose of TNF (1 mg/kg), co-treatment with TNF plus ETX was just as lethal if not more so. This data suggests that the combined effects of a low dose of TNF (0.1 mg/kg) with a low dose of ETX (1.55 mg/kg) may be more detrimental early on than a highly lethal dose of either agent given alone.

3. TNF- and ETX- induced mortality after glucan pretreatment

It was hypothesized that an agent which increased an animal's sensitivity to the lethal effects of ETX, such as glucan, also may sensitize an animal to the lethal effects of TNF. This hypothesis was tested in the following manner. Fed rats were pretreated with saline or glucan 72 hours prior to the injection of saline, 0.1 mg/kg TNF, or 1.25 mg/kg ETX (table 4). Doses of 0.1 mg/kg TNF and 1.25 mg/kg ETX injected into these rats were sublethal, as indicated by 0% mortality observed for the saline pretreated groups. Glucan pretreatment greatly enhanced the animals' sensitivity to ETX as demonstrated by 7 out of 7 deaths after the injection of 1.25 mg/kg ETX. However, animals pretreated with glucan

TABLE 4

EFFECTS OF GLUCAN PRETREATMENT ON TNF- OR ETX- INDUCED LETHALITY

TREATMENT 1	TREATMENT 2	DOSE (mg/kg)	LETHALITY
SALINE	SALINE	--	0/6
	TNF	0.1	0/6
	ETX	1.25	0/6
GLUCAN	SALINE	--	0/5
	TNF	0.1	0/5
	ETX	1.25	7/7*

Rats were injected i.v. either with saline or 10 mg/kg particulate glucan and returned to their cages with ample food and water. After 72 hours, they were challenged with saline, TNF (0.1 mg/kg), or ETX (1.25 mg/kg) and were observed for 24 hours to determine survival. $^{*}p \leq 0.05$

were not more sensitive to the lethal effects of TNF: 1.25 mg/kg of TNF was a sublethal dose for the glucan pretreated animals as well as the saline pretreated animals. Glucan pretreatment enhanced ETX sensitivity such that a 24x smaller dose given to rats was just as lethal as a 30 mg/kg dose given to non-sensitized rats. However, this was not the case for TNF: a 10x smaller dose of TNF did not have the same lethal effects in glucan pretreated rats (table 4) as a 1.0 mg/kg dose had in normal rats (table 3).

4. Hemodynamic effects of ETX, TNF, and ETX plus TNF co-treatment: mean blood pressure

As a simplified index of overall hemodynamic status, mean blood pressure (MBP) was recorded from the carotid artery cannula of conscious, unrestrained rats. MBP was recorded and compared for the following groups of animals: 1) high dose ETX and high dose TNF treated fasted rats; 2) low dose ETX and low dose TNF treated fasted rats; and 3) low dose ETX plus low dose TNF co-treated fasted rats. High dose TNF was tested also in fed cannulated rats.

ETX administration resulted in significant modifications of MBP in fasted animals. Sixty minutes after a low dose of ETX (1.55 mg/kg), MBP had dropped to 83 mmHg from a pre-injection level of 128 mmHg (figure 3). MBP rebounded by 90 minutes to 98 mmHg, even though it was still significantly lower than the saline control MBP. The MBP for ETX treated rats also was significantly lower than those for the low dose TNF treated animals at 60, 90, and 120 minutes, respectively.

Administration of a high dose of ETX (30 mg/kg) to fasted rats (figure 1) resulted in a significant hypotensive response at 30, 60, and

90 minutes post-injection. MBP dropped to levels of 82, 76, and 88 mmHg by 30, 60, and 90 minutes, respectively, from a pre-injection level of 127 mmHg. These pressures at 30, 60, and 90 minutes also were significantly lower than those observed in the saline and high dose TNF treated groups. By 120 minutes, the ETX-induced hypotension had subsided and the MBP was up to 106 mmHg, same as the saline control values.

Contrary to the effects observed after ETX, treatment with either low (0.1 mg/kg; figure 3) or high (1 mg/kg; figure 1) doses of TNF produced no significant alterations in MBP in fasted rats as compared to the saline controls. MBP for the low dose TNF group averaged 117 mmHg (figure 3) which was consistant with that recorded for the saline control animals. A high dose of TNF given to fasted cannulated rats elicited no observable change in MBP, which averaged 108 mmHg (figure 1). In fed rats, however, a 1 mg/kg dose of TNF resulted in a statistically significant elevation in mean blood pressure to 137 and 131 mmHg at 90 and 120 minutes post-injection, respectively (figure 2). These values were significantly higher than 124 and 115 mmHg observed in the saline control animals at 90 and 120 minutes, respectively. This was a transient increase in MBP; by 210 minutes the TNF treated animals had a MBP identical to the saline controls.

Co-treatment with low dose ETX plus low dose TNF in fasted rats resulted in a significant depression in MBP within 30 minutes (figure 3). The MBP remained significantly depressed relative to the saline control MBP throughout the study. By 30 minutes, co-treatment with ETX plus TNF resulted in a significant drop in MBP to 98 mmHg, which was significantly lower than the saline control value of 118 mmHg. More importantly,

FIGURE 1

EFFECTS OF HIGH DOSE TNF AND ETX ON MEAN BLOOD PRESSURE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=3), heat-inactivated TNF (H.I.-TNF; n=5), 1.0 mg/kg TNF (n=6), or 30 mg/kg ETX (n=6). Values reported are mean blood pressures ± SEM (mmHg) in surviving rats. Mean pressures reported at 240-330 minutes for the TNF treated group are the average of 2 values. The 360 minute value reported for the TNF treated group is the mean blood pressure in the single surviving rat. Pressures reported at 330 and 360 minutes for the ETX treated group represent the means of 2 values. ★:$p \leq 0.05$ treatment vs saline. ☆:$p \leq 0.05$ TNF vs. ETX.

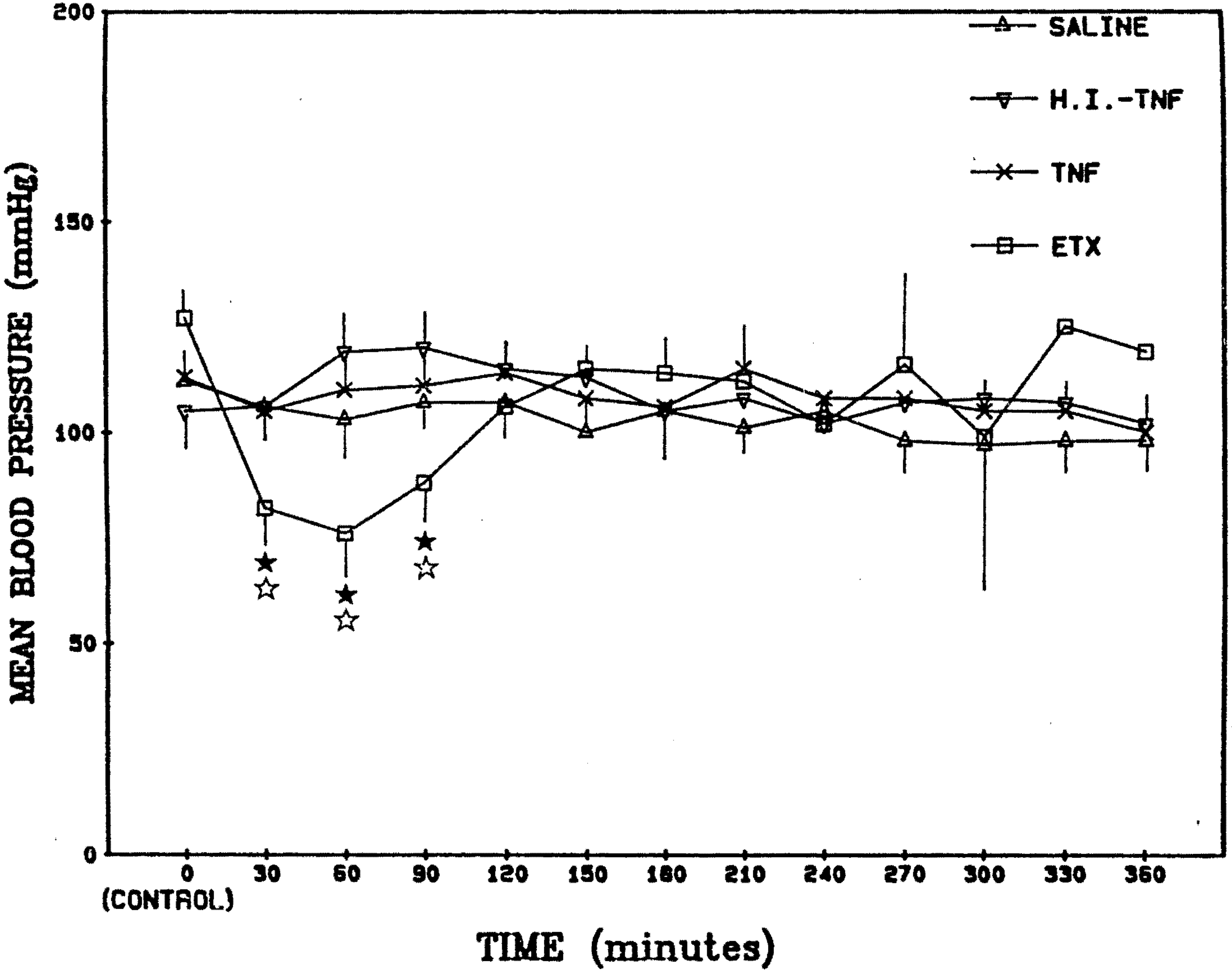

SALINE
H.I.-TNF
TNF
ETX
MEAN BLOOD PRESSURE (mmHg)
200
150
100
50
0
0
(CONTROL)
30
60
90
120
150
180
210
240
270
300
330
360
TIME (minutes)
★
☆

FIGURE 2

EFFECTS OF HIGH DOSE TNF ON MEAN BLOOD PRESSURE IN FED CANNULATED RATS

Rats were given an i.v. bolus injection of saline (n=5) or 1 mg/kg TNF (n=5). Values reported are mean blood pressures ± SEM (mmHg) in surviving rats. ★:$p \leq 0.05$ TNF vs. saline.

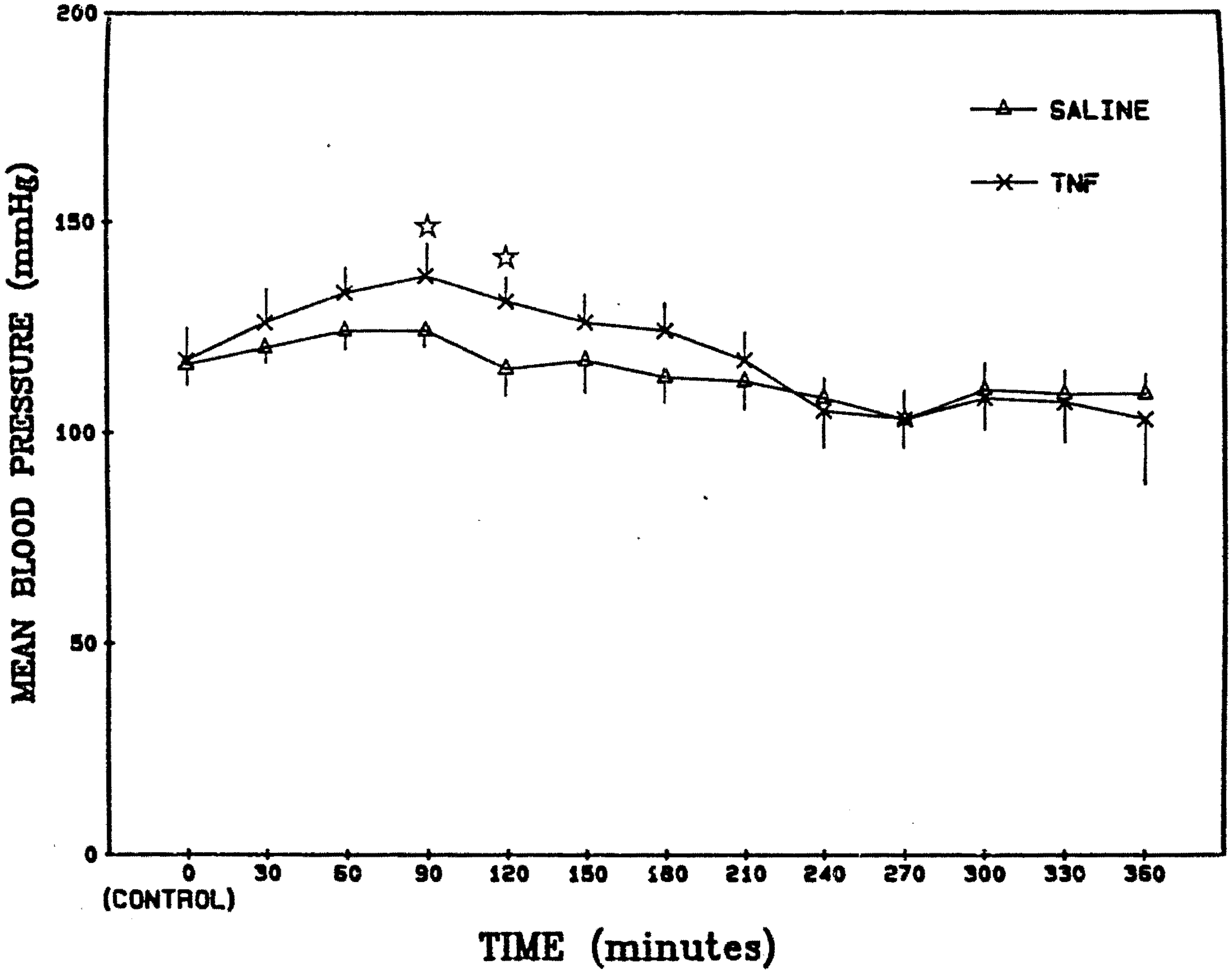
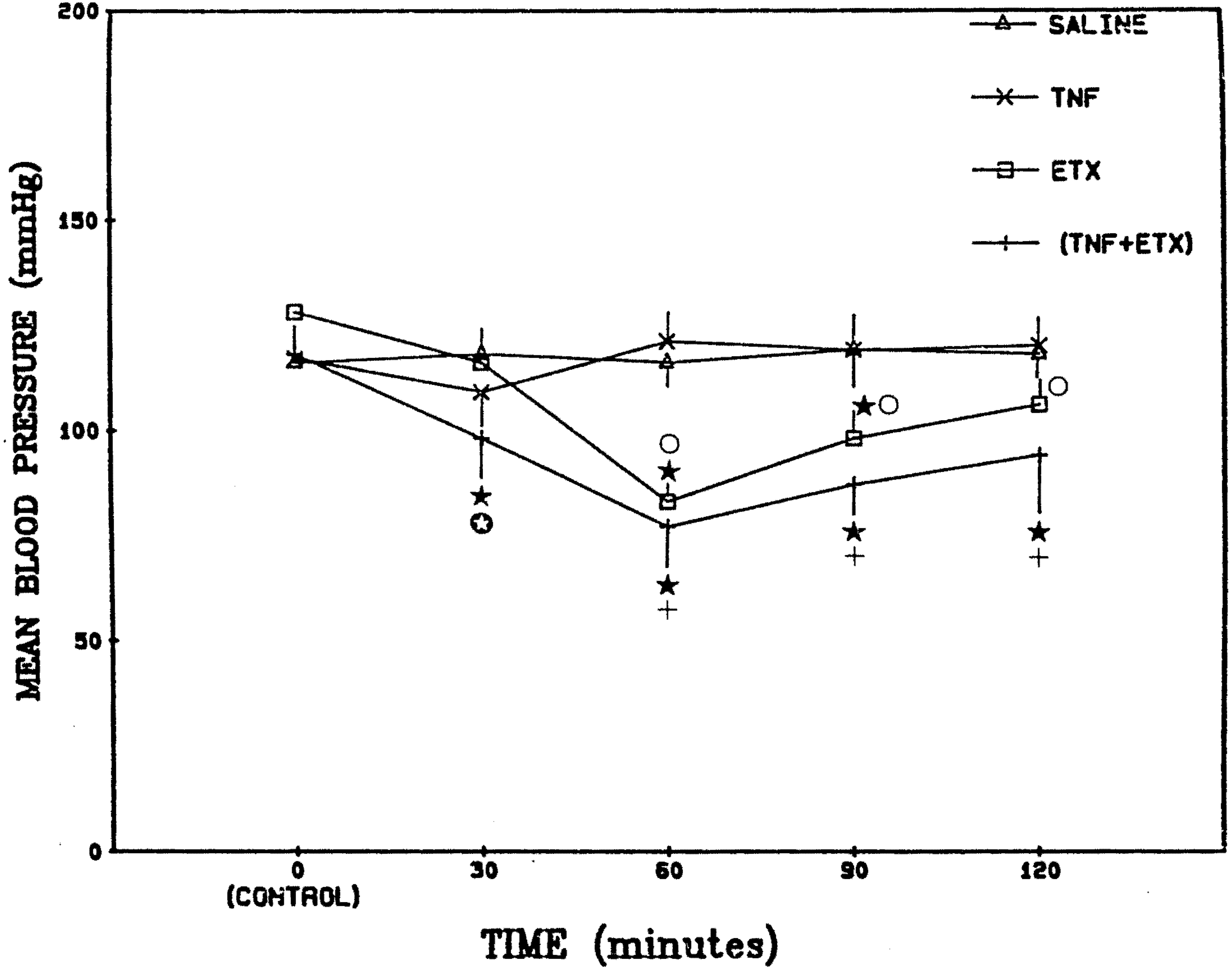

200
150
100
50
0
MEAN BLOOD PRESSURE (mmHg)
SALINE
TNF
0
(CONTROL)
30
60
90
120
150
180
210
240
270
300
330
360
TIME (minutes)

FIGURE 3

EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON MEAN BLOOD PRESSURE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=7), 0.1 mg/kg TNF (n=6), 1.55 mg/kg ETX (n=4), or co-treatment with 0.1 mg/kg TNF plus 1.55 mg/kg ETX (n=6). Values reported are mean blood pressures ± SEM (mmHg) in surviving rats. ★:$p \leq 0.05$ treatment vs saline. ○:$p \leq 0.05$ ETX vs. TNF. ✚:$p \leq 0.05$ TNF plus ETX co-treatment vs. TNF. ✪:$p \leq 0.05$ TNF plus ETX co-treatment vs. ETX.


MEAN BLOOD PRESSURE (mmHg)
200
150
100
50
0
SALINE
TNF
ETX
(TNF+ETX)
0
(CONTROL)
30
60
90
120
TIME (minutes)

co-treatment with ETX plus TNF elicited a drop in MBP at 30 minutes which was significantly lower than the 116 mmHg pressure observed for the low dose ETX (1.55 mg/kg) treated rats. Co-treatment with ETX plus TNF resulted in significantly lower MBP than observed in the low dose TNF treated rats at 60, 90, and 120 minutes. Co-treatment with ETX plus TNF appeared to augment the hypotensive effect of ETX alone at 60, 90 and 120 minutes, even though the pressures were not significantly different statistically. Co-treatment with ETX plus TNF also appeared to result in a slower recovery of MBP than observed after low dose ETX treatment alone.

5. Hemodynamic effects of ETX, TNF, and ETX plus TNF co-treatment: pulse rate

Pulse rates were counted as an indicator of heart rates from the carotid artery pulsatile blood pressure tracings recorded with a Grass polygraph. Pulse rates were counted and compared for the following groups of conscious, unrestrained rats: 1) high dose ETX and high dose TNF treated fasted rats; 2) low dose ETX and low dose TNF treated fasted rats; and 3) low dose ETX and low dose TNF co-treated fasted rats. Additionally, the influence of high dose TNF on pulse rates in fed, conscious rats was evaluated. Saline treated animals for each treatment group maintained fairly steady pulse rates throughout the course of these studies. H.I.-TNF treated rats had a slight but physiologically insignificant elevation in pulse rate (figure 4).

Dose dependent changes in pulse rate were observed with ETX treated rats. A low dose of ETX (1.55 mg/kg) injected into rats resulted in a transient decrease in pulse rate relative to saline control rats by

60 minutes (figure 6). At 90 minutes, the pulse rate in ETX treated animals was 360 ppm compared to 423 ppm in saline control rats. This pulse rate observed in the ETX treated rats at 90 minutes was also significantly lower than the pulse rates of 426 ppm and 415 ppm determined for low dose TNF and ETX plus TNF co-treated rats, respectively. Quite opposite results were observed with rats given a high dose of ETX, ie. 30 mg/kg (figure 4). Pulse rates increased from a pre-injection value of 392 ppm to a peak value of 480 ppm 180 minutes later. Within 30 minutes after the injection of a high dose of ETX, pulse rates in these rats increased to 443 ppm, which was significantly higher than the corresponding pulse rate for saline controls and high dose TNF treated rats, 361 and 404 ppm, respectively. By 90 minutes, the tachycardia induced with ETX was no longer significantly different from that demonstrated after 1 mg/kg TNF.

A low dose of TNF (0.1 mg/kg) produced no distinguishable changes in pulse rate (figure 6). Mean pulse rates remained relatively steady at an average rate of 420 ppm. However, a ten fold greater dose of TNF resulted in significant elevations in pulse rates (figures 4 and 5). The tachycardia induced with high dose TNF in fasted, cannulated rats was evident by 30 minutes and was statistically significant, relative to saline control rates, by 90 minutes after TNF injection. Ninety minutes after TNF (1 mg/kg) injection, the pulse rate was 466 ppm, approximately 100 ppm greater than the saline control rate. The tachycardia increased to 518 ppm by 180 minutes and remained elevated. A similar profile was observed with fed rats given 1 mg/kg TNF (figure 5): TNF-induced elevation in pulse rate was evident at 30 minutes and continued to

FIGURE 4

EFFECTS OF HIGH DOSE TNF AND ETX ON PULSE RATE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=3), heat-inactivated TNF (H.I.-TNF; n=5), 1.0 mg/kg TNF (n=5), or 30 mg/kg ETX (n=6). Values reported are mean pulses per minute ± SEM (ppm) in surviving rats. Values reported at 360 minutes for the TNF and ETX treated groups represent the average of 2 determinations. ★:$p \leq 0.05$ treatment vs. saline. ✪:$p \leq 0.05$ TNF vs. H.I.-TNF. ☆:$p \leq 0.05$ ETX vs. TNF.

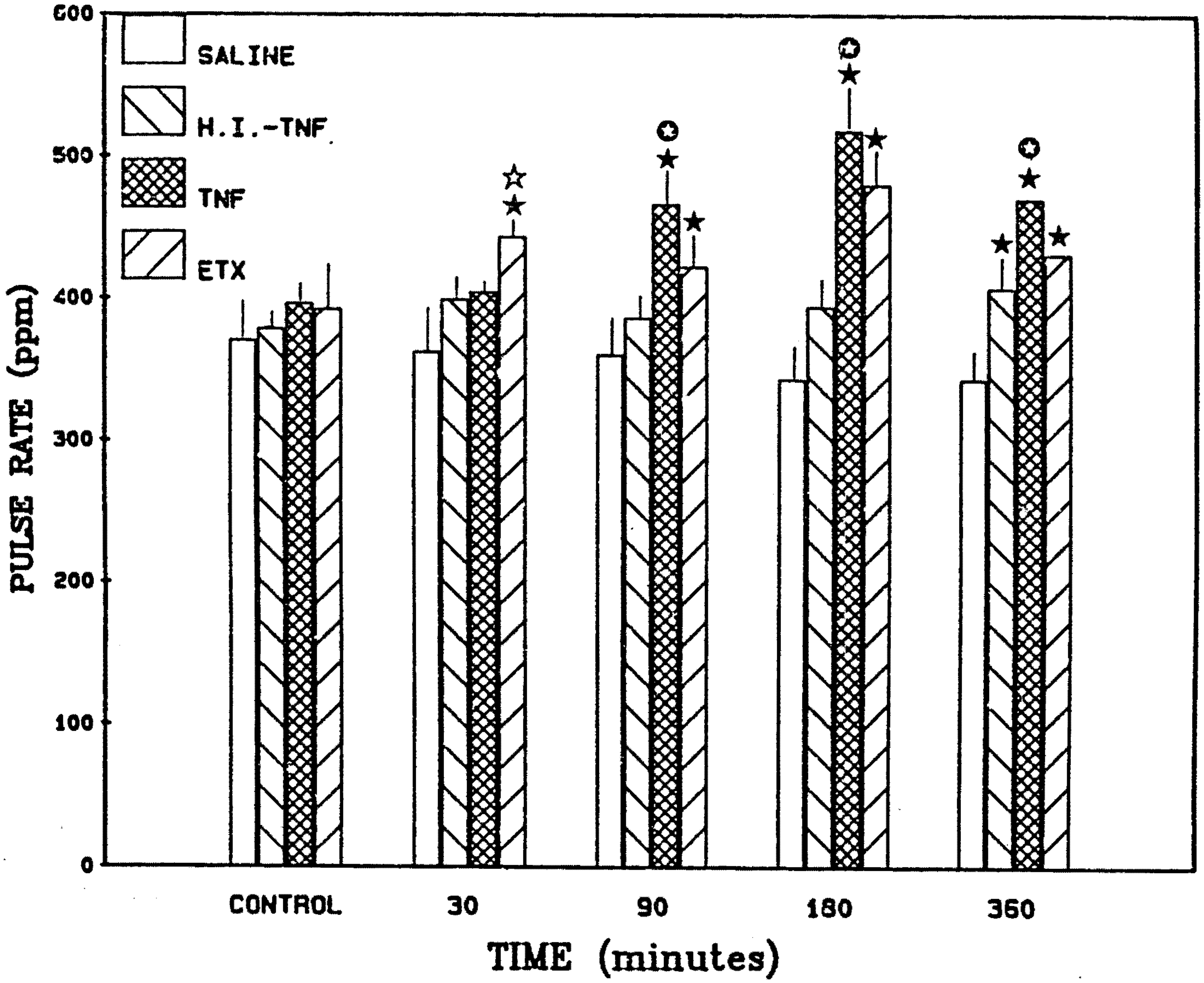

600
500
400
300
200
100
0
PULSE RATE (ppm)
SALINE
H.I.-TNF
TNF
ETX
CONTROL
30
90
180
360
TIME (minutes)

FIGURE 5

EFFECTS OF HIGH DOSE TNF ON PULSE RATE IN FED CANNULATED RATS

Rats were given an i.v. bolus injection of saline (n=5) or 1 mg/kg TNF (n=5). Values reported are mean pulses per minute ± SEM (ppm) measured in surviving rats. ★:$p \leq 0.05$ TNF vs saline.

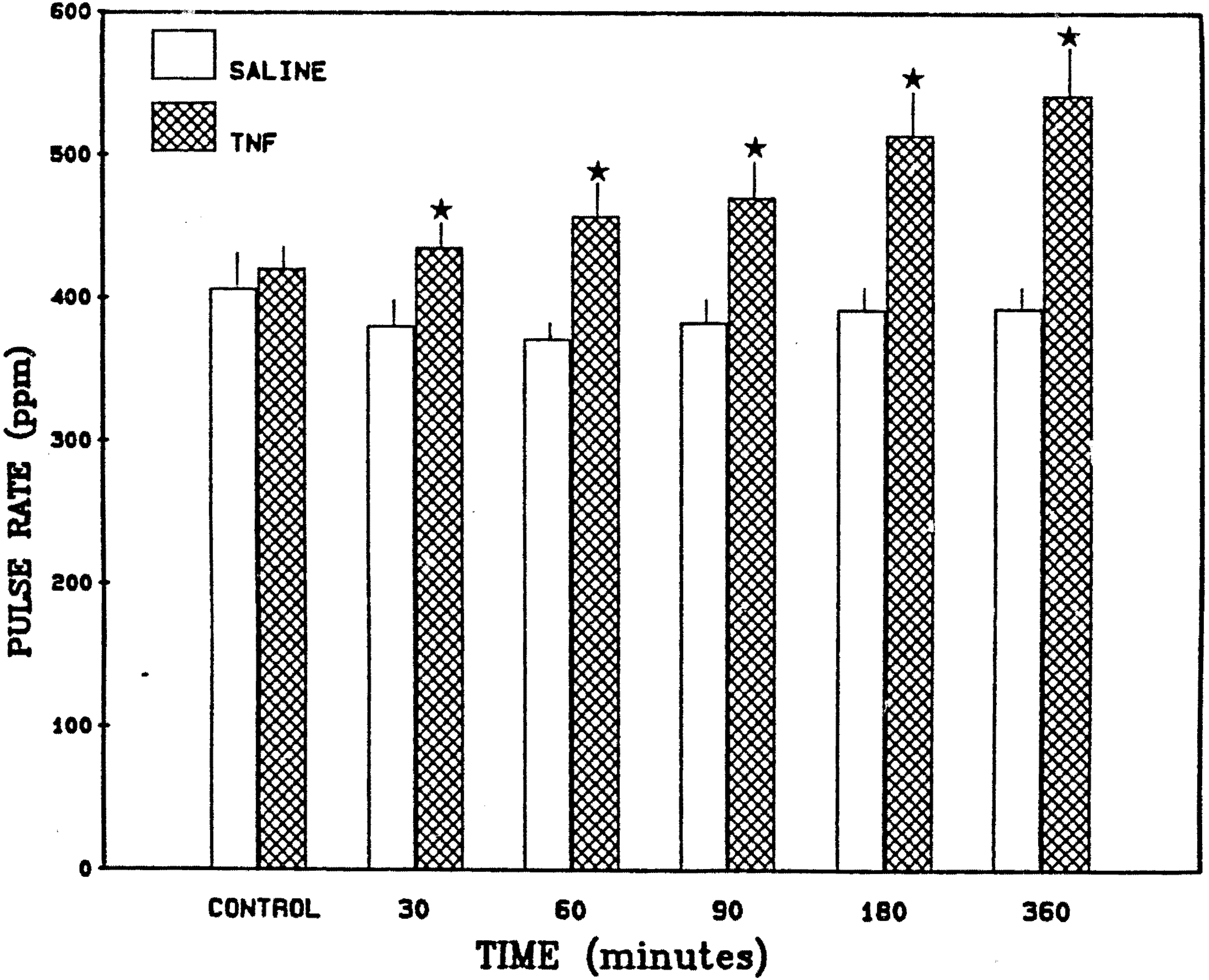
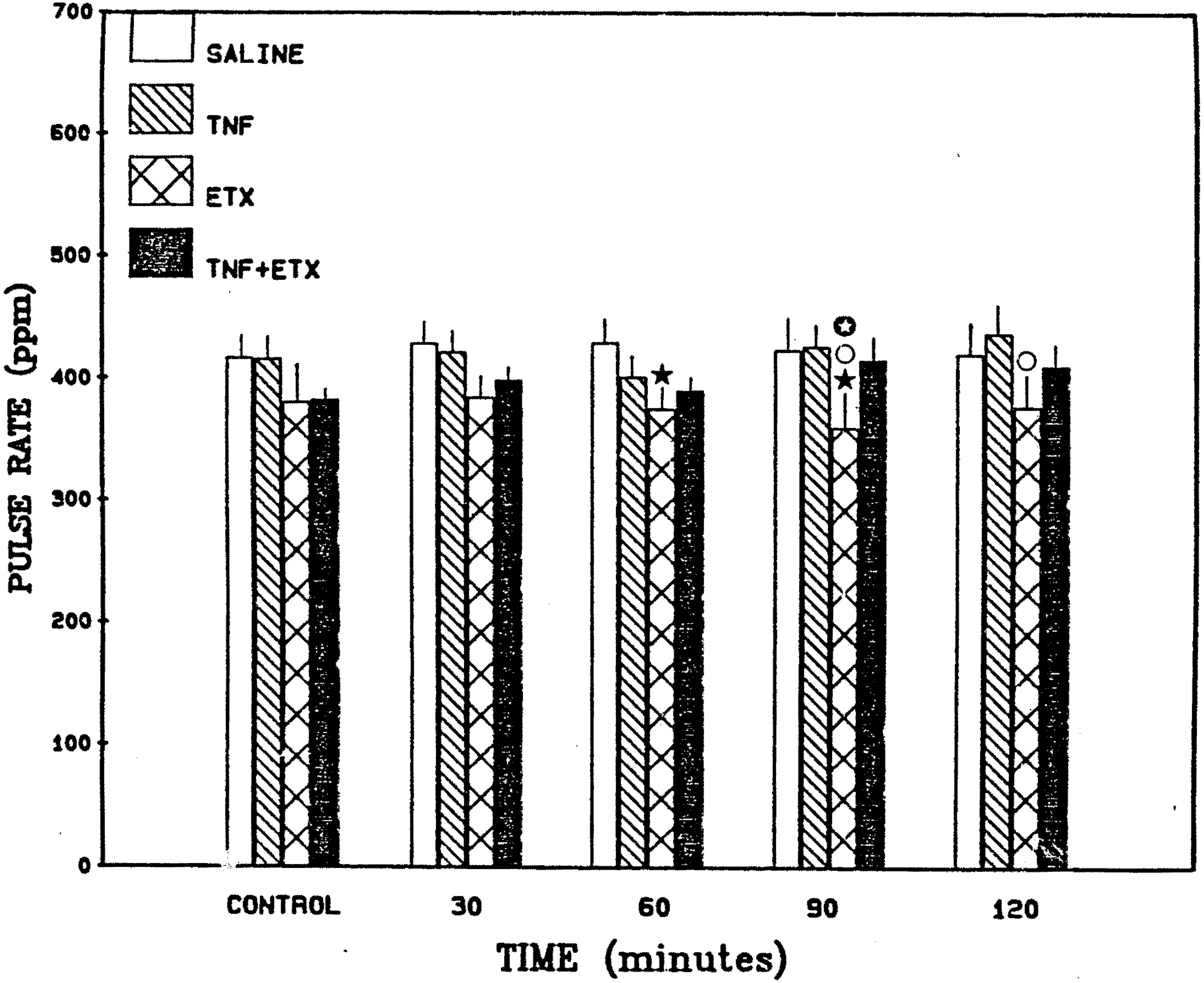

SALINE
TNF
PULSE RATE (ppm)
600
500
400
300
200
100
0
CONTROL
30
60
90
180
360
TIME (minutes)
★

FIGURE 6

EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PULSE RATE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=7), 0.1 mg/kg TNF (n=6), 1.55 mg/kg ETX (n=4), or co-treatment with 0.1 mg/kg TNF plus 1.55 mg/kg ETX (n=6). Values reported are mean pulses per minute ± SEM (ppm) measured in surviving rats. ★:$p \leq 0.05$ treatment vs. saline. ○:$p \leq 0.05$ ETX vs. TNF. ✪:$p \leq 0.05$ ETX vs. TNF plus ETX co-treatment.


700
600
500
400
300
200
100
0
PULSE RATE (ppm)
SALINE
TNF
ETX
TNF+ETX
★
○
CONTROL
30
60
90
120
TIME (minutes)

increase further during the subsequent 6 hours. Pulse rates in the fed rats given TNF increased from a pre-injection rate of 420 ppm to 542 ppm by 6 hours. Resultant TNF-induced tachycardia was not the consequence of contaminating ETX, as demonstrated by the pulse rates reported for H.I.-TNF rats in figure 4. Mean pulse rates determined at 90, 180, and 360 minutes for the high dose TNF treated animals were significantly greater than the pulse rates in H.I.-TNF treated controls.

Co-treatment with low dose ETX plus low dose TNF resulted in neither an increase in pulse rate as observed after a high dose of ETX or TNF alone, nor a decrease in pulse rate as observed after a low dose of ETX alone. There were no significant changes in pulse rates measured in low dose ETX plus low dose TNF co-treated rats relative to both the respective pre-injection rate of 382 ppm and the saline controls (figure 6). It appeared that the bradycardia inducing effect of low dose ETX was obliterated by co-treatment with low dose TNF.

6. Metabolic effects of TNF, ETX, and TNF plus ETX co-treatment: plasma glucose

Arterial blood samples were taken from the indwelling carotid artery cannula of conscious, unrestrained rats in order to assess changes in plasma glucose concentration as a result of the following treatments: 1) high dose TNF and high dose ETX in fasted rats; 2) low dose TNF and low dose ETX in fasted rats; and 3) co-treatment with low dose TNF plus low dose ETX in fasted rats. The glucoregulatory changes induced by high dose TNF were assessed also in fed, conscious, unrestrained rats.

The *in vivo* glucoregulatory effect of TNF was dose dependent. A low dose of TNF (0.1 mg/kg) elicited no measurable changes in plasma glucose concentrations in fasted rats (figure 9). However, a significant glucoregulatory effect was demonstrated with 1 mg/kg TNF in both fasted and fed rats. As illustrated in figure 7, a high dose of TNF induced a slight hyperglycemia, reflected in the increase in fasted plasma glucose concentrations by 90 minutes. Plasma glucose concentration increased to 153 mg/dl by 90 minutes, which was significantly greater than the plasma glucose concentration of 115 mg/dl in the H.I.-TNF group. It was postulated that the hyperglycemia-inducing effect of TNF might be better illustrated in fed rats as opposed to fasted rats. Therefore, fed cannulated rats were challenged with a 1 mg/kg dose of TNF, as illustrated in figure 8. A significant hyperglycemia was measured 60 minutes after a high dose of TNF: plasma glucose concentration increased to 195 mg/dl, a significant elevation above the saline control value of 161 mg/dl.

The decrease in plasma glucose concentrations observed 360 minutes after high dose TNF administration was of a larger magnitude than the initial increase in plasma glucose observed at 90 minutes (figure 7). When fasted rats were injected with 1 mg/kg TNF, plasma glucose levels dropped to 76.3 mg/dl, significantly lower than the 136 mg/dl concentration observed in the saline controls. The glucose concentration reported at 360 minutes for high dose TNF treated fasted rats was the average value obtained from two rats; 133 and 19 mg/dl. The second value, 19 mg/dl, was obtained from a rat in the agonal stages after TNF treatment. Similar degrees of hypoglycemia were observed in the fed rats that died after treatment with 1 mg/kg TNF (data not shown).

As a comparison to the changes induced by TNF, dose and time dependent alterations in plasma glucose concentrations induced by ETX administration are shown in figures 7 and 9. Rats injected with a low dose of ETX (1.55 mg/kg) demonstrated a significant hyperglycemia as early as 30 minutes after injection, which increased further by 60 and 90 minutes (figure 9). Plasma glucose concentrations increased to 201, 273, and 290 mg/dl by 30, 60, and 90 minutes, respectively, after ETX injection. By 120 minutes, plasma glucose concentration dropped to 245 mg/dl. These concentrations were significantly higher than the average glucose concentrations observed for the saline and low dose TNF treated rats, 126 and 141 mg/dl, respectively.

High dose ETX induced a biphasic change in plasma glucose concentrations (figure 7). Plasma glucose concentration almost doubled by 30 minutes after ETX (30 mg/kg) injection, increasing from a pre-injection value of 134 mg/dl to 257 mg/dl. Peak hyperglycemia induced with high dose ETX occurred earlier than with low dose ETX: peak elevation occurred by 30 and 90 minutes after injection of 30 mg/kg and 1.55 mg/kg of ETX, respectively. The hyperglycemia elicited by high dose ETX was followed by significant hypoglycemia at 360 minutes. Plasma glucose concentrations at 360 minutes for the two surviving rats were 69 and 64.5 mg/dl. Analogous to the severe hypoglycemia observed during the agonal phase after high dose TNF administration, high dose ETX induced a severe hypoglycemia in the rats that succumbed to the lethal effects of ETX (data not shown).

Co-treatment with TNF plus ETX induced changes in plasma glucose concentration which closely parallel the changes in plasma glucose

FIGURE 7

EFFECTS OF HIGH DOSE TNF AND ETX ON PLASMA GLUCOSE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=4), heat-inactivated TNF (H.I.-TNF; n=5), 1.0 mg/kg TNF (n=6), or 30 mg/kg ETX (n=6). Values reported are mean plasma glucose concentrations ± SEM (mg/dl) in surviving rats. Values reported at 360 minutes for the TNF and ETX treated group represent the average of 2 determinations.

★:$p \leq 0.05$ treatment vs saline. ✪:$p \leq 0.05$ TNF vs. H.I.-TNF.

☆:$p \leq 0.05$ ETX vs. TNF.

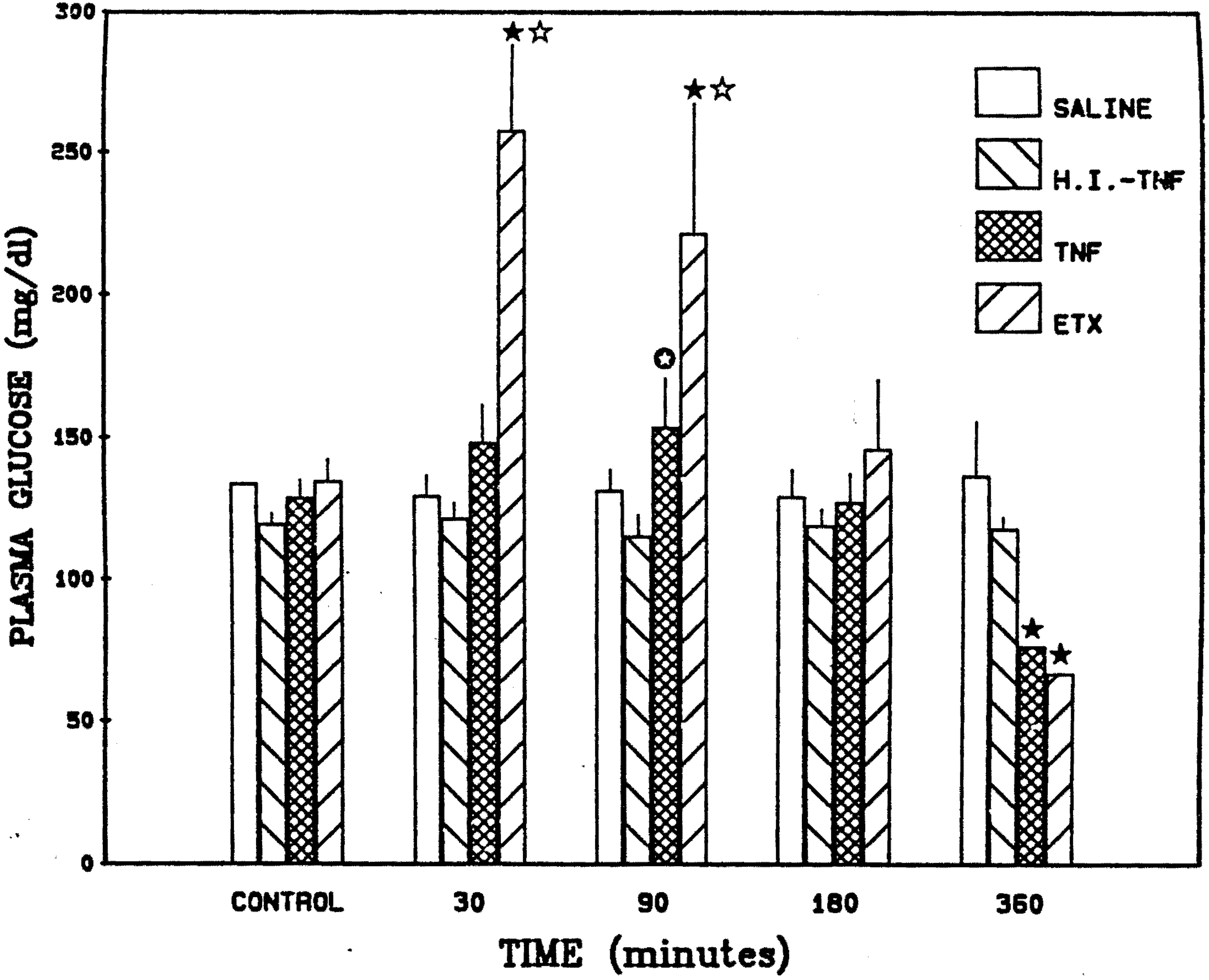

PLASMA GLUCOSE (mg/dl)
300
250
200
150
100
50
0
CONTROL
30
90
180
360
TIME (minutes)
SALINE
H.I.-TNF
TNF
ETX

FIGURE 8

EFFECTS OF HIGH DOSE TNF ON PLASMA GLUCOSE IN FED CANNULATED RATS

Rats were injected i.v. with saline (n=5) or 1 mg/kg TNF (n=5). Values reported are mean plasma glucose concentrations ± SEM (mg/dl) in surviving rats. ★:$p \leq 0.05$ TNF vs. saline.

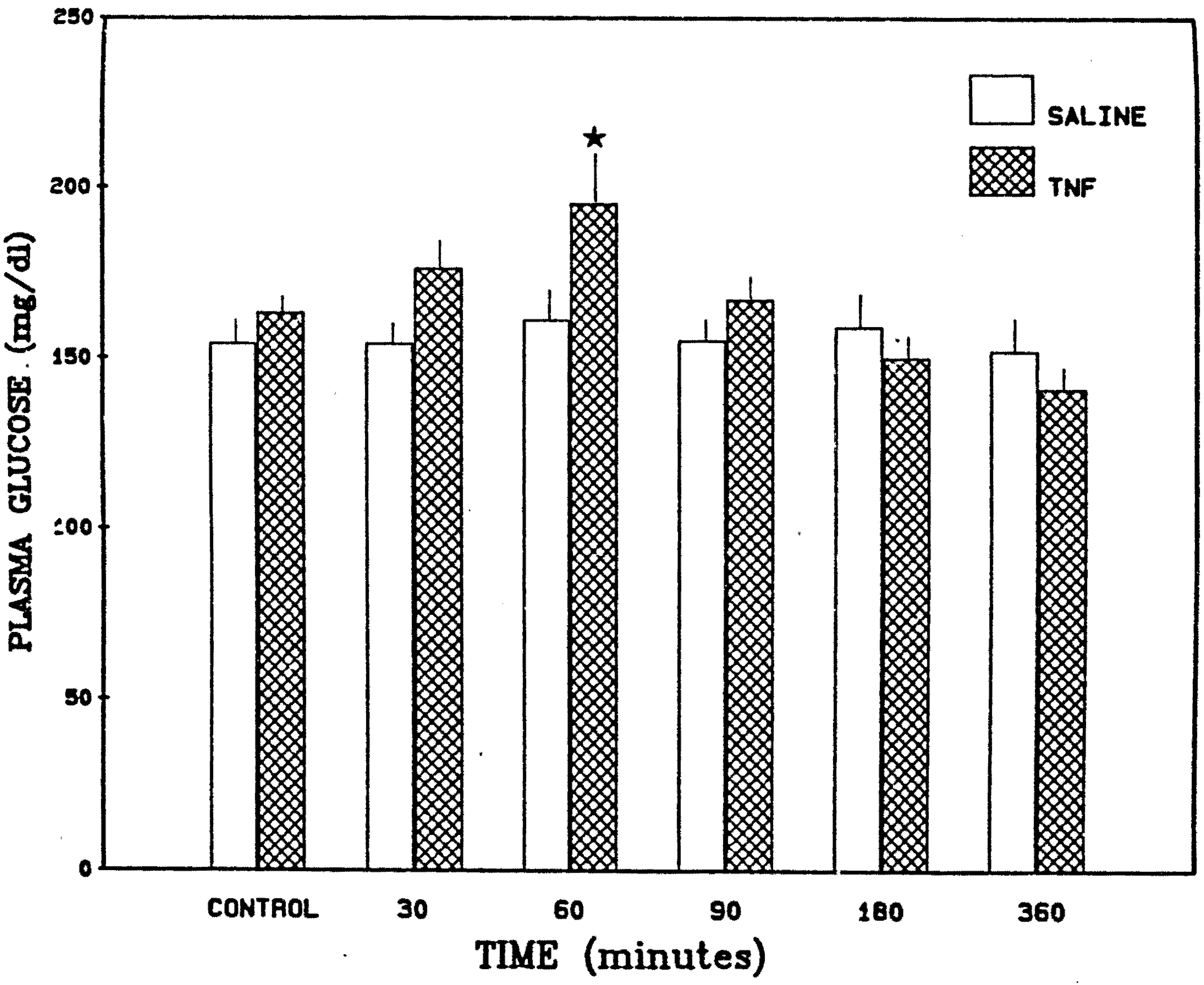
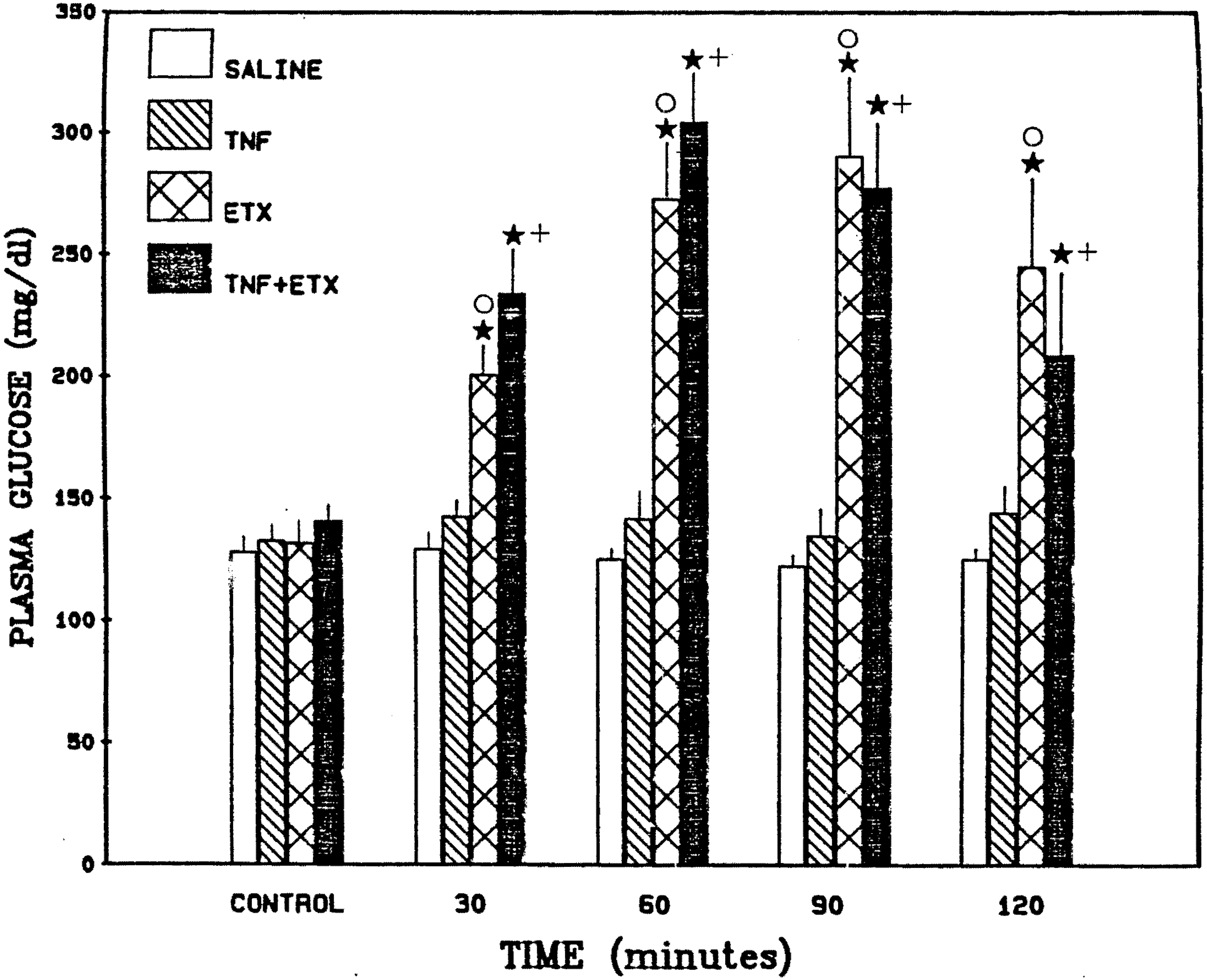

250
200
150
100
50
0
PLASMA GLUCOSE (mg/dl)
SALINE
TNF
★
CONTROL
30
60
90
180
360
TIME (minutes)

FIGURE 9

EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PLASMA GLUCOSE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=7), 0.1 mg/kg TNF (n=6), 1.55 mg/kg ETX (n=5), or co-treatment with 0.1 mg/kg TNF plus 1.55 mg/kg ETX (n=7). Values reported are mean plasma glucose concentrations ± SEM (mg/dl) in surviving rats. ★:$p \leq 0.05$ treatment vs saline. ○:$p \leq 0.05$ ETX vs. TNF. ✚:$p \leq 0.05$ TNF plus ETX co-treatment vs. TNF.


350
300
250
200
150
100
50
0
PLASMA GLUCOSE (mg/dl)
SALINE
TNF
ETX
TNF+ETX
CONTROL
30
60
90
120
TIME (minutes)
○
★
★+

observed after a low dose of ETX alone (figure 9). A significant hyperglycemia was observed as early as 30 minutes after TNF plus ETX co-treatment. The plasma glucose concentration reported at 30 minutes for the TNF plus ETX co-treated group, ie. 234 mg/dl, was higher than the 201 mg/dl concentration determined for the low dose ETX treated group, even though the difference was not statistically significant. Peak plasma glucose concentrations occurred 60 minutes after co-treatment; plasma glucose increased from a pre-injection concentration of 141 mg/dl to 304 mg/dl by 60 minutes. Plasma glucose concentrations at 90 and 120 minutes, 277 and 209 mg/dl respectively, indicated that the hyperglycemia induced with TNF plus ETX co-treatment was transient. Just as observed during the agonal phases after high dose ETX or high dose TNF treatment, rats that died after co-treatment with TNF plus ETX had plasma glucose concentrations as low as 19 mg/dl.

7. Metabolic effects of TNF, ETX, and TNF plus ETX co-treatment: plasma lactate

Changes in arterial plasma lactate concentrations of conscious, unrestrained, cannulated rats were determined for the following treatment groups: 1) high dose TNF and high dose ETX in fasted rats; 2) low dose TNF and low dose ETX in fasted rats; 3) co-treatment with low dose TNF plus low dose ETX in fasted rats. Additionally, the effect of high dose TNF on plasma lactate concentration was examined in fed, conscious, unrestrained, cannulated rats.

Alterations in plasma lactate concentration induced by TNF were dose dependent. A low dose of TNF, 0.1 mg/kg, resulted in an insignificant increase in plasma lactate concentration from 0.65 to 1.07 mM by

120 minutes post-injection (figure 12). However, a high dose of TNF (1 mg/kg) induced a significant increase in plasma lactate concentration in fasted cannulated rats. By 90 minutes post-injection, plasma lactate had increased from a pre-injection value of 0.63 to 1.7 mM (figure 10). Plasma lactate concentrations continued to increase, so that by 180 minutes, plasma lactate concentrations were elevated to 3.79 mM, which was significantly higher than the corresponding lactate concentrations in saline and H.I.-TNF treated rats. At 360 minutes, the plasma lactate concentration reported for the high dose TNF treated rats was an average obtained from two rats; 7.85 mM for the dying animal and 0.9 mM for the 24 hour survivor.

Significant hyperlactacidemia was elicited also in fed rats administered a high dose of TNF. As shown in figure 11, a significant hyperlactacidemia was demonstrated as early as 30 minutes after a 1 mg/kg dose of TNF. Plasma lactate increased from a pre-injection value of 0.87 to 1.49 mM by 30 minutes. Plasma lactate concentration continued to increase and reached 2.30 mM by 360 minutes post-injection. The significant hyperlactacidemia produced after a high dose of TNF in fed rats was not as severe as the hyperlactacidemia produced in fasted rats.

Rats injected with ETX demonstrated similar changes in plasma lactate concentrations as described for TNF treated animals. Low dose ETX induced a statistically significant increase in plasma lactate relative to saline treated rats by 60 minutes (figure 12). Lactate concentrations increased from a pre-injection value of 0.58 mM to 2.6 mM by 60 minutes. Plasma lactate concentrations continued to increase to 3.64 mM by 90 minutes and then remained elevated. High dose ETX administration elicited an even greater hyperlactacidemia than that observed after low dose ETX (figure 10). Plasma lactate concentrations increased significantly from a pre-injection value of 0.58 to 4.35 mM by 30 minutes post-injection and continued to increase to 5.43 mM by 360 minutes. The plasma lactate concentration reported at 360 minutes was the average of 2 determinations; 5.65 and 5.20 mM. Peak hyperlactacidemia elicited after a high dose of ETX was similar to that produced after a high dose of TNF at 180 and 360 minutes. However, the initial increase in plasma lactate occurred earlier and to a greater degree after a high dose of ETX than after a high dose of TNF.

Co-treatment with low dose TNF plus low dose ETX resulted in a greater hyperlactacidemia than that reported after high dose ETX or high dose TNF alone. Plasma lactate increased from a pre-injection concentration of 0.73 mM to 1.87 mM by 30 minutes after co-treatment with low dose TNF plus ETX. The elevation in plasma lactate produced by TNF plus ETX co-treatment was significantly greater than the plasma lactate concentrations observed in saline control and low dose TNF treated rats at 30 minutes post-injection. Although the initial increase in plasma lactate reported after co-treatment with low dose TNF plus ETX was not significantly different from that observed after a low dose of ETX alone, by 90 minutes the increase in lactate observed after TNF plus ETX co-treatment was significantly greater than that observed after low dose ETX. Plasma lactate concentration increased to 8.72 mM by 120 minutes after TNF plus ETX co-treatment, which was higher than the values reported for saline controls, low dose TNF, low dose ETX, and the peak values reported after treatment with high dose TNF or ETX.

FIGURE 10

EFFECTS OF HIGH DOSE TNF AND ETX ON PLASMA LACTATE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=4), heat-inactivated TNF (H.I.-TNF; n=5), 1.0 mg/kg TNF (n=6), or 30 mg/kg ETX (n=6). Values reported are mean plasma lactate concentrations ± SEM (mM) in surviving rats. Plasma lactate concentrations reported at 360 minutes for the TNF and ETX treated groups represent the average of 2 values.

★:$p \leq 0.05$ treatment vs saline. ✪:$p \leq 0.05$ TNF vs. H.I.-TNF.

☆:$p \leq 0.05$ ETX vs. TNF.

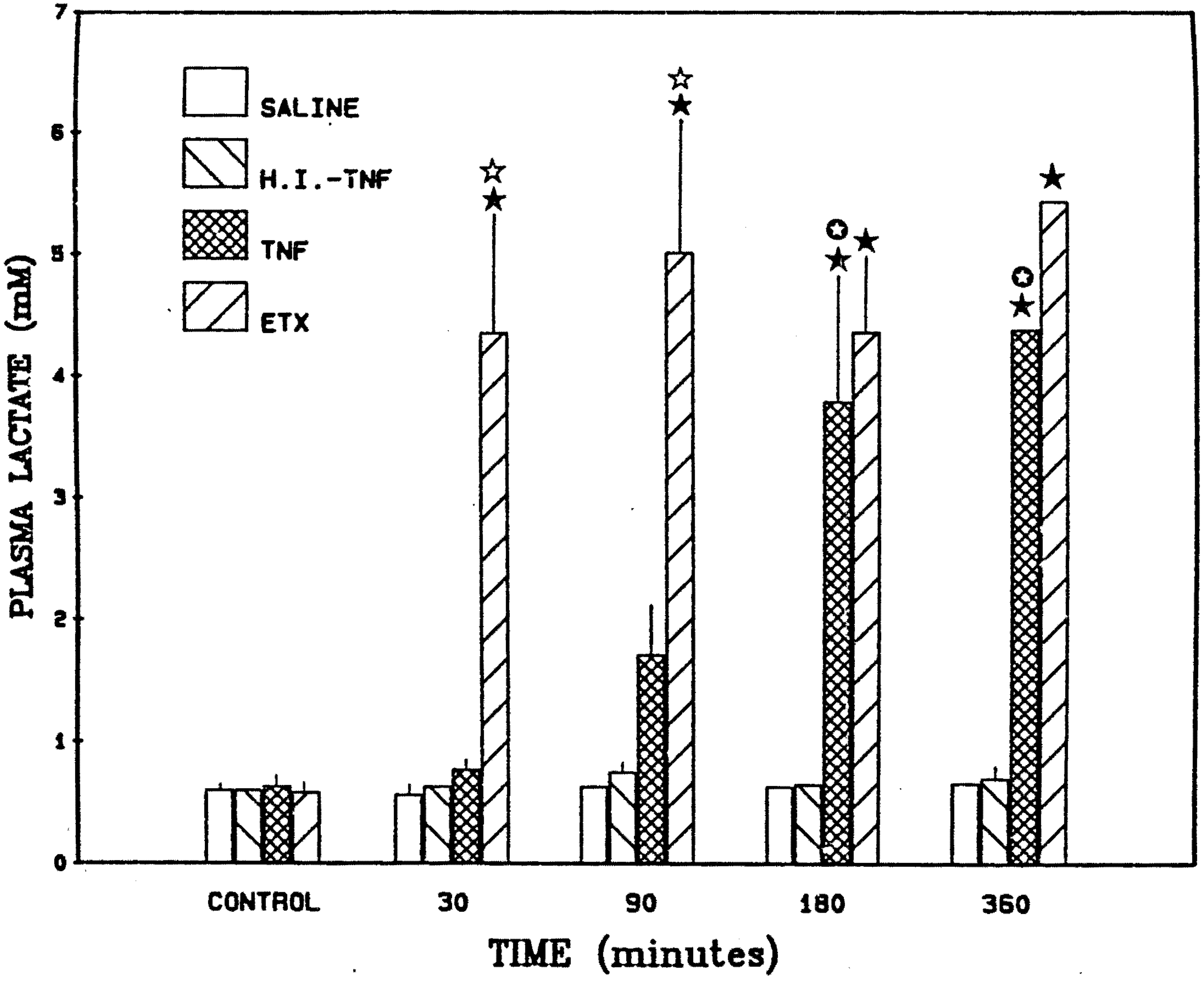
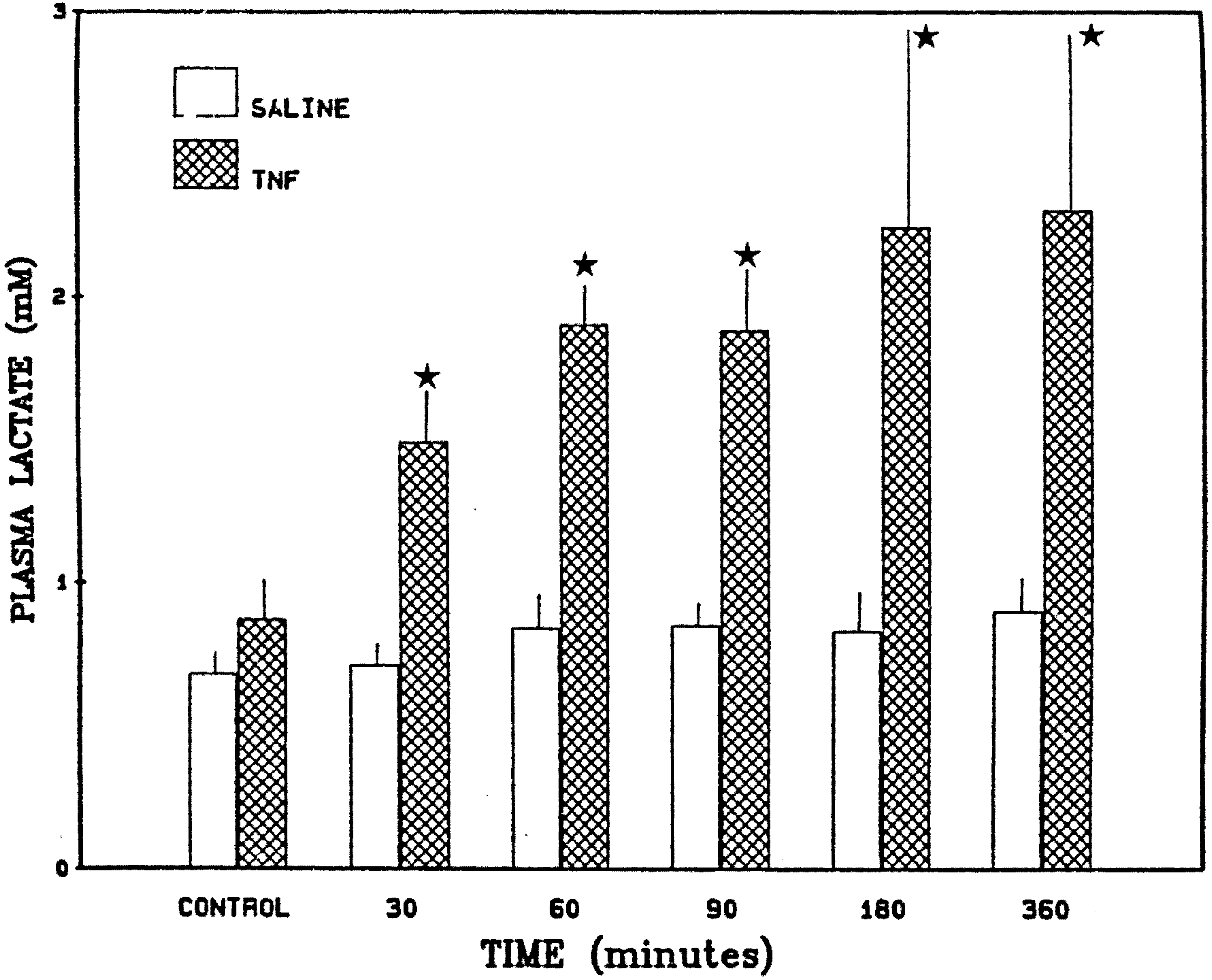

SALINE
H.I.-TNF
TNF
ETX
PLASMA LACTATE (mM)
0
1
2
3
4
5
6
7
CONTROL
30
90
180
360
TIME (minutes)

FIGURE 11

EFFECTS OF HIGH DOSE TNF ON PLASMA LACTATE IN FED CANNULATED RATS

Rats were given an i.v. bolus injection of saline (n=5) or 1 mg/kg TNF (n=5). Values are mean plasma lactate concentrations ± SEM (mM) in surviving rats. ★:$p \leq 0.05$ TNF vs. saline.

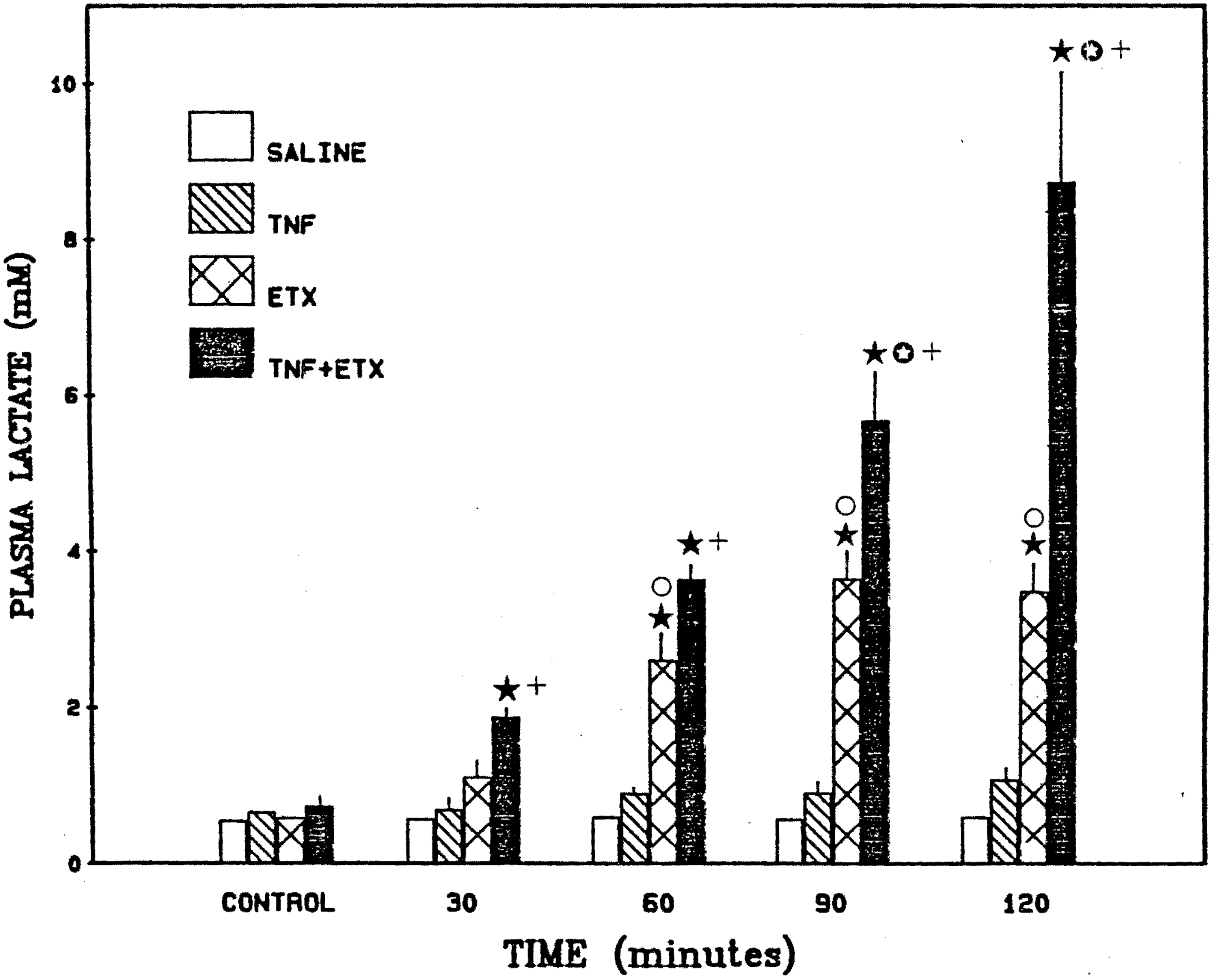


3
2
1
0
SALINE
TNF
PLASMA LACTATE (mM)
CONTROL
30
60
90
180
360
TIME (minutes)
★
★
★
★
★

FIGURE 12

EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PLASMA LACTATE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=7), 0.1 mg/kg TNF (n=6), 1.55 mg/kg ETX (n=5), or co-treatment with 0.1 mg/kg TNF plus 1.55 mg/kg ETX (n=7). Values reported are mean plasma lactate concentrations ± SEM (mM) in surviving rats. ★:$p \leq 0.05$ treatment vs saline. ○:$p \leq 0.05$ ETX vs. TNF. +:$p \leq 0.05$ TNF plus ETX co-treatment vs. TNF. ✪:$p \leq 0.05$ TNF plus ETX co-treatment vs. ETX.


PLASMA LACTATE (mM)
0
2
4
6
8
10
SALINE
TNF
ETX
TNF+ETX
CONTROL
30
60
90
120
TIME (minutes)
★ +
★ ○
★ +
★ ○
★ ✪ +
★ ○
★ ✪ +

8. Endocrine effects of TNF, ETX, and TNF plus ETX co-treatment: plasma insulin

Arterial blood samples from conscious, unrestrained, cannulated rats were analyzed to determine the endocrine modulatory effects, as assessed by changes in plasma insulin concentration, of the following treatments: 1) high dose TNF and high dose ETX in fasted rats; 2) low dose TNF and low dose ETX in fasted rats; and 3) co-treatment with low dose TNF plus low dose ETX in fasted rats. Moreover, the influence of high dose TNF on plasma insulin was evaluated also in fed, conscious, unrestrained, cannulated rats.

The ability of TNF to influence plasma insulin concentrations was investigated in fasted and fed rats. From the data in figure 15, it was difficult to determine if low dose TNF (0.1 mg/kg) had a direct effect on plasma insulin in fasted rats. Because the pre-injection plasma insulin concentration for the TNF treated rats was significantly elevated relative to the saline control value, the concentration reported 30 minutes after TNF injection was not statistically different from the pre-injection control value. However, the 30 minute value for the TNF treated rats was significantly greater than the corresponding value reported for the saline injected rats; 25.3 μU/ml versus 18.8 μU/ml, respectively. Plasma insulin concentrations for the TNF treated animals were not different from those determined in the saline control rats at 60, 90, and 120 minutes. When compared to the respective pre-injection value of 27.5 μU/ml, however, plasma insulin concentrations appeared to decrease at 90 and 120 minutes as a result of TNF treatment; 18.0 and 14.3 μU/ml, respectively.

Significant changes in plasma insulin concentration were observed in fasted and fed rats after treatment with high dose TNF (1 mg/kg). Plasma insulin concentrations were significantly depressed relative to the saline controls at 90 and 360 minutes after high dose TNF administration to fasted rats (figure 13). By 90 minutes, the plasma insulin concentration determined for the TNF treated animals was approximately half the value determined for the saline treated rats, 12.9 versus 24.5 μU/ml, respectively. The hypoinsulinemic effect of TNF also was evident at 360 minutes post-injection: 22.3 versus 10.0 μU/ml for the saline control and high dose TNF treated rats, respectively. The value reported at 360 minutes for the TNF treated group was the average from two rats; 9.51 and 10.56 μU/ml. When tested in fed rats, a high dose of TNF elicited a biphasic change in plasma insulin concentrations (figure 14). A significant hyperinsulinemia was reported at 60 minutes, with plasma insulin concentration elevated to 62.0 μU/ml for the TNF treated rats. By 360 minutes, a significant hypoinsulinemia was demonstrated in the TNF treated group: plasma insulin concentration dropped to 20.5 μU/ml. Hence, a significant TNF-induced hyperinsulinemia was demonstrated only in fed rats, whereas significant hypoinsulinemia was observed by 360 minutes post-injection in both fed and fasted rats.

Dose dependent changes were observed in plasma insulin concentration after ETX administration. No significant changes in plasma insulin concentration were elicited after low dose ETX administration, as shown in figure 15. Although the pre-injection plasma insulin concentration for the low dose ETX treated rats appeared to be greater than the values reported for the other groups, the difference was not

FIGURE 13

EFFECTS OF HIGH DOSE TNF AND ETX ON PLASMA INSULIN IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=4), heat-inactivated TNF (H.I.-TNF; n=5), 1.0 mg/kg TNF (n=6), or 30 mg/kg ETX (n=6). Values reported are mean plasma insulin concentrations ± SEM (μU/ml) in surviving rats. Plasma insulin concentrations reported at 360 minutes for the TNF and ETX treated groups represent the average of 2 determinations. ★:$p \leq 0.05$ treatment vs saline. ☆:$p \leq 0.05$ TNF vs. ETX.

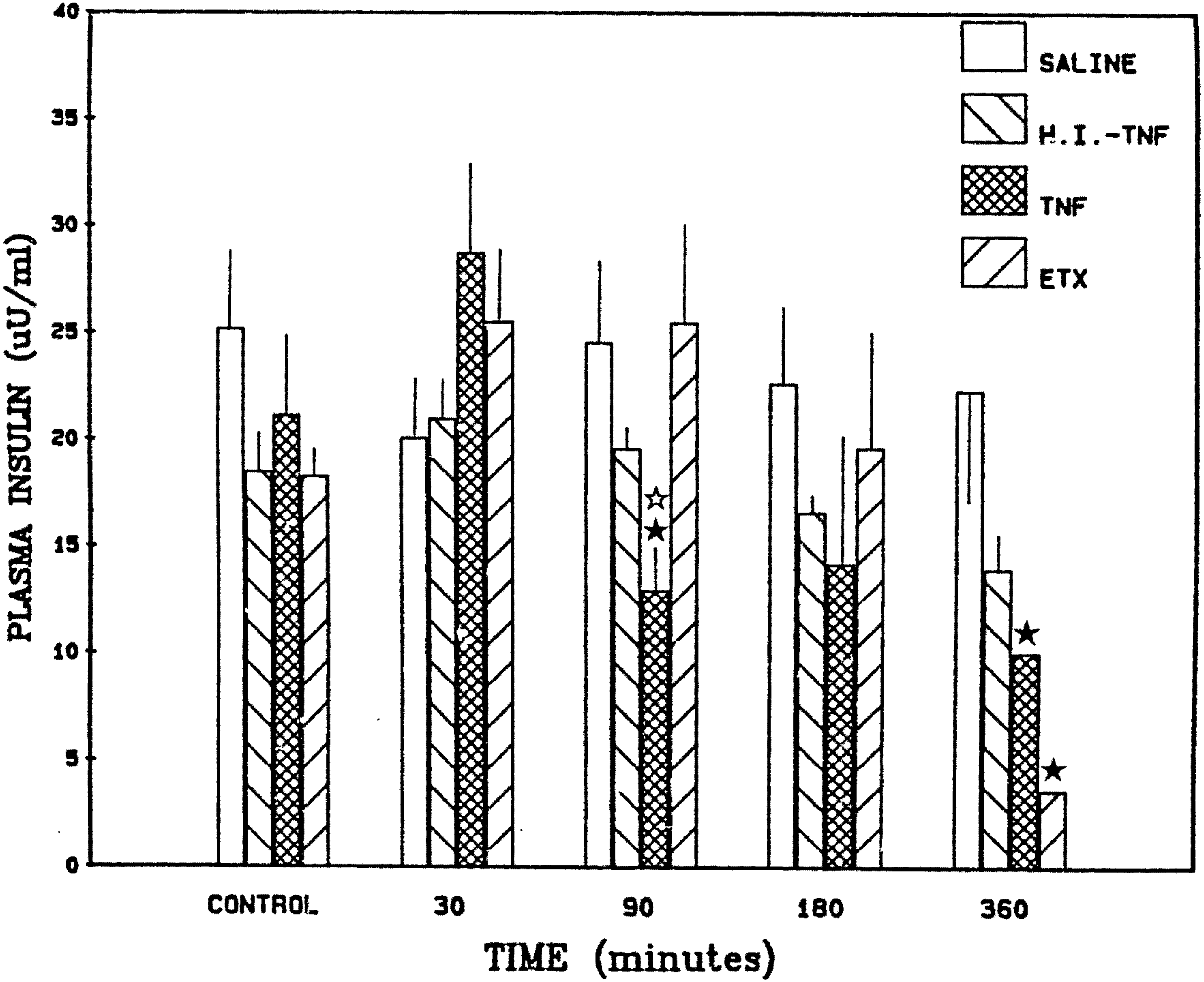

40
35
30
25
20
15
10
5
0
PLASMA INSULIN (uU/ml)
SALINE
H.I.-TNF
TNF
ETX
☆
★
★
★
CONTROL
30
90
180
360
TIME (minutes)

FIGURE 14

EFFECTS OF HIGH DOSE TNF ON PLASMA INSULIN IN FED CANNULATED RATS

Rats were given an i.v. bolus injection of saline (n=5) or 1 mg/kg TNF (n=5). Values reported are mean plasma insulin concentrations ± SEM (μU/ml) in surviving rats. ★:$p \leq 0.05$ TNF vs. saline.

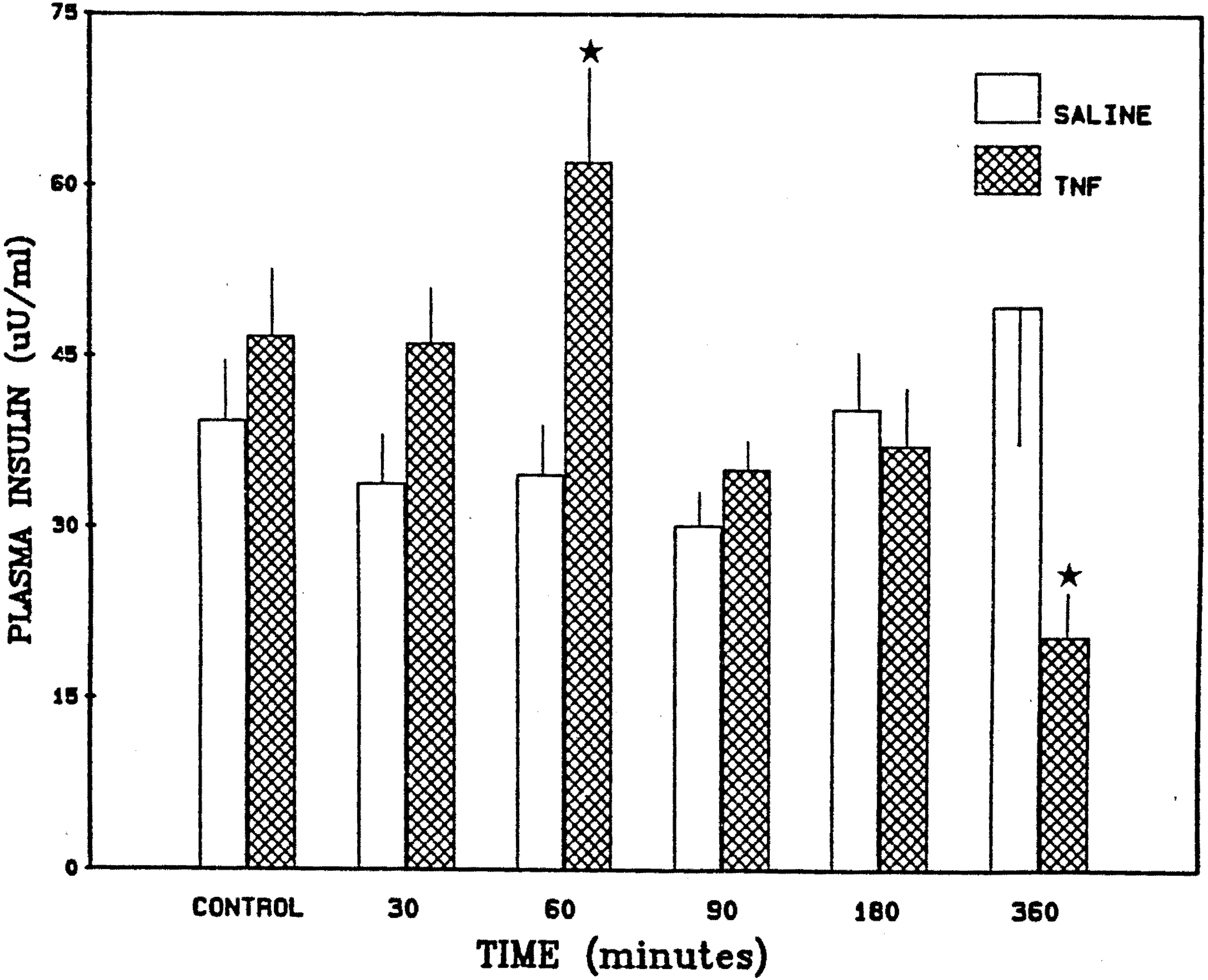
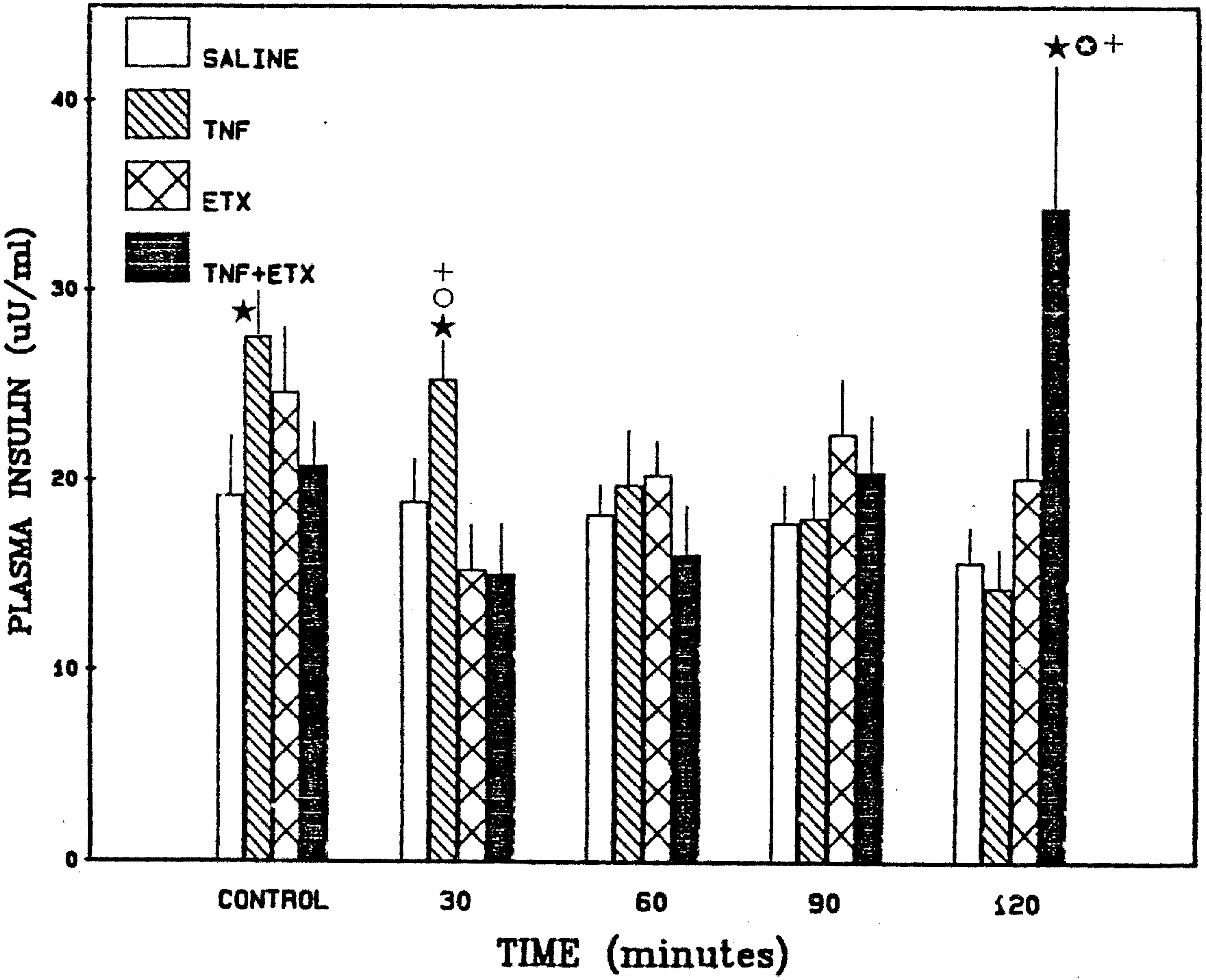

75
60
45
30
15
0
PLASMA INSULIN (uU/ml)
SALINE
TNF
★
★
CONTROL
30
60
90
180
360
TIME (minutes)

FIGURE 15

EFFECTS OF LOW DOSE TNF, LOW DOSE ETX, AND LOW DOSE TNF PLUS ETX CO-TREATMENT ON PLASMA INSULIN IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=7), 0.1 mg/kg TNF (n=6), 1.55 mg/kg ETX (n=5), or co-treatment with 0.1 mg/kg TNF plus 1.55 mg/kg ETX (n=7). Values reported are mean plasma insulin concentrations ± SEM (μU/ml) in surviving rats. ★:$p \leq 0.05$ treatment vs saline. ○:$p \leq 0.05$ TNF vs. ETX. ✚:$p \leq 0.05$ TNF vs. TNF plus ETX co-treatment. ✪:$p \leq 0.05$ TNF plus ETX co-treatment vs. ETX.


SALINE
TNF
ETX
TNF+ETX
PLASMA INSULIN (uU/ml)
0
10
20
30
40
CONTROL
30
60
90
120
TIME (minutes)
★
+
○
★
★ ✪ +

statistically significant. High dose ETX administration, however, induced an observable but not statistically significant increase in plasma insulin concentrations by 30 and 90 minutes relative to the respective pre-injection control value (figure 13). A significant hypoinsulinemia was elicited 360 minutes after a high dose of ETX. The plasma insulin concentration reported at 360 minutes for the high dose ETX treated rats, 3.60 μU/ml, was the average for 2 rats; 3.96 and 3.23 μU/ml.

Co-treatment with low dose TNF plus ETX resulted in a significant change in plasma insulin which was unlike that reported for rats treated with either TNF or ETX alone. No significant alterations in plasma insulin concentration occurred within the first 90 minutes after co-treatment with TNF plus ETX (figure 15). By 120 minutes, however, there was a significant increase in plasma insulin concentration to 34.3 μU/ml, which was significantly greater than the values reported for the saline control, low dose TNF, and low dose ETX treated groups; 15.7, 14.3, and 20.1 μU/ml, respectively.

9. Neuro-endocrine effects of TNF: plasma catecholamines

The effects of high dose TNF on plasma epinephrine and norepinephrine concentrations were evaluated in fasted, conscious, unrestrained, cannulated rats. Saline treated rats had no measurable changes in plasma epinephrine or norepinephrine concentrations; plasma epinephrine and norepinephrine remained steady throughout the experiment at average mean values of 72.1 and 82.1 pg/ml, respectively. Because changes in plasma epinephrine and norepinephrine concentrations are sensitive indicators of stress, the failure to demonstrate a significant

FIGURE 16

EFFECTS OF HIGH DOSE TNF ON PLASMA EPINEPHRINE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=3), heat-inactivated TNF (H.I.-TNF; n=4), or 1.0 mg/kg TNF (n=6). Values reported are mean plasma epinephrine concentrations ± SEM (pg/ml) in surviving rats. The value reported at 360 minutes for the TNF treated group represents the average of 2 determinations. ★:$p \leq 0.05$ treatment vs saline. ✪:$p \leq 0.05$ TNF vs. H.I.-TNF.

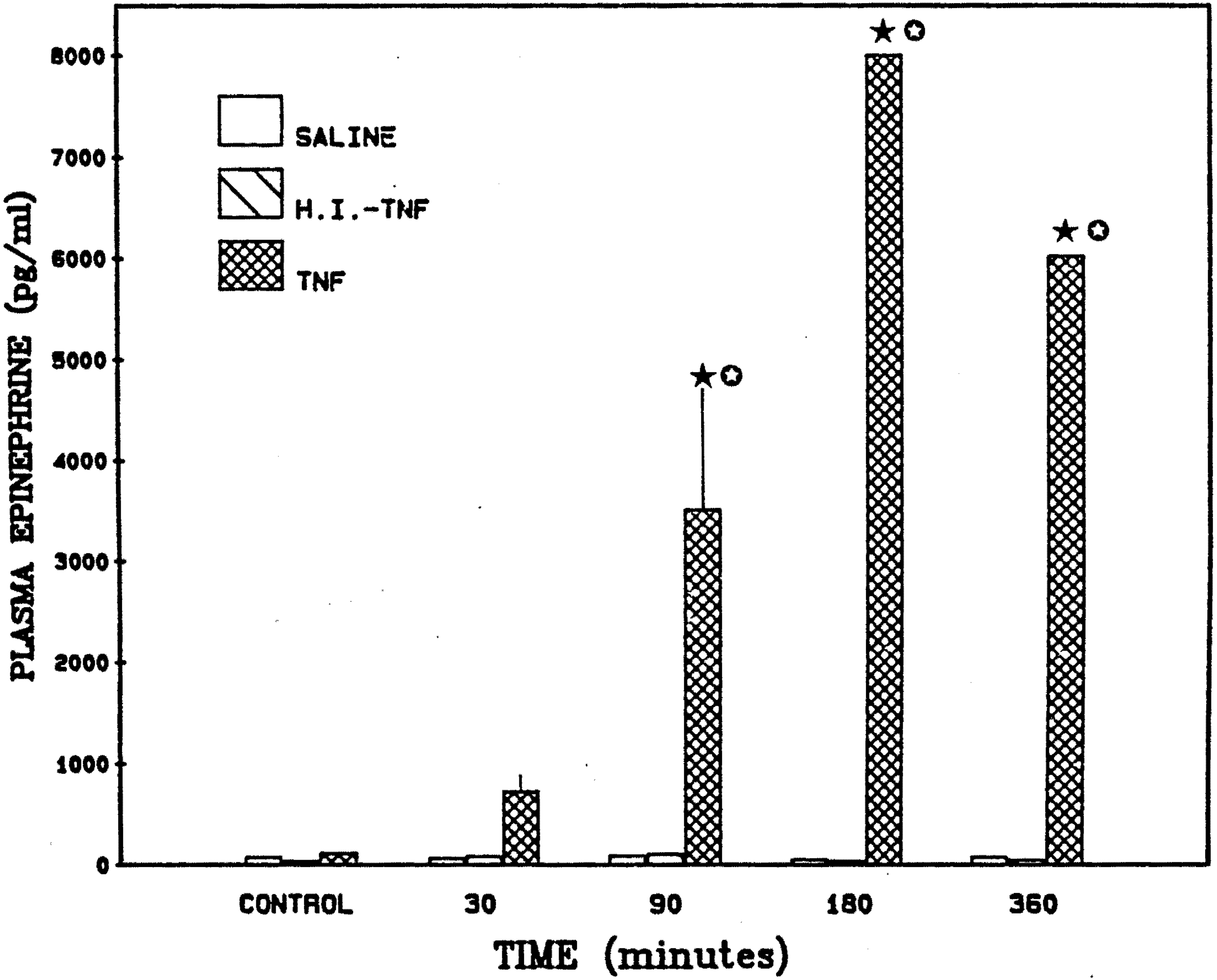
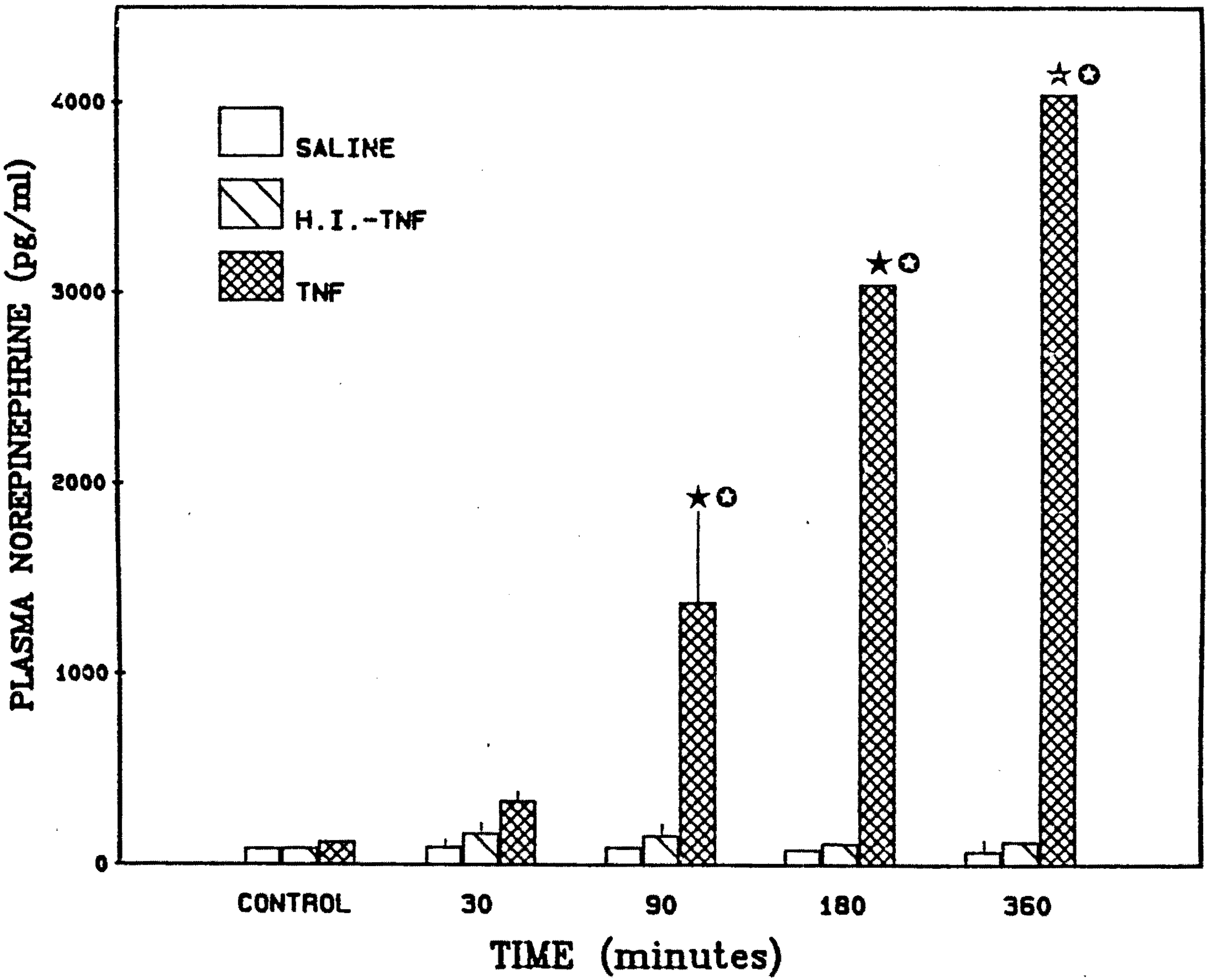

PLASMA EPINEPHRINE (pg/ml)
8000
7000
6000
5000
4000
3000
2000
1000
0
SALINE
H.I.-TNF
TNF
★✪
★✪
★✪
CONTROL
30
90
180
360
TIME (minutes)

FIGURE 17

EFFECTS OF HIGH DOSE TNF ON PLASMA NOREPINEPHRINE IN FASTED CANNULATED RATS

Rats were injected i.v. with either saline (n=3), heat-inactivated TNF (H.I.-TNF; n=4), or 1.0 mg/kg TNF (n=6). Values reported are mean plasma norepinephrine concentrations ± SEM (pg/ml) in surviving rats. The value reported at 360 minutes for the TNF group represents the average of 2 values. ★:$p \leq 0.05$ treatment vs saline. ✪:$p \leq 0.05$ TNF vs. H.I.-TNF.


SALINE
H.I.-TNF
TNF
PLASMA NOREPINEPHRINE (pg/ml)
4000
3000
2000
1000
0
★✪
★✪
☆✪
CONTROL
30
90
180
360
TIME (minutes)

elevation in their concentrations after saline injection supported the belief that the experimental procedure per se was not stressing the animals.

Significant elevations in plasma epinephrine and norepinephrine concentrations occurred by 90 minutes after high dose TNF administration. Plasma epinephrine increased to 3509 pg/ml from a pre-injection value of 116 pg/ml (figure 16). Peak elevation in plasma epinephrine was demonstrated at 180 minutes with a value of 8004 ± 3674 pg/ml. By 360 minutes, only 2 animals remained: the 24 hour survivor had a plasma epinephrine concentration of 459 pg/ml, whereas the dying animal had a value of 11587 pg/ml. Plasma norepinephrine concentrations increased significantly from a pre-injection value of 120 pg/ml to 1374 pg/ml by 90 minutes after high dose TNF administration (figure 17). Norepinephrine concentrations continued to increase to 3042 ± 1528 and 4039 pg/ml by 180 and 360 minutes, respectively. The value reported at 360 minutes was the average of 2 determinations; 544 pg/ml determined for the 24 hour survivor and 7534 pg/ml determined for the agonal stage rat.

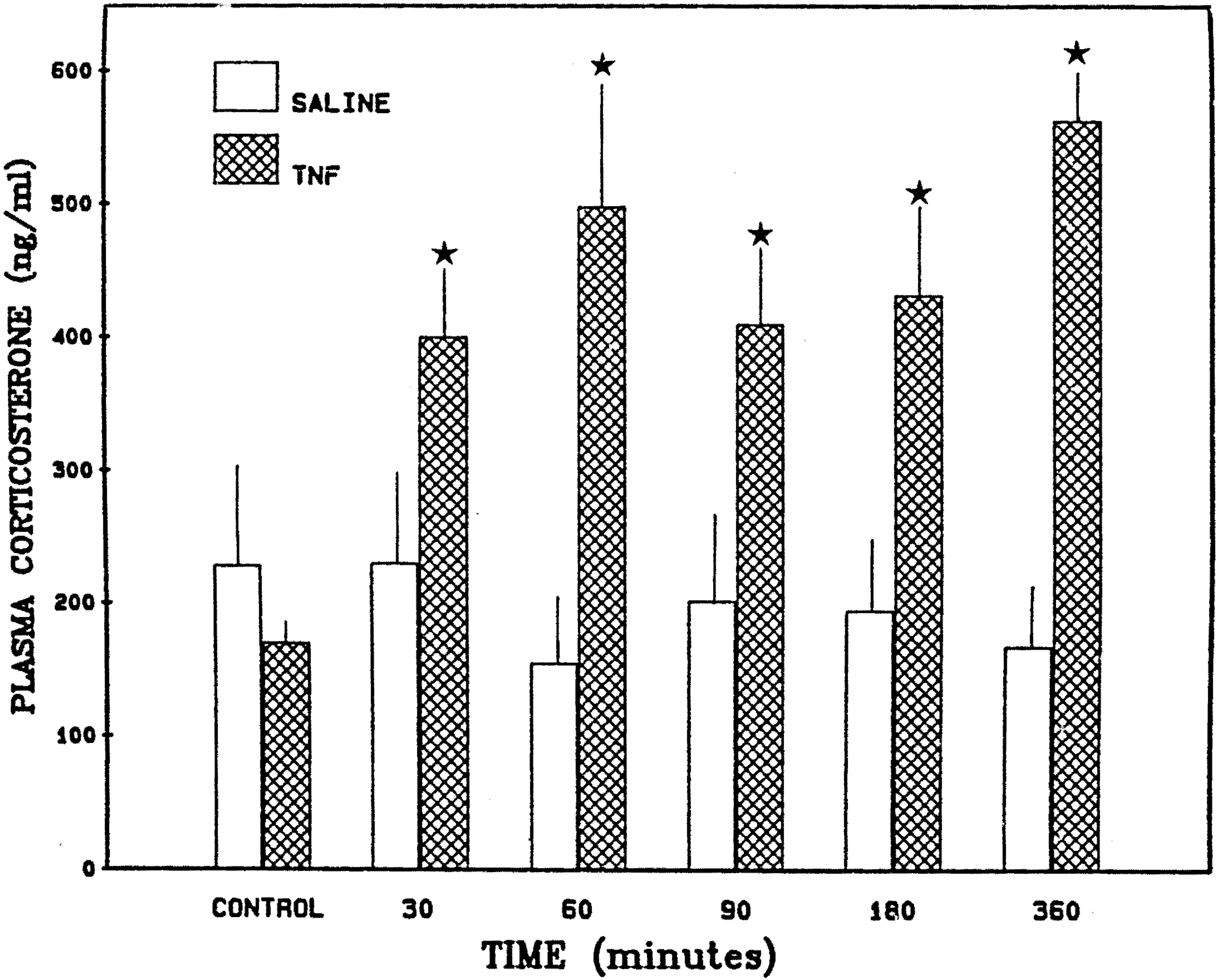
10. Endocrine effects of TNF: plasma corticosterone

Plasma corticosterone concentrations were measured after high dose TNF treatment in fed, conscious, unrestrained, cannulated rats (figure 18). Saline injected rats had no measurable change in plasma corticosterone concentration; mean plasma corticosterone concentration averaged 196 ng/ml. However, high dose TNF induced a significant increase in plasma corticosterone: by 30 minutes, plasma corticosterone increased to 400 ng/ml versus 230 ng/ml for the saline control rats. TNF treated animals maintained significantly elevated plasma corticosterone

FIGURE 18

EFFECTS OF HIGH DOSE TNF ON PLASMA CORTICOSTERONE IN FED CANNULATED RATS

Rats were injected i.v. with saline (n=5) or 1 mg/kg TNF (n=5). Values reported are mean plasma corticosterone concentrations ± SEM (ng/ml) in surviving rats. ★:$p \leq 0.05$ TNF vs. saline.


SALINE
TNF
PLASMA CORTICOSTERONE (ng/ml)
0
100
200
300
400
500
600
CONTROL
30
60
90
180
360
TIME (minutes)
★
★
★
★
★

concentrations relative to saline treated animals for 360 minutes. Final plasma corticosterone concentrations were 563 ng/ml in the TNF treated group and 168 ng/ml in saline treated controls.

B. IN VITRO EFFECTS OF TNF: ISOLATED PERFUSED RAT LIVERS

TNF induced significant alterations in plasma glucose concentration comparable to those induced after ETX administration in conscious, cannulated rats (figure 8). These observations suggested that the changes in hepatic glucoregulation observed after ETX administration might be mediated by endogenous TNF, released from ETX-activated Kupffer cells, directly influencing neighboring hepatocyte function. To evaluate this hypothesis initially, TNF was tested for direct hepatic glucoregulatory effects using the isolated perfused rat liver model. Livers were excised from fed male rats under sodium pentobarbital anesthesia and perfused in a recirculating liver perfusion apparatus with glucose-free KRB containing 2 mg/ml albumin, as described in chapter III. Because the perfusion buffer was glucose-free, the glucose concentration measured in the buffer during the perfusion was the net result of hepatic glucose mobilization from endogenous stores of glycogen.

The liver was allowed to stabilize in the perfusion system for 30 minutes. During that time, net hepatic glucose production stimulated by the absence of glucose in the perfusion buffer and oxygen consumption reached steady state levels of 92.6 μmoles glucose/gm liver wet weight/hour and 56.4 μl of oxygen/gm liver wet weight/minute, respectively.

As reported in table 5, the direct effect of TNF on net liver glucose production rates was examined using the isolated perfused rat liver model. Control glucose production rates determined at 30 minutes were similar for each group of livers; 94.2, 93.0, and 90.7 μmoles of glucose/gm liver wet weight/ hour. At 31 minutes, either 1 ml of PBS (n=6), 150 μg of TNF (n=5), or 300 μg TNF (n=4) was added directly to the perfusion reservoir. During the subseqeunt 15 minutes of perfusion, there was a slight reduction in glucose production rate observed in the PBS treated livers: net glucose production rate decreased from a value of 94.2 to 72.8 μmoles glucose/gm liver wet weight/hour by 46 minutes. Livers administered 150 μg of TNF (1 μg/ml final concentration) demonstrated a greater reduction in net glucose output by 46 minutes than was observed for the PBS treated livers. After 15 minutes of perfusion with 1 μg/ml TNF, net glucose production rate dropped to 56.6 μmoles glucose/gram liver wet weight/hour, which appeared to be lower than the rate reported for the PBS treated controls ($p \leq 0.10$).

In order to pursue further the possibility that TNF might directly modulate hepatic glucose production, livers were challenged at 46 minutes with a maximal stimulatory dose of glucagon (1 μM final concentration). As reported in table 5, net glucose production rate increased approximately 4 fold for the PBS treated control group to 294 μmoles glucose/gm liver wet weight/hour. Glucagon challenge of the TNF (1 μg/ml) treated livers resulted in an increase in net glucose production rate to 202 μmoles glucose/gm liver wet weight/hour, which was less than that observed with the PBS treated livers ($p \leq 0.10$).

The basal and glucagon stimulated net glucose production rates for livers perfused with 1 μg/ml TNF suggested that a higher dose of TNF might significantly ($p \leq 0.05$) modulate net hepatic glucose production. Therefore, a group of livers was tested in the same manner as just

TABLE 5

EFFECTS OF TNF ON GLUCOSE PRODUCTION RATES BY ISOLATED PERFUSED RAT LIVERS

CONTROL	N	TREATMENT	BASAL	GLUCAGON (1 uM)
94.2±10	6	PBS	72.8±5.1	294±23
93.0±6.7	5	TNF(1μg/ml)	56.6±6.3*	202±39*
90.7±18	4	TNF(2μg/ml)	65.5±9.3	222±46

Values reported are mean net glucose production rates ± SEM by isolated perfused rat livers (μmoles glucose/gm liver wet weight/hour). Livers were excised from pentobarbital anesthetized rats and were perfused with KRB + 2 mg/ml albumin. The control values represent the net glucose production rates after 30 minutes of perfusion. Either PBS (1 or 2 ml) or TNF (150 or 300 μg) was added directly to the perfusion reservoir after 31 minutes of perfusion. After 46 minutes of perfusion, maximum glucose production was achieved with the administration of glucagon (1 μM final concentration). Total perfusion time was 64 minutes. *$p \leq 0.10$ TNF vs PBS.

described except that a 2 fold greater dose of TNF was added directly to the perfusion reservoir after 31 minutes of perfusion, ie. 300 μg. However, there was no significant difference between the net glucose production rate of TNF treated livers (2 μg/ml) and PBS treated livers; 65.5 vs. 72.8 μmoles glucose/gm liver wet weight/hour, respectively. Furthermore, TNF treatment (2 μg/ml) failed to alter the glucagon stimulated increase in net hepatic glucose production. After challenge with 1 μM glucagon for 15 minutes, net glucose production rates increased to 222 μmoles glucose/gm liver wet weight/hour in the TNF treated livers, which was not significantly different from the rate reported for the PBS treated livers, 294 μmoles glucose/gm liver wet weight/hour.

Net hepatic lactate production was continuously monitored during the perfusion and the calculated rates are reported in table 6. Net lactate production rates determined during the 30 minute control period were similar for all 3 groups of livers; 26.3, 20.9, and 11.5 μmoles lactate/gram liver wet weight/hour, respectively. Perfusion with 1 or 2 μg/ml of TNF for 15 minutes did not influence net lactate production rates. The rates determined after 15 minutes of treatment with 1 and 2 μg/ml TNF, 24.0 and 21.1 μmoles lactate/gm liver wet weight/hour, were not significantly different from the rate reported for the PBS treated group, 17.7 μmoles lactate/gm liver wet weight/hour. After challenge with 1 μM glucagon, livers removed lactate from the perfusate rather than release lactate into the perfusion buffer. Therefore, net lactate uptake rates, presented as negative values for net lactate production rates, are reported in table 6. TNF treatment did not influence hepatic lactate uptake in response to 1 μM glucagon. Fifteen minutes after challenge

TABLE 6

EFFECTS OF TNF ON LACTATE PRODUCTION RATES BY ISOLATED PERFUSED RAT LIVERS

CONTROL	N	TREATMENT	BASAL	GLUCAGON (1 uM)
26.3±5.6	6	PBS	17.7±7.5	-12.7±6.7
20.9±10	5	TNF(1μg/ml)	24.0±12	-13.1±5.9
11.5±17	4	TNF(2μg/ml)	21.1±3.9	-14.9±8.3

Values reported are mean net lactate production rates ± SEM by isolated perfused rat livers (μmoles lactate/gm liver wet weight/hour). The addition of glucagon resulted in a net uptake of lactate by the livers as indicated by the negative values.

TABLE 7

EFFECTS OF TNF ON OXYGEN CONSUMPTION RATES BY ISOLATED PERFUSED RAT LIVERS

CONTROL	N	TREATMENT	BASAL	GLUCAGON (1 uM)
58.3±3.0	6	PBS	59.8±2.8	62.9±4.2
57.4±2.6	5	TNF(1μg/ml)	58.1±1.2	60.8±1.9
53.6±2.5	4	TNF(2μg/ml)	57.6±2.7	60.2±1.7

Values reported are mean net oxygen consumption rates ± SEM by isolated perfused rat livers (μl of oxygen/gm liver wet weight/min). Liver oxygen consumption rates were calculated from the difference in oxygen saturation of the perfusion fluid going into and coming out of the perfused liver.

with 1 μM glucagon, net lactate uptake rates were 12.7, 13.1, and 14.9 μmoles lactate/gm liver wet weight/hour for PBS, 1 and 2 μg/ml TNF treated livers, respectively.

In addition to net hepatic glucose and lactate production, liver oxygen consumption was measured during the perfusion, as discussed in chapter III. During the control period, liver oxygen consumption rates were similar for each group of livers: 58.3, 57.4, and 53.6 μl oxygen/gm liver wet weight/minute, table 7. After 15 minutes of continuous perfusion with 1 or 2 μg/ml TNF, there was no measurable change in oxygen consumption rate relative to the control rate determined for PBS treated livers. The slight increase in oxygen consumption rate observed after challenge with 1 μM glucagon was not altered by treatment with TNF. Fifteen minutes after challenge with 1 μM glucagon, oxygen consumption rates reported for PBS, 1 μg/ml TNF, and 2 μg/ml TNF treated livers were 62.9, 60.8, and 60.2 μl oxygen/gm liver wet weight/minute, respectively.

There were no other observable changes in livers perfused with 1 or 2 μg/ml TNF. Flow rate to the livers remained steady at 50 ml/minute for both PBS and TNF treated livers. The outward appearance of the livers during perfusion with 1 or 2 μg/ml TNF was unaltered: there was no hepatic swelling or discoloration after TNF administration.

C. IN VITRO EFFECTS OF TNF: ISOLATED LIVER PARENCHYMAL CELLS

As a final step in determining the direct, short-term, hepatic glucoregulatory effects of TNF, isolated hepatocytes from fed rats were incubated with various doses of TNF. Unlike the isolated perfused liver model, hepatocytes were in direct contact with TNF without significant interference from other non-parenchymal liver cells, such as Kupffer cells and endothelial cells. Furthermore, the dose dependent influence of TNF on hepatic glucoregulation was easily evaluated with the cells isolated from each individual liver, rather than different cell preparations for each dose of TNF tested.

TNF at doses of 0.01-5.0 μg/ml had no effect on net glucose production rates, as reported in table 8. Basal rates of glucose production in the presence of 0.01-5.0 μg/ml TNF averaged 0.970 μmoles glucose/mg protein/hour, which was not significantly different from the basal rate of 0.939 μmoles glucose/mg protein/hour reported for the PBS treated control cells. Just as done with the isolated perfused livers, isolated hepatocytes were maximally stimulated with 1 μM glucagon to determine if TNF treatment modulated the hormonal response. Glucose production stimulated by the cAMP-dependent hormone glucagon was tested in cells incubated with PBS or 0.01-5.0 μg/ml TNF (table 8). PBS treated control cells stimulated with 1 μM glucagon demonstrated an increase in net glucose production rate to 1.45 μmoles glucose/mg protein/hour from a basal rate of 0.939. TNF treatment failed to modify the cellular response to 1 μM glucagon, as reflected by the net glucose production rates. The average glucose production rate observed with hepatocytes stimulated with 1 μM glucagon in the presence of 0.01-5.0 μg/ml TNF was 1.39 μmoles glucose/mg protein/hour, a rate not significantly different from the control rate of 1.45 reported for the PBS treated cells.

Since hepatic glucose production also is stimulated by a Ca^{++}-dependent pathway as well as a cAMP-dependent pathway, the Ca^{++}-dependent glucoregulatory hormone, vasopressin, was tested in PBS and 0.01-5.0 μg/ml TNF treated cells. Under the same conditions as just described,

TABLE 8

EFFECTS OF TNF ON BASAL AND GLUCAGON-STIMULATED GLUCOSE PRODUCTION RATES BY ISOLATED HEPATOCYTES

TREATMENT	DOSE (ug/ml)	BASAL	GLUCAGON(1 uM)
PBS	--	0.939±0.040	1.45±0.28
TNF	5	0.975±0.082	1.42±0.27
TNF	1	0.963±0.071	1.44±0.28
TNF	0.5	0.958±0.064	1.41±0.28
TNF	0.1	0.951±0.074	1.38±0.29
TNF	0.05	0.986±0.11	1.38±0.27
TNF	0.01	0.986±0.10	1.33±0.28

Values reported are mean net glucose production rates ± SEM determined from four different cell preparations (μmoles glucose/mg protein/hr.). For each preparation, cells were isolated and incubated in triplicate for 60 minutes at 37°C in KRB + 2 mg/ml albumin in the absence or presence of the indicated doses of TNF ± glucagon.

TABLE 9

EFFECTS OF TNF ON BASAL AND VASOPRESSIN - STIMULATED GLUCOSE PRODUCTION RATES BY ISOLATED HEPATOCYTES

TREATMENT	DOSE (ug/ml)	BASAL	VASOPRESSIN (1 uM)
PBS	--	0.681±0.021	0.780±0.078
TNF	5	0.650±0.046	0.767±0.072
TNF	1	0.592±0.011	0.794±0.079
TNF	0.5	0.647±0.054	0.774±0.065
TNF	0.1	0.644±0.059	0.743±0.072
TNF	0.05	0.607±0.048	0.759±0.073
TNF	0.01	0.644±0.053	0.780±0.072

Values reported are mean net glucose production rates ± SEM for three separate cell preparations (μmoles glucose/mg protein/hr). For each preparation, cells were isolated and were incubated in triplicate for 60 minutes at 37°C in KRB + 2 mg/ml albumin in the absence or presence of the indicated doses of TNF ± vasopressin.

TNF had no effect on the basal rate of glucose production by isolated hepatocytes (table 9). The average glucose production rate in response to TNF was 0.631 μmoles glucose/mg protein/hour, which was not significantly different from the PBS control rate of 0.681 μmoles glucose/mg protein/hour. In response to maximal stimulation with 1 μM vasopressin, PBS treated control cells and TNF treated cells responded similarly, as assessed by net hepatic glucose production rates. Glucose production rates increased to 0.780 and an average value of 0.770 μmoles glucose/mg protein/hour for PBS treated and TNF treated cells, respectively. Hence, under short-term conditions, TNF failed to directly influence hepatic glucoregulation under both basal and hormone stimulated conditions.

In conjunction with evaluating the direct effects of TNF on net hepatic glucose production, TNF also was evaluated for a direct effect on net lactate production by isolated hepatocytes. As reported in table 10, TNF did not influence the basal rate of lactate production by isolated hepatocytes. The average rate of lactate production under the influence of TNF was 0.354 μmoles lactate/mg protein/hour, which was insignificantly different from the PBS control rate of 0.343 μmoles lactate/mg protein/hour. Similar to the results obtained with the isolated perfused liver model, stimulation with 1 μM glucagon blunted the net lactate production by isolated hepatocytes. In the presence of 1 μM glucagon, net lactate production rate dropped to 0.073 μmoles lactate/mg protein/hour from a basal value of 0.343 μmoles lactate/mg protein/hour for the PBS treated control cells. And just as observed with the isolated perfused livers, TNF treatment did not interfere with the hepatocytes' ability to use lactate as a gluconeogenic substrate after stimulation

TABLE 10

EFFECTS OF TNF ON LACTATE PRODUCTION RATES BY ISOLATED HEPATOCYTES

TREATMENT	DOSE (ug/ml)	BASAL	GLUCAGON (1uM)
PBS	--	0.343±0.050	0.073±0.005
TNF	5	0.333±0.050	0.085±0.006
TNF	1	0.333±0.040	0.093±0.016
TNF	0.5	0.350±0.051	0.098±0.018
TNF	0.1	0.365±0.046	0.133±0.050
TNF	0.05	0.373±0.037	0.073±0.012
TNF	0.01	0.370±0.071	0.113±0.040

Values reported are mean net lactate production rates ± SEM for four separate cell preparations (μmoles lactate/mg protein/hr). For each preparation, cells were isolated and were incubated in triplicate for 60 minutes at 37°C in KRB + 2 mg/ml albumin in the absence or presence of the indicated doses of TNF ± glucagon.

TABLE 11

EFFECTS OF TNF ON LACTATE PRODUCTION RATES BY ISOLATED HEPATOCYTES IN THE ABSENCE OR PRESENCE OF VASOPRESSIN

TREATMENT	DOSE (ug/ml)	BASAL	VASOPRESSIN (1 uM)
PBS	--	0.244±0.053	0.358±0.092
TNF	5	0.276±0.022	0.321±0.066
TNF	1	0.215±0.063	0.287±0.048
TNF	0.5	0.301±0.038	0.323±0.007
TNF	0.1	0.232±0.053	0.307±0.072
TNF	0.05	0.217±0.044	0.319±0.065
TNF	0.01	0.270±0.070	0.323±0.052

Values reported are mean net lactate production rates ± SEM for three separate cell preparations (μmoles lactate/mg protein/hr). For each preparation, cells were isolated and were incubated in triplicate in KRB plus 2 mg/ml albumin for 60 minutes at 37°C in the absence or presence of the indicated doses of TNF ± vasopressin.

TABLE 12

EFFECTS OF TNF ON GLUCOSE UTILIZATION BY ISOLATED HEPATOCYTES

TREATMENT	DOSE (ug/ml)	GLUCOSE OXIDATION
CONTROL	--	333±54
TNF	5	344±15
TNF	1	341±55
TNF	0.5	354±52
TNF	0.1	338±27
TNF	0.05	356±27
TNF	0.01	298±18

Values reported are mean ^{14}C counts ± SEM from four separate cell preparations (DPM/mg protein). For each preparation, cells were isolated and were incubated in triplicate for 120 minutes at 37°C in KRB containing 2 mg/ml albumin, 1 mg/ml D-glucose, and 0.1 μCi/ml ^{14}C-D-glucose in the absence or presence of TNF. Expired $^{14}CO_2$ was collected for 60 minutes on methlybenzethonium hydroxide saturated filter paper after the addition of 62.5% citric acid.

with glucagon (table 10). Hepatocytes treated with 0.01-5.0 μg/ml TNF responded to the glucagon challenge in a manner similar to the PBS treated control cells. Net lactate production rates dropped as a result of glucagon-stimulated hepatic uptake to an average value of 0.099 μmoles lactate/mg protein/hour for the TNF treated hepatocytes, which was not significantly different from the rate of 0.073 μmoles lactate/mg protein/hour reported for the control cells. Moreover, TNF also failed to influence the net lactate production rate of hepatocytes stimulated with 1 μM vasopressin (table 11). Just as reported in table 10, TNF did not modulate the basal lactate production rate reported in table 11. The average net lactate production rate in the presence of 0.01-5.0 μg/ml TNF was 0.252 μmoles lactate/mg protein/hour, which was not different from the control value of 0.244 μmoles lactate/mg protein/hour. TNF also failed to alter the net lactate production rate determined after treatment with 1 μM vasopressin. Net lactate production rates determined in the presence of 1 μM vasopressin were 0.358 for PBS treated cells and an average rate of 0.313 μmoles lactate/mg protein/hour for the TNF treated cells.

Finally, TNF was tested to determine if its direct hepatic glucoregulatory influence was on hepatocyte glucose utilization rather than net glucose or lactate production. Since net hepatic glucose and lactate production are crude indices of more subtle cellular changes, TNF was evaluated for a dose dependent influence on glucose oxidation by isolated hepatocytes. Hepatocytes were incubated for 2 hours x 37°C in KRB containing 2 mg/ml albumin, 1 mg/ml D-glucose, 0.1 μCi/ml ^{14}C-glucose and either PBS or 0.01-5.0 μg/ml TNF. Released $^{14}CO_2$ was collected for 1 hour as a measure of ^{14}C-glucose oxidation. As reported in table 12, TNF did not significantly influence glucose utilization by isolated hepatocytes. The DPM/mg protein released by PBS treated control cells was 333, which was not significantly different from the average value of 338 DPM/mg protein reported for the TNF treated groups.

CHAPTER V

DISCUSSION

A. *IN VIVO* EVALUATION OF TNF AS A MEDIATOR OF ENDOTOXIC SHOCK

This study compared select pathophysiological changes observed after comparably lethal, i.v. bolus injections of ETX vis-a-vis TNF in unanesthetized, unrestrained, cannulated rats. This model was implemented for a number of reasons. First, conscious, unrestrained, cannulated adult rats were used so that hemodynamic monitoring and blood samplings were easily obtained and analyzed without the use of anesthesia during the experimental protocol. Secondly, since exogenous ETX results in a "spike-like" increase in endogenous plasma TNF concentrations (103,174), exogenous TNF was administered as an i.v. bolus injection such that peak blood levels of exogenous TNF were reached quickly (31,82) and were followed by a rapid decline (31,82). Although a continuous infusion of exogenous TNF achieves the level in plasma TNF elicited after exogenous ETX administration (175), the rise and subsequent decline in plasma TNF concentration are slower. Since both the magnitude of TNF release (103,224) as well as the duration (127) of the spike may be important in the severity of the TNF insult, an i.v. bolus injection of exogenous TNF was used for this study rather than a continuous infusion. Additionally, the doses of exogenous TNF administered resulted in levels of plasma TNF comparable to those measured after an ETX challenge. Assuming that exogenous TNF is confined to the extracellular space (82), high dose TNF (1.0 mg/kg) administered to 350 gram rats resulted in a calculated initial plasma TNF concentration equivalent to 5 μg/ml, which was similar to the 5.5 μg/ml TNF produced in vivo in BCG-primed rabbits stimulated with ETX (1). Low dose TNF (0.1 mg/kg) administration resulted in a calculated initial plasma concentration of 0.5 μg/ml, which was greater than the sub-nanogram per milliliter peak concentrations reported for humans either infused with non-lethal doses of TNF (175) or given a non-lethal bolus injection of ETX (103,174). Sub-microgram per milliliter peak concentrations were observed in baboons (103) and rabbits (161) following a lethal injection of Escherichia coli and ETX, respectively.

1. Mortality and morbidity comparisons

Comparison between the pathophysiological changes induced by ETX and TNF in vivo demonstrated important similarities as well as salient differences. Both TNF and ETX had dose dependent lethal effects in conscious, unrestrained, cannulated rats; however, on a milligram per kilogram basis, rats were more sensitive to the lethal effects of TNF than the lethal effects of ETX. TNF at doses of 1 and 1.8 mg/kg resulted in mortality rates of 83% in 24 hours (table 2) and 64% in 12 hours (249), respectively. Comparable doses of ETX, ie. 1.55 mg/kg, produced only a 20% mortality in 24 hours; a 30 mg/kg dose of ETX was required for 100% mortality rate during 24 hours. When rats were pretreated with glucan, which enhanced the reticuloendothelial system (78), sensitivity to the lethal effects of ETX increased whereas sensitivity to the lethal effects of TNF remained unaltered. The differential sensitivity to TNF and ETX under normal and RES stimulated conditions provide useful insights in the pathogenesis of endotoxic shock. Since greater quantities of TNF are presumably released in response to ETX challenge under RES stimulated conditions than under normal conditions, the enhanced sensitivity to ETX observed after glucan pretreatment is correlated with the enhanced TNF activity. Failure to demonstrate an augmentation in the sensitivity to TNF under RES stimulated conditions suggests that the pathological changes elicited by TNF were not mediated through TNF stimulated macrophage cytotoxicity (202) or mediator secretion (12,38, 67). Other agents, such as actinomycin D (34,46) and D-galactosamine (145) which interfere with cellular function rather than stimulate the RES, increase the sensitivity to both ETX and TNF, therefore supporting the hypothesis that the lethal effects induced by ETX and TNF are related.

The dose dependent morbidity induced by TNF and ETX was indistinguishable: rats exhibited piloerection, labored breathing, hunched-over posture, and progressive cyanosis subsequent to either ETX or TNF injection. Diarrhea was consistently observed in the ETX treated rats but not in the TNF treated rats, even though TNF-induced diarrhea had been reported by other investigators (13,125,214,249). These symptoms in rats are analogous to the flu-like symptoms, such as chills, headache, myalgia, nausea, and fever, reported by humans given either a bolus injection of ETX (174,175) or an infusion of TNF (175).

Neutralization of endogenous TNF by either rabbit antiserum (26) or specific monoclonal antibodies (250) reduced the lethal effects of ETX and live gram negative bacteria. Furthermore, the inability to produce TNF in response to an ETX challenge likewise reduces the mortality and morbidity associated with lethal endotoxicosis. For instance, endotoxin-resistant mice fail to produce TNF in response to an ETX challenge as a result of pre- and post-translational defects (24). Moreover, the therapeutic effect of steroid treatment prior to an ETX challenge is related to the steroid-induced block in macrophage TNF production (24). These findings indeed implicate a role for endogenous TNF in the mortality and morbidity observed during endotoxicosis and septic shock.

2. Hemodynamic comparisons: mean blood pressure and pulse rate

Even though comparably lethal doses of ETX and TNF were used in this study, the pattern of blood pressure changes induced by each agent differed. ETX repeatedly produced a significant hypotensive response, both in this study and in other studies (107,116). TNF, on the other hand, did not produce any significant change in MBP as demonstrated in this study using conscious rats and in other studies using phase I cancer patients (31,276) or conscious rats administered a non-lethal dose of TNF (13). In contrast, studies performed by other investigators using anesthetized dogs (253) and rats (249) demonstrated significant TNF-induced hypotension. Since anesthesia is known to interfere with cardiovascular function, the hypotension observed after TNF administration in anesthetized animals suggests that the response to TNF was altered by the state of the animals.

Even though TNF did not significantly alter MBP under conscious conditions, TNF did induce a significant tachycardia in conscious rats. Similarly, previous studies have demonstrated a significant elevation in heart rate after peak blood TNF levels were reached subsequent to an ETX injection (174,175) or during a continuous infusion of TNF (175) in conscious humans. The tachycardia-inducing property of TNF implicates TNF as a mediator of the persistant tachycardia observed in ETX treated rats long after the MBP returned to control levels after the initial hypotensive response.

Thus, a comparison between the hemodynamic changes induced by ETX and those induced by TNF suggest the following: 1) the transient hypotension elicited by ETX is not mediated by endogenously produced TNF since exogenous TNF did not elicit a hypotensive response; and 2) the persistant tachycardia observed in ETX treated rats long after the MBP has returned to control values is possibly mediated by endogenously produced TNF.

3. Metabolic comparisons: plasma glucose and lactate

Both ETX and TNF elicited an initial hyperglycemia. However, the initial hyperglycemia observed during endotoxicosis (73,106,137,282) most likely is not mediated by the hyperglycemia-inducing ability of TNF (125,249). This conclusion is based on the following observations. First, the hyperglycemia induced by ETX occurred more rapidly than the rise in plasma glucose induced by exogenous TNF. Second, the hyperglycemia induced with high dose TNF was only a fraction of the hyperglycemia elicited by both low and high dose ETX. Since the TNF induced effects are delayed and reduced compared to those induced by ETX, it is unlikely that endogenous TNF mediated the ETX induced hyperglycemia. Third, the increase in plasma glucose observed after ETX administration occurred coincident with rather than subsequent to the production of endogenous TNF (28,90,95,103,161,174,175,266). Although the hyperglycemia inducing property of TNF probably was not involved in the initial hyperglycemia of endotoxicosis, it might be involved in delaying, but not preventing, the hypoglycemia which is observed late in the course of endotoxicosis.

More dramatic was the profound hypoglycemia demonstrated during both ETX- and TNF-induced shock (figure 7). Rats that succumbed to the lethal effects of ETX (73,77,106,137,187,282) and TNF (16,125,249) did so with accompanying hypoglycemia and convulsive episodes. It may be that endogenous TNF is responsible for the hypoglycemic effect of ETX either by interfering with hepatic glucose production through modulation of glycogenolysis and/or gluconeogenesis or by enhancing glucose utilization. The direct effect of TNF on hepatic glucoregulation will be discussed in section C. However, TNF may participate in enhanced peripheral glucose utilization by increasing glucose uptake (142) and utilization by muscle (142) and macrophage-rich tissues (173). Moreover, since chronic exposure to TNF modulates the utilization of two important energy sources, fat and protein (254), it may be reasonable to suspect that TNF also interferes with the regulation of the third energy substrate, glucose.

The hyperlactacidemia which accompanies endotoxicosis (137,187, 282) may be mediated, in part, by endogenous TNF (16,249,253). Since the hyperlactacidemia induced by low and high dose ETX was significantly earlier than that elicited by high dose TNF, it suggests that the initial increase in plasma lactate probably is not mediated by endogenous TNF activity. The initial rise in plasma lactate observed after ETX administration probably results from the increased availability of glucose under conditions of hypoperfusion or hypoxia due to the ETX-induced hypotension. However, the progressive elevation in plasma lactate observed subsequent to high dose TNF administration suggests that TNF is more likely involved in the persistant elevation in plasma lactate observed after ETX administration. The progressive hyperlactacidemia which develops after TNF administration probably is the result of TNF-induced tissue hypoxia. TNF-induced endothelial cell procoagulant activity (29,186) and endothelial cell disruption (242) compromise organ blood flow and oxygen delivery, which leads to an elevation in plasma lactate concentrations and organ damage.

Thus, the comparison between the metabolic changes observed during endotoxicosis to those induced by TNF showed the following: 1) endogenous TNF most likely is not involved in the initial hyperglycemia observed after ETX administration, although it may participate in maintaining plasma glucose concentrations at elevated levels; 2) endogenous TNF activity is more likely to participate in eliciting the profound hypoglycemia observed late in the course of endotoxicosis; and 3) endogenous TNF activity contributes to the hyperlactacidemia observed during the latter stages of endotoxicosis as a result of TNF-induced tissue hypoxia.

4. Neuro-endocrine comparisons: alterations in plasma insulin, catecholamines, and corticosterone

The hormonal changes observed after TNF and ETX administration strongly support the role of TNF as a mediator of these endocrine alterations. The hypoinsulinemia observed late after ETX administration, as reported here and by Kelleher, _et al_. (123), may result from the accompanying hypoglycemia. However, as observed in this study, the magnitude of the hypoinsulinemic responses induced by ETX and TNF were larger than warranted based on the accompanying plasma glucose concentrations, thus suggesting that TNF may contribute to the severity of the hypoinsulinemia of sepsis. This conclusion is supported not only by the in vivo comparison, but also by the in vitro studies which demonstrated that TNF reduced insulin secretion by 40% (18) without damaging islet integrity (153) or insulin content (18).

Additionally, although not dramacically demonstrated in this study, a significant basal hyperinsulinemia usually occurs within the first 90 minutes after ETX administration (35,117,123) as a result of pancreatic hypersecretion (283). TNF also may contribute to the hyperinsulinemia of endotoxicosis, as demonstrated by the slight increase in plasma insulin concentrations reported after high dose TNF (figures 13 and 14) and by the 3 fold increase in plasma insulin reported by Bagby, et al. (13). Their conclusions, however, are limited since the preinjection control insulin concentrations were not reported. While the data supporting the role of TNF in the hypoinsulinemia of endotoxic shock is credible, further studies need to be done in order to ascertain the ability of TNF to modulate the hyperinsulinemic response observed early during endotoxicosis.

The elevations in plasma epinephrine and norepinephrine concentrations induced by exogenous TNF suggest that TNF partially mediates the increase in plasma catecholamine concentrations reported during endotoxicosis. Although the effects of ETX on plasma catecholamines were not evaluated in this study, Jones and Romano (116) documented the changes in conscious, cannulated rats: plasma epinephrine and norepinephrine concentrations increased dramatically within the first 30 minutes after the bolus injection of ETX and remained significantly elevated for 360 minutes. The initial elevation in plasma catecholamine levels coincided with the hypotensive effect of ETX, thereby suggesting that the initial elevations in plasma epinephrine and norepinephrine were a reflex response to the ETX-induced hypotension. However, plasma catecholamine concentrations remained elevated for 360 minutes, which was long after the mean blood pressure had returned to control levels at 120 minutes. In light of the increase in plasma catecholamine concentrations induced by exogenous TNF administration both in this study and in studies performed by other investigators (13,253), the persistent elevation in plasma epinephrine and norepinephrine observed during endotoxicosis may result from endogenous TNF activity. Although the mechanism responsible for the TNF-induced elevation in plasma catecholamine concentrations is unknown, there are three possible explanations for this finding. First, TNF may increase sympathetic activity by acting directly on the hypothalamus to increase sympathetic outflow. Second, TNF may increase circulating levels of plasma catecholamines by interfering with peripheral sympathetic activity. TNF may directly increase norepinephrine release at the nerve terminals and/or interfere with norepinephrine reuptake and degradation. Third, the elevation in plasma epinephrine, primarily, may result from TNF-stimulated release from the adrenal medulla. Even though TNF's mechanism of action was not examined in this study, the findings suggest that the elevated catecholamine concentrations observed during endotoxicosis may result from two stimuli: 1) an initial baroreceptor reflex response to the ETX induced hypotension resulting in the immediate elevation in plasma catecholamine concentration; and 2) endogenous TNF induced increase in plasma epinephrine and norepinephrine by, as yet, an unknown mechanism.

Finally, elevation in the stress hormone corticosterone during endotoxicosis may result from endogenous TNF activity. Although it was not determined in this study, plasma corticosterone concentrations are generally increased during endotoxicosis (107,123): plasma levels increase approximately 45% two hours after a bolus injection of ETX. As shown in this study and in other studies (253,276), TNF induced a significant increase in plasma corticosterone concentrations within 30 minutes. This may have resulted directly from a stimulatory effect of TNF on the adrenal glands or indirectly by TNF increasing plasma adrenocorticotrophic hormone levels (174,175). The time course for the elevation in plasma corticosterone concentrations observed after ETX administration compared to the response elicited with exogenous TNF suggests that endogenous TNF produced in response to an ETX challenge may mediate the increase in plasma corticosterone reported during endotoxicosis.

These observations indicate that TNF may be a primary mediator of the following neuro-endocrine alterations observed during endotoxic shock: 1) hypoinsulinemia; 2) persistant elevation of plasma epinephrine and norepinephrine; and 3) elevated corticosterone concentrations.

5. Additional pathophysiological effects

In addition to the lethal, hemodynamic, metabolic, and neuroendocrine effects examined in this study, TNF may mediate some additional pathophysiological changes observed during endotoxicosis, such as: fever (31,66,126,154,156,276); stimulation of acute phase protein synthesis

(59,151,178,181,199,236,276); muscle protein degradation (276); depressed plasma iron concentrations (175); complement activation (181); increased procoagulant activity (29,186); decreased lipogenesis (194,196,248); and PMN infiltration (58,176,229). Exogenous TNF also induced histological changes in the lungs, liver, kidneys, and gastrointestinal tract which were similar to those observed during endotoxicosis, such as PMN infiltration, focal areas of necrosis, and vascular congestion (personal observations) (88,195,210,249,253,254). All these changes induced by TNF administration alone further support the belief that endogenous TNF activity is a primary mediator of pathophysiological changes observed during septic shock.

6. Other mediators and interactions

Significant concentrations of TNF are measurable not only after ETX challenge, but also during bacterial meningitis (147), visceral leishmaniasis (227), malaria (227), meningococcal meningitis, septicemia, or both meningitis and septicemia (269). As discussed previously, there appears to be a positive correlation between the severity of the insult and the amount of TNF detected (103,224). However, detection of plasma TNF alone is not enough to determine the severity of the insult (127,179), therefore suggesting that additional inflammatory mediators may be just as important in the progression of lethal infections (103). One such mediator, interleukin 1, might have an important role in the pathophysiology observed after TNF and ETX administration. TNF stimulates IL-1 production and release both in vivo (66) and in vitro (12,62,150,185,202) as does ETX (57,65). As reviewed by Dinarello (65), IL-1 mediates many of the responses prevalent during inflammation such as fever, neutrophilia, alterations in plasma divalent cations, synthesis of acute phase proteins, stimulation of muscle proteolysis, and stimulation of B and T cell function. IL-1 also induces hypoglycemia (63,104) and enhances the rate of glucose clearance after a glucose challenge (219). Endocrine alterations resulting from IL-1 administration include hyperinsulinemia (63,219), hyperglucagonemia (63) and elevated serum corticosterone levels (213). These findings suggest that endogenous IL-1 may be an important mediator in both ETX- and TNF-induced shock, since many of the pathophysiological changes observed after ETX and TNF administration can also be produced by IL-1.

More importantly, perhaps, may be the interaction between TNF and IL-1. IL-1 increases the lethal effects of TNF (268): the LD_{50} dose for TNF is reduced 3 fold when administered in conjunction with a non-lethal dose of IL-1. The shock-like (190) and lethal effects (268) observed after co-treatment with TNF and IL-1 were more than additive. Furthermore, TNF and IL-1 synergize in stimulating PMN infiltration (274), islet cell destruction (206), and tumor cell cytostasis (192).

Other mediators, such as arachidonic acid metabolites, oxygen derived free radicals, and platelet activating factor, also may contribute to the pathogenesis of TNF and ETX. Prostaglandin E_2 and prostacyclin production and release are stimulated by TNF (12,61,120, 125,182) and ETX (84,124,144) and may mediate the fever, glucose alterations, gastrointestinal damage, and mortality observed after either agent (66,84,124,125,144,182,235,247). Oxygen derived free radicals released from infiltrating PMNs in response to TNF (11,140,166,260,277) and ETX (84) challenge contribute to the tissue damage elicited by each agent. Platelet-activating factor, likewise, is released in response to TNF (38,245) and ETX (44,99,138,271) and may contribute to the hemodynamic changes as well as the tissue injury demonstrated after their administration. Furthermore, macrophage released mediators such as macrophage insulin-like activity (74,284) and macrophage insulin releasing activity (79) may be important in mediating the glucose and endocrine alterations observed during endotoxicosis.

IFN-γ is also involved in the pathogenesis of endotoxicosis. It is released after ETX stimulation (264) and subsequently increases TNF production (27,36,52,91,92) and TNF binding sites (5,217,257,259). Moreover, IFN-γ synergizes with TNF in causing mortality (247), cytotoxicity (45,244), and tissue damage to organs such as liver, lung, gastrointestinal tract, spleen, and uterus (247). The sequential production of TNF, IL-1, and IFN-γ in response to an ETX or bacterial challenge may be crucial in the development of irreversible shock (103): all three mediators are produced sequentially after a lethal bacterial challenge, whereas only TNF and IL-1 are sequentially produced after a non-lethal endotoxin challenge.

C. IN VIVO EFFECTS OF TNF PLUS ETX CO-TREATMENT

1. Mortality and morbidity

A further assessment of TNF as a mediator of endotoxic shock was performed using a co-treatment model to determine whether low doses of each agent given within seconds of each other would produce a highly lethal shock-like effect similar to that observed after challenge with highly lethal doses of either agent given alone. In this study, the effects of TNF plus ETX co-treatment on mortality, hemodynamic, metabolic, and endocrine parameters were evaluated. As reported in this study and by Rothstein and Schreiber (214), exogenous TNF plus exogenous ETX co-treatment produces a highly lethal response within a matter of hours. TNF not only synergizes with exogenous ETX (214), but also enhances the lethal effects of endogenous bacteria released subsequent to cecal ligation and puncture (263).

The enhanced lethality observed after TNF plus ETX co-treatment may be mediated by the dual elevations in plasma TNF concentrations produced in response to both agents. As demonstrated by Mathison, et al. (161), infusion of exogenous TNF followed by a bolus injection of ETX produced 2 peaks in blood TNF concentrations: the initial peak corresponded to the infused exogenous TNF and the second peak corresponded to endogenous TNF production and release in response to the ETX challenge. This response is unique to stimulation with TNF and ETX, for repeated stimulation with a single agent, such as ETX or TNF, did not produce such a response. Stimulation with repeated doses of ETX (90,161) or repeated doses of TNF (272) failed to elicit repeated elevations in plasma TNF concentration. Macrophages produce a limited quantity of TNF in response to ETX challenge, therefore repeated stimulation with ETX does not increase the amount of TNF produced (25,81,90,91). The inability to repeatedly elevate plasma TNF concentrations may be the basis for the desensitization which occurs relative to the respective stimulus.

The synergism observed between TNF and ETX may be a major component in the pathophysiology of TNF- and ETX-induced shock. Significant gastrointestinal damage is induced by both agents, which may result in the release of gut-derived bacteria and endotoxin. This, in turn, may exacerbate the condition in two ways. First, the gut-derived bacteria and ETX can synergize with circulating TNF, thus augmenting the mortality and pathophysiological changes elicited by each agent. Secondly, the gut-derived bacteria and ETX may stimulate the macrophages to release TNF, thereby maintaining or further increasing circulating levels of TNF. Moreover, ETX interacts with the macrophages, perhaps through the myristoylation of macrophage receptors (2,3), and increases intracellular protein kinase C levels (218,279). Elevation of intracellular protein kinase C stimulates TNF mRNA production (102) as well as reduces TNF receptor binding (4,109,115,262), thereby further increasing circulating levels of TNF. Low concentrations of circulating TNF appear to have beneficial effects which destroy invasive agents and protect the host. However, it appears that extremely high concentrations of TNF and/or prolonged elevation of TNF may mediate the eventual demise of the host.

2. Hemodynamic, metabolic, and endocrine effects

The hemodynamic, metabolic, and endocrine changes induced by TNF plus ETX co-treatment were evaluated with the expectation that they might provide some insight in how these agents combine to produce a highly lethal shock-like effect. TNF plus ETX co-treatment may compromise cardiac function, as evidenced by the failure to elicit a significant tachycardia in response to the significant hypotension observed 30 minutes afer TNF plus ETX co-treatment. Highly lethal doses of either TNF or ETX induced significant elevations in heart rate even though high dose TNF did not induce an initial hypotension. However, co-treatment prevented such a response, suggesting that ETX plus TNF together may be directly toxic to the heart or that the reflex stimulation of catecholamine release was somehow compromised. However, since plasma epinephrine and norepinephrine levels were not determined for the TNF plus ETX co-treated animals, these suggestions are all speculative.

The possibility that TNF plus ETX co-treatment compromises cardiac function is supported by the greater degree of hyperlactacidemia produced after co-treatment than produced after treatment with high doses of either agent alone. Elevated lactate concentrations are indicative of anaerobic metabolism due to inadequate oxygen supply. Insufficient oxygen delivery to the vital organs may result from inadequate cardiac function and/or inadequate blood flow due to enhanced procoagulant activity.

The delayed increase in insulin secretion relative to the hyperglycemia observed after TNF plus ETX co-treatment indicates that the responsiveness of the islets to a glucose stimulus was compromised or that the blood supply to the pancreas was altered. Normally, insulin levels increase in less than 10 minutes after a glucose stimulus (96), whereas after TNF plus ETX co-treatment, 60 minutes passed before a significant hyperinsulinemia was elicited in response to the hyperglycemic stimulus.

The lethal hypoglycemia observed after treatment with high doses of TNF or ETX alone was also induced in the TNF plus ETX co-treated rats. The hypoglycemia accompanying the death of the rats occurred earlier, as evidenced by the 100% mortality rate in 4 hours. These extremely low plasma glucose concentrations suggest that the fuel delivery to the vital organs was probably inadequate to meet metabolic demands and therefore contributed to the demise of the animals.

The enhanced lethality and pathophysiological changes induced by TNF plus ETX co-treatment may be caused not only by the dual elevation in plasma TNF levels butt also by additional mediators known to be released in response to TNF and ETX. Perhaps it is the combination of mediators at various concentrations which induce irreversible shock since TNF synergizes with ETX, IL-1 and IFN-γ. Experiments with "mediator cocktails" analogous to Coley's cocktails may provide some important insights. By controlling the levels of a particular mediator in question with specific antibodies, the mediator cocktails can be given in the hopes of ascertaining which mediators at what concentrations are required to induce a shock like state.

C. IN VITRO EFFECTS OF TNF ON HEPATIC GLUCOREGULATION

The *in vivo* changes induced by exogenous TNF suggested that it may actively mediate some of the pathophysiological changes in plasma glucose and lactate concentrations observed during endotoxic shock. Because the liver functions as the major producer of plasma glucose, TNF was tested *in vitro* for direct hepatic glucoregulatory activity using two *in vitro* models: isolated perfused rat livers and isolated liver cells. The perfused rat liver model was used to examine the direct effects of TNF on whole organ function vis-a-vis alterations in net glucose and lactate production, oxygen consumption, and organ perfusion. This *in vitro* model of hepatic function would demonstrate: 1) if TNF altered plasma glucose and lactate production by interfering with organ blood flow via changes in vascular resistance; and 2) if TNF influenced hepatic glucose and lactate production by stimulating mediator release, such as prostaglandins, from the lining endothelial and Kupffer cells (48,93). The isolated hepatocytes demonstrated only the direct effects of TNF on parenchymal cell glucose and lactate production, since the preparations were virtually devoid of contaminating non-parenchymal cells. Together, these two in vitro models would ascertain whether or not TNF influences hepatic glucoregulation and if the response was a direct TNF-induced alteration in hepatocyte glucogenesis or a secondary response due to TNF-induced mediator release and/or vascular tone alteration.

The direct hepatic glucoregulatory effect of TNF was initially examined using the isolated perfused rat liver model. Initial exogenous TNF concentrations in the perfusate were comparable to plasma TNF concentrations found circulating in vivo after a lethal ETX challenge. Exogenous TNF failed to modulate net glucose production by isolated perfused livers, suggesting that the slight hyperglycemia observed after TNF administration was not due to TNF-stimulated hepatic glucose production. If TNF either directly stimulated liver glucose production or indirectly stimulated glucose production through the production of prostaglandins, changes in net glucose production by the isolated perfused rat livers would have occurred within the time frame of the perfusions (148). Furthermore, the slight hyperglycemia observed after TNF administration was not stimulated by TNF-induced hepatic hypoperfusion or inadequate oxygen consumption since such alterations in flow rate and oxygen consumption were not demonstrated with the isolated perfused livers.

When applied to the ETX model of septic shock, these in vitro observations suggest that endogenous TNF probably is not directly responsible for the hyperglycemia observed shortly after ETX administration. However, our inability to directly stimulate glucose production in vitro with ETX using isolated perfused livers suggested that a mediator was responsible for the hyperglycemic effect of ETX (data not shown). Only one study conducted by Casteleijn, et al. (42) demonstrated a direct glycogenolytic effect of ETX using perfused livers. The perfusion flow rate, however, was insufficient for adequate oxygen delivery to the perfused livers, thus implying that the perfused livers in their study were hypoxic. The data in this study proposes that the hyperglycemia observed during ETX- and TNF-induced shock is mediated at least in part by the elevated catecholamine levels produced indirectly by the reflex response to ETX-induced hypotension and directly by the administration of exogenous TNF. The elevated corticosterone levels observed at 2 hours and 30 minutes after ETX and TNF challenge, respectively, also contributed to the plasma glucose concentration by stimulating glucose production by the liver.

The hypothesis that a hepatic action of TNF may be responsible for mediating the hypoglycemia observed late in the course of ETX- and TNF-induced shock is not supported by the perfused liver data. TNF failed to significantly reduce net glucose production rate under both basal and glucagon-stimulated conditions. If TNF directly prevented the mobilization of glucose or interfered with glucose mobilization in response to hormone stimulation, then the perfused liver model would have demonstrated such a response. Perhaps, longer treatment with TNF is required to elicit such an effect, since treatment with TNF for more than 12 hours was required to demonstrate a direct in vitro effect of TNF on hepatic protein synthesis (59,151,181,199). Additionally, other mediators released by TNF and ETX administration may be involved in the hypoglycemia. For instance, a significant hypoglycemic response was demonstrated after IL-1 administration (63,104), but preliminary studies from this laboratory failed to demonstrate a direct glucoregulatory effect of IL-1 on perfused rat livers.

The hyperlactacidemia observed after ETX and TNF administration suggested that TNF may be responsible for the hyperlactacidemia of septic shock. When examined using perfused rat livers, TNF did not induce any changes in hepatic lactate production under basal or hormone stimulated conditions, thus suggesting that the elevated lactate concentrations observed after TNF and ETX treatment were not produced as a result of altered hepatic function. This hypothesis is supported by the following observations. First, TNF did not directly stimulate lactate production by the perfused rat livers, indicating that the hyperlactacidemic effect of TNF was not the result of enhanced basal lactate production by the liver. Second, TNF did not interfere with glucagon-stimulated hepatic uptake of lactate, suggesting that the gluconeogenic ability of the liver was not compromised by treatment with TNF. Third, TNF did not stimulate lactate production by altering hepatic perfusion flow or oxygen consumption, thus suggesting that liver function is not compromised by TNF treatment in a blood-free media. However, since the livers were perfused with a KRB media and not with whole blood, this system was devoid of the procoagulant activity induced by TNF (29,186). Since TNF strongly stimulates blood coagulation, our data suggests that the hyperlactacidemia observed during TNF- and ETX-induced shock probably resulted from tissue hypoxia due to inadequate oxygen delivery rather than a direct TNF-induced alteration in liver function.

In order to assure that the results obtained using the perfused liver model were a true indication of the short term effects of TNF on hepatic function, isolated liver parenchymal cells were challenged with TNF directly. In this manner, the initial concentration of TNF directly stimulating the hepatic parenchymal cells was dependent on the concentration of TNF in the incubation medium. Unlike the perfused liver model, these isolated cell preparations were virtually devoid of contaminating non-parenchymal cells such as endothelial and Kupffer cells which may interfere with the concentration of TNF directly in contact with the parenchymal cells. Since the concentration of TNF directly in contact with hepatocytes after ETX stimulation _in vivo_ has not been determined, this model allowed for a range of TNF concentrations to be tested directly. Isolated hepatocytes were incubated directly with TNF, at concentrations ranging from 0.01 to 5.0 μg/ml. In agreement with the isolated perfused liver data reported in this study and the isolated hepatocyte data of Rofe, _et al_. (212), TNF did not enhance the basal glucose production by isolated hepatocytes. Thus, the hyperglycemia induced with TNF does not result from a direct hepatic action of TNF. Instead, the hyperglycemia observed during TNF- and ETX-induced shock is most likely mediated by the elevated catcholamine as well as corticosterone levels, as discussed previously.

TNF also did not interfere with hormone-stimulated glucose production using two glucogenic hormones; glucagon and vasopressin. Glucagon and vasopressin stimulate glucose production via two different intracellular mechanisms: glucagon stimulates hepatic glucose production by a cAMP-dependent mechanism whereas vasopressin stimulates glucose production by a Ca^{++}-dependent mechanism. TNF did not interfere with the ability of either hormone to stimulate hepatic glucose production, supporting the hypothesis that TNF treatment does not alter the intracellular second messengers involved in hepatic glucose production.

TNF not only failed to influence basal and hormone-stimulated glucose production, but also failed to alter the gluconeogenic capability of the liver as assessed by net glucose production (212). Although not shown in this dissertation, TNF at concentrations of 0.01-5 μg/ml did not alter the gluconeogenic capability of isolated hepatocytes. A similar finding was demonstrated by the depressed lactate production observed with glucagon-stimulated hepatocytes. TNF did not inhibit the uptake of the gluconeogenic substrate lactate by the isolated hepatocytes stimulated with the gluconeogenic-stimulatory hormone, glucagon. Thus, this data supports the hypothesis that the hypoglycemia observed during the agonal stages of TNF- and ETX-induced shock was not mediated by a direct TNF-induced inhibition of hepatic gluconeogenesis. Furthermore, increased hepatic glucose utilization by the parenchymal cells of the liver does not seem to be involved in the hypoglycemia induced by TNF and ETX since TNF failed to directly enhance glucose utilization by isolated hepatocytes _in_ _vitro_. Perhaps, as proposed by Meszaros, _et al_. (173), enhanced glucose utilization by the macrophage-rich organs may contribute to the hypoglycemia of TNF- and ETX-induced shock. Since glucose utilization by the isolated perfused liver was not assessed in this study, that may be a direct hepatic effect of TNF. Additional studies evaluating the role of TNF on hepatic glucoregulation may require longer treatment with TNF or more sensitive indices of altered hepatic function such as changes in key glucoregulatory enzyme levels and activity.

The _in vitro_ data obtained using isolated perfused rat livers and isolated liver parenchymal cells suggests that TNF does not mediate the hyper- and hypoglycemia observed during TNF- and ETX-induced shock by directly altering hepatic glucoregulation. However, previous studies from this laboratory (76) demonstrated that the glucoregulatory changes observed during endotoxic shock could not be reproduced _in vitro_ using isolated hepatocytes stimulated with ETX. Therefore, mediators released from the sinusoidal Kupffer cells were proposed to be responsible for the glucoregulatory changes observed after ETX administration (41,171). In light of the results reported in this study and by Rofe _et al_. (212), the search for a direct hepatic glucoregulatory mediator is not over. Additional _in vitro_ studies using a number of important inflammatory mediators such as IL-1, IFN-γ, and TNF, as well as the development of techniques to measure the concentrations of inflammatory mediators in contact with hepatic parenchymal cells _in vivo_, may provide further data concerning the role of inflammtory mediators in hepatic glucoregulation.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study examined the role of TNF in the pathophysiology of septic shock. In specific, the role of TNF as an important mediator of the endotoxic syndrome was evaluated in three ways. First, the hemodynamic, metabolic, and endocrine alterations induced by bolus injections of ETX were compared to the hemodynamic, metabolic, and neuro-endocrine alterations induced by comparably lethal injections of TNF using conscious, unrestrained, cannulated, adult rats. Second, the lethal, hemodynamic, metabolic, and endocrine alterations induced by the co-administration of low doses of TNF plus ETX were examined. Third, the direct glucoregulatory effects of TNF on liver glucose and lactate production were examined using two different in vitro models -- isolated perfused rat livers and isolated liver parenchymal cells. The following conclusions were derived from the data in this study.

1) TNF induced a dose-dependent, lethal, shock-like state in conscious, unrestrained, cannulated rats similar to that induced by ETX.

2) The morbidity induced after TNF administration was identical to that induced after ETX administration: rats exhibited such signs as piloerection, hunched-over posture, labored breathing, and progressive cyanosis.

3) The initial hypotension induced after ETX administration was not mediated by endogenous TNF activity, for exogenous TNF did not induce any significant changes in mean blood pressure.

4) Endogenous TNF may mediate the prolonged elevation in heart rate observed after the mean blood pressure returned to control values following the hypotensive response to ETX administration: high dose exogenous TNF induced a progressive increase in pulse rate.

5) The elevated pulse rates observed after TNF administration may be a secondary response due to the elevation in plasma epinephrine and norepinephrine concentrations induced by TNF.

6) Both TNF and ETX induced a hyperglycemic response. However, the hyperglycemia observed after ETX and TNF administration did not result from a direct hepatic effect of TNF, as evidenced from the inability to demonstrate an increase in the net glucose production rates by isolated perfused livers and isolated hepatocytes treated with TNF in vitro.

7) The hyperglycemia induced after ETX and TNF administration was probably mediated by the elevated catecholamine and corticosterone concentrations observed following ETX and TNF administration.

8) Both TNF and ETX elicited a profound hypoglycemia coincident with the death of the rats. The hypoglycemia observed during the agonal stages of TNF- and ETX-induced shock was probably not mediated by a direct effect of TNF on hepatic function: TNF did not interfere with the net glucose production rates of isolated perfused livers or isolated liver cells.

9) Endogenous TNF activity may contribute to the hyperlactacidemia that developed after ETX administration, for a progressive hyperlactacidemia developed subsequent to exogenous TNF administration.

10) Tissue hypoxia and organ damage which develop as a result of TNF-induced procoagulant activity and vascular disruption may be responsible

for the hyperlactacidemia observed after TNF and ETX administration.

11) TNF may mediate the hyperinsulinemia reported during endotoxicosis.

12) The hypoinsulinemia observed after TNF administration suggested that TNF may be responsible for the hypoinsulinemia observed during the latter stages of endotoxicosis by reducing insulin release in response to a glucose stimulus.

13) Endogenous TNF-induced elevation in plasma epinephrine and norepinephrine concentrations may contribute to the persistant elevation in plasma catecholamines observed during endotoxicosis after the mean blood pressure returned to control values. Exogenous TNF induced a progressive increase in plasma epinephrine and norepiniephrine concentrations by acting centrally, peripherally, or on the adrenal medulla to increase the circulating concentrations of catecholamines.

14) Endogenous TNF may mediate the elevation in plasma corticosterone concentrations observed after ETX administration: exogenous TNF induced a significant elevation in plasma corticosterone.

15) Co-treatment with low doses of TNF plus ETX augmented the lethal effects of both agents.

16) The mechanism responsible for the enhanced lethality observed after co-treatment with low doses of TNF plus ETX remains to be determined, however, compromised cardiac function may be a possibility.

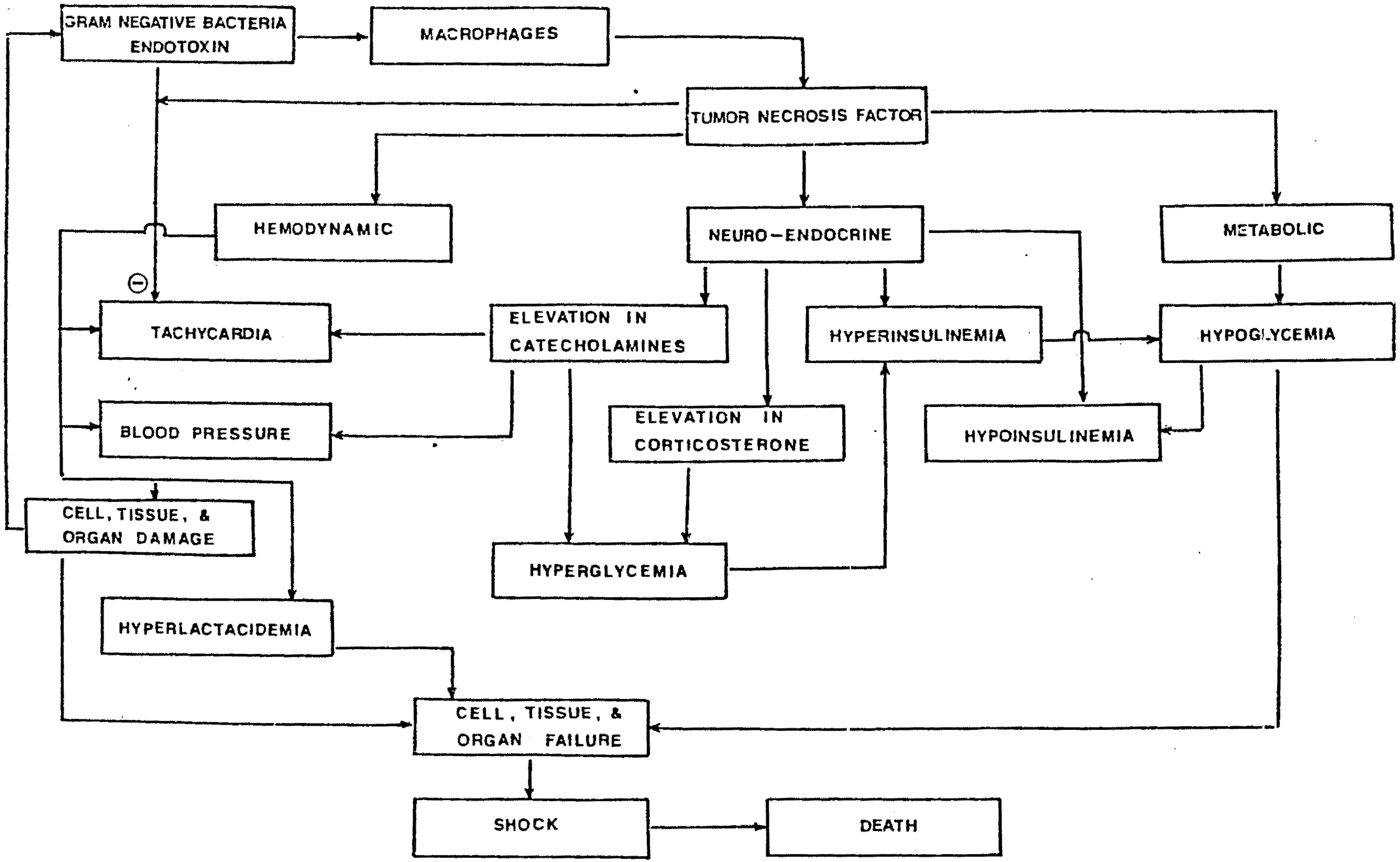
17) TNF does not directly influence short-term hepatic glyogenolysis, gluconeogenesis, or glucose utilization under both basal and hormone-stimulated conditions _in vitro_.

18) TNF does not directly interfere with _in vitro_ liver perfusion flow or oxygen consumption.

Figure 19 is a schematic representation of TNF's role in the pathogenesis of septic shock, as caused by invading gram negative bacteria and its constituent ETX. TNF is surely an important mediator of the hemodynamic, metabolic, and neuro-endocrine alterations associated with septic shock. However, to conclude that TNF is the major mediator of the lethal effects of septic shock would be naive in light of the other inflammatory mediators known to be released and to interact with TNF, such as IL-1 and IFN-γ. Moreover, the interaction between TNF and ETX suggests that the organ pathology induced _in vivo_, in particular the hemorrhagic necrosis of the gastrointestinal tract, may be a crucial factor contributing to the lethal demise of the host.

Further studies examining the role of inflammatory mediators in the pathophysiology accompanying gram negative bacterial infection may provide helpful insights into the sequence of events which are responsible for causing a gram negative infection to progress to a lethal state of septic shock. In this way, therapeutic intervention can be better directed toward the lethal agent(s) and thus decrease the mortality associated with this modern day health problem.

FIGURE 19: TUMOR NECROSIS FACTOR AND THE PATHOPHYSIOLOGY OF SEPTIC SHOCK


GRAM NEGATIVE BACTERIA ENDOTOXIN
MACROPHAGES
TUMOR NECROSIS FACTOR
HEMODYNAMIC
NEURO-ENDOCRINE
METABOLIC
⊖
TACHYCARDIA
ELEVATION IN CATECHOLAMINES
HYPERINSULINEMIA
HYPOGLYCEMIA
BLOOD PRESSURE
ELEVATION IN CORTICOSTERONE
HYPOINSULINEMIA
CELL, TISSUE, & ORGAN DAMAGE
HYPERGLYCEMIA
HYPERLACTACIDEMIA
CELL, TISSUE, & ORGAN FAILURE
SHOCK
DEATH

REFERENCES

1. ABE, S., T. GATANAGA, M. YAMAZAKI, G. SOMA, and D. MIZUNO. Purification of rabbit tumor necrosis factor. FEBS. Lett. 180:203-206, 1985.

2. ADEREM, A.A., K.A. ALBERT, M.M. KEUM, J.K.T. WANG, P. GREENGARD, and Z.A. COHN. Stimulus-dependent myristoylation of a major substrate for protein kinase C. Nature. 332:362-364, 1988.

3. ADEREM, A.A., M.M. KEUM, E. PURE, and Z.A. COHN. Bacterial lipopolysaccharides, phorbol myristate acetate, and zymosan induce the myristoylation of specific macrophage proteins. Proc. Natl. Acad. Sci. USA. 83:5817-5821, 1986.

4. AGGARWAL, B.B., and T.E. EESSALU. Effect of phorbol esters on down-regulation and redistribution of cell surface receptors for tumor necrosis factor-alpha. J. Biol. Chem. 262:16450-16455, 1987.

5. AGGARWAL, B.B., T.E. EESSALU, and P.E. HASS. Characterization of receptors for human tumour necrosis factor and their regulation by gamma-interferon. Nature 318:665-667, 1985.

6. AGGARWAL, B.B., and W.J. KOHR. Human tumor necrosis factor. Methods. Enzymol. 116:448-456, 1985.

7. AGGARWAL, B.B., W.J. KOHR, P.E. HASS, B. MOFFAT, S.A. SPENCER, W.J. HENZEL, T.S. BRINGMAN, G.E. NEDWIN, D.V. GOEDDEL, and R.N. HARKINS. Human tumor necrosis factor. Production, purification, and characterization. J. Biol. Chem. 260:2345-2354, 1985.

8. AGGARWAL, B.B., P.R. TRAQUINA, and T.E. EESSALU. Modulation of receptors and cytotoxic response of tumor necrosis factor-alpha by various lectins. J. Biol. Chem. 261:13652-13656, 1986.

9. ARMSTRONG, D., L.S. YOUNG, R.D. MEYER, and A.H. BLEVINS. Infectious complications of neoplastic disease. Med. Clin. North. Am. 55:729-745, 1971.

10. ATKINSON, Y.H., W.A. MARASCO, A.F. LOPEZ, and M.A. VADAS. Recombinant human tumor necrosis factor-alpha. Regulation of N-formylmethionylleucylphenylalanine receptor affinity and function on human neutrophils. J. Clin. Invest. 81:759-765, 1988.

11. BABIOR, B.M. Oxidants from phagocytes: agents of defense and destruction. Blood 64:959-966, 1984.

12. BACHWICH, P.R., S.W. CHENSUE, J.W. LARRICK, and S.L. KUNKEL. Tumor necrosis factor stimulates interleukin-1 and prostaglandin E2 production in resting macrophages. Biochem. Biophys. Res. Commun. 136:94-101, 1986.

13. BAGBY, G.J., C.H. LANG, D.M. HARGROVE, J.J. THOMPSON, L.A. WILSON, and J.J. SPITZER. Glucose kinetics in rats infused with endotoxin-induced monokines or tumor necrosis factor. Circ. Shock. 24:111-121, 1988.

14. BAGLIONI, C., S. MCCANDLESS, J. TAVERNIER, and W. FIERS. Binding of human tumor necrosis factor to high affinity receptors on HeLa and lymphoblastoid cells sensitive to growth inhibition. J. Biol. Chem. 260:13395-13397, 1985.

15. BARTHOLEYNS, J., M. FREUDENBERG, and C. GALANOS. Growing tumors induce hypersensitivity to endotoxin and tumor necrosis factor. Infect. Immun. 55:2230-2233, 1987.

16. BAUSS, F., W. DROGE, and D.N. MANNEL. Tumor necrosis factor mediates endotoxic effects in mice. Infect. Immun. 55:1622-1625, 1987.

17. BEISEL, W.R. Metabolic response to infection. Ann. Rev. Med. 26:9-20, 1975.

18. BENDTZEN, K., T. MANDRUP-POULSEN, J. NERUP, J.H. NIELSEN, C.A. DINARELLO, and M. SVENSON. Cytototoxicity of human pI7 interleukin-1 for pancreatic Islets of Langerhans. Science 232:1545-1547, 1986.

19. BERESINI, M.H., M.J. LEMPERT, and L.B. EPSTEIN. Overlapping polypeptide induction in human fibroblasts in response to treatment with interferon-alpha, interferon-gamma, interleukin 1 alpha, interleukin 1 beta, and tumor necrosis factor. J. Immunol. 140:485-493, 1988.

20. BERKOW, R.L., D. WANG, J.W. LARRICK, R.W. DODSON, and T.H. HOWARD. Enhancement of neutrophil superoxide production by preincubation with recombinant human tumor necrosis factor. J. Immunol. 139:3783-3791, 1987.

21. BERRY, M.N., and D.S. FRIEND. High-yield preparation of isolated rat liver parenchymal cells. J. Cell. Biol. 43:506-520, 1969.

22. BEUTLER, B., and A. CERAMI. Cachectin (tumor necrosis factor): a macrophage hormone governing cellular metabolism and inflammatory response. Endo. Rev. 9:57-66, 1988

23. BEUTLER, B., D. GREENWALD, J.D. HULMES, M. CHANG, Y.C. PAN, J. MATHISON, R. ULEVITCH, and A. CERAMI. Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature 316:552-554, 1985.

24. BEUTLER, B., N. KROCHIN, I.W. MILSARK, C. LUEDKE, and A. CERAMI. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science 232:977-980, 1986.

25. BEUTLER, B., J. MAHONEY, N. LE TRANG, P. PEKALA, and A. CERAMI. Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. J. Exp. Med. 161:984-995, 1985.

26. BEUTLER, B., I.W. MILSARK, and A.C. CERAMI. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 229:869-871, 1985.

27. BEUTLER, B., V. TKACENKO, I. MILSARK, N. KROCHIN, and A. CERAMI. Effect of gamma interferon on cachectin expression by mononuclear phagocytes. Reversal of the lpsd (endotoxin resistance) phenotype. J. Exp. Med. 164:1791-1796, 1986.

28. BEUTLER, B.A., I.W. MILSARK, and A. CERAMI. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. J. Immunol. 135:3972-3977, 1985.

29. BEVILACQUA, M.P., J.S. POBER, G.R. MAJEAU, W. FIERS, R.S. COTRAN, and M.A. GIMBRONE. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. Proc. Natl. Acad. Sci. USA. 83:4533-4537, 1986.

30. BLANCHARD, D.K., J.Y. DJEU, T.W. KLEIN, H. FRIEDMAN, and W.E. STEWART. Protective effects of tumor necrosis factor in experimental Legionella pneumophila infections of mice via activation of PMN function. J. Leukocyte. Biol. 43:429-435, 1988.

31. BLICK, M., S.A. SHERWIN, M. ROSENBLUM, and J. GUTTERMAN. Phase I study of recombinant tumor necrosis factor in cancer patients. Cancer Res. 47:2986-2989, 1987.

32. BROUDY, V.C., J.M. HARLAN, and J.W. ADAMSON. Disparate effects of tumor necrosis factor-alpha/cachectin and tumor necrosis factor-beta/lymphotoxin on hematopoietic growth factor production and neutrophil adhesion molecule expression by cultured human endothelial cells. J. Immunol. 138:4298-4302, 1987.

33. BROUDY, V.C., K. KAUSHANSKY, G.M. SEGAL, J.M. HARLAN, and J.W. ADAMSON. Tumor necrosis factor type alpha stimulates human endothelial cells to produce granulocyte/macrophage colony-stimulating factor. Proc. Natl. Acad. Sci. USA. 83:7467-7471, 1986.

34. BROWN, D.E., and D.C. MORRISON. Possible alteration of normal mechansisms of endotoxin toxicity in vivo by actinomycin D. J. Inf. Dis. 146:746-750, 1982.

35. BUCHANAN, B.J., and J.P. FILKINS. Insulin secretion and the carbohydrate metabolic alterations of endotoxemia. Circ. Shock. 3:267-280, 1976.

36. BURCHETT, S.K., W.M. WEAVER, J.A. WESTALL, A. LARSEN, S. KRONHEIM, and C.B. WILSON. Regulation of tumor necrosis factor/cachectin and IL-1 secretion in human mononuclear phagocytes. J. Immunol. 140:3473-3481, 1988.

37. CAMPBELL, I.L., L. OXBROW, J. WEST, and L.C. HARRISON. Regulation of MHC protein expression in pancreatic beta-cells by interferon-gamma and tumor necrosis factor-alpha. Mol. Endocrinol. 2:101-107, 1988.

38. CAMUSSI, G., F. BUSSOLINO, G. SALVIDIO, and C. BAGLIONI. Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. J. Exp. Med. 166:1390-1404, 1987.

39. CAPUT, D., B. BEUTLER, K. HARTOG, R. THAYER, S. BROWN-SHIMER, and A. CERAMI. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. Proc. Natl. Acad. Sci. USA. 83:1670-1674, 1986.

40. CARSWELL, E.A., L.J. OLD, R.L. KASSEL, S. GREEN, N. FIORE, and B. WILLIAMSON. An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA. 72:3666-3670, 1975.

41. CASTELEIJN, E., J. KUIPER, H.C.J. VAN ROOIJ, J.A.A.M. KAMPS, J.F. KOSTER, and T.J.C. VAN BERKEL. Hormonal control of glycogenolysis in parenchymal liver cells by Kupffer and endothelial cells. J. Biol. Chem. 263:2699-2703, 1988.

42. CASTELEIJN, E., J. KUIPER, H.C.J. VAN ROOIJ, J.A.A.M. KAMPS, J.F. KOSTER, and T.J.C. VAN BERKEL. Endotoxin stimulates glycogenolysis in the liver by means of intercellular communication. J. Biol. Chem. 263:6953-6955, 1988.

43. CAVENDER, D., Y. SAEGUSA, and M. ZIFF. Stimulation of endothelial cell binding of lymphocytes by tumor necrosis factor. J. Immunol. 139:1855-1860, 1987.

44. CHANG, S.-W., C.O. FEDDERSEN, P.M. HENSON, and N.F. VOELKEL. Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rats. J. Clin. Invest. 79:1498-1509, 1987.

45. CHAPEKAR, M.S., and R.I. GLAZER. The synergistic cytocidal effect produced by immune interferon and tumor necrosis factor in HT-29 cells is associated with inhibition of rRNA processing and (2',5') oligo (A) activation of RNase L. Biochem. Biophys. Res. Commun. 151:1180-1187, 1988.

46. CHEN, A.R., K.P. MCKINNON, and H.S. KOREN. Lipopolysaccharide (LPS) stimulates fresh human monocytes to lyse actinomycin D-treated WEHI-164 target cells via increased secretion of a monokine similar to tumor necrosis factor. J. Immunol. 135:3978-3987, 1985.

47. CHRISTMAS, S.E., A. MEAGER, and M. MOORE. Production of interferon and tumour necrosis factor by cloned human natural cytotoxic lymphocytes and T cells. Clin. Exp. Immunol. 69:441-450, 1987.

48. CLARK, M.A., M.J. CHEN, S.T. CROOKE, and J.S. BOMALASKI. Tumour necrosis factor (cachectin) induces phospholipase A2 activity and synthesis of a phospholipase A2-activating protein in endothelial cells. Biochem. J. 250:125-132, 1988.

49. COLEY, W.B. Contribution to the knowledge of sarcoma. Ann. Surg. 14:199-220, 1891.

50. COLEY, W.B. The treatment of malignant tumors by repeated inoculations of erysipelas, with a report of original cases. Am. J. Med. Sc. 105:487-511, 1893.

51. COLEY, W.B. Treatment of inoperable malignant tumors with toxins of erysipelas and the bacillus prodigiosus. Trans. Amer. Surg. Assn. 12:183-212, 1894.

52. COLLART, M.A., D. BELIN, J.D. VASSALLI, S. DE KOSSODO, and P. VASSALLI. Gamma interferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase genes, which are controlled by short-lived repressors. J. Exp. Med. 164:2113-2118, 1986.

53. COLLINS, T., L.A. LAPIERRE, W. FIERS, J.L. STROMINGER, and J.S. POBER. Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A,B antigens in vascular endothelial cells and dermal fibroblasts in vitro. Proc. Natl. Acad. Sci. USA. 83:446-450, 1986.

54. CREASEY, A.A., R. YAMAMOTO, and C.R. VITT. A high molecular weight component of the human tumor necrosis factor receptor is associated with cytotoxicity. Proc. Natl. Acad. Sci. USA. 84:3293-3297, 1987.

55. CURRIE, G.A., and C. BASHAM. Activated macrophages release a factor which lyses malignant cells but not normal cells. J. Exp. Med. 142:1600-1605, 1975.

56. CUTURI, M.C., M. MURPHY, M.P. COSTA GIOMI, R. WEINMANN, B. PERUSSIA, and G. TRINCHIERI. Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. J. Exp. Med. 165:1581-1594, 1987.

57. CYBULSKY, M.I., M.K. CHAN, and H.Z. MOVAT. Acute inflammation and microthrombosis induced by endotoxin, interleukin-1, and tumor

necrosis factor and their implication in gram-negative infection. Lab. Invest. 58:365-378, 1988.

58. CYBULSKY, M.I., D.J. MCCOMB, and H.Z. MOVAT. Neutrophil leukocyte emigration induced by endotoxin. Mediator roles of interleukin 1 and tumor necrosis factor alpha. J. Immunol. 140:3144-3149, 1988.

59. DARLINGTON, G.J., D.R. WILSON, and L.B. LACHMAN. Monocyte-conditioned medium, interleukin-1, and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. J. Cell Biol. 103:787-793, 1986.

60. DAVIS, J.M., M.A. NARACHI, N.K. ALTON, and T. ARAKAWA. Structure of human tumor necrosis factor alpha derived from recombinant DNA. Biochemistry. 26:1322-1326, 1987.

61. DAYER, J.M., B. BEUTLER, and A. CERAMI. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J. Exp. Med. 162:2163-2168, 1985.

62. DEBS, R.J., H.J. FUCHS, R. PHILIP, A.B. MONTGOMERY, E.N. BRUNETTE, D. LIGGITT, J.S. PATTON, and J.E. SHELLITO. Lung-specific delivery of cytokines induces sustained pulmonary and systemic immunomodulation in rats. J. Immunol. 140:3482-3488, 1988.

63. DEL REY, A., and H. BESEDOVSKY. Interleukin 1 affects glucose homeostasis. Am. J. Physiol. 253:R794-R798, 1987.

64. DILUZIO, N.R., and C.G. CRAFTON. Influence of altered reticulo-endothelial function on vascular clearance and tissue distribution of S. enteritidis endotoxin. Proc. Soc. Exp. Biol. Med. 132:686-690, 1969.

65. DINARELLO, C.A. Interleukin-1. Rev. Infect. Dis. 6:51-95, 1984.

66. DINARELLO, C.A., J.G. CANNON, S.M. WOLFF, H.A. BERNHEIM, B. BEUTLER, A. CERAMI, I.S. FIGARI, M.A. PALLADINO, and J.V. O'CONNOR. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J. Exp. Med. 163:1433-1450, 1986.

67. DROGE, W., B. BENNINGHOFF, and V. LEHMANN. Tumor necrosis factor augments the immunogenicity and the production of L-ornithine by peritoneal macrophages. Lymphokine. Res. 6:111-117, 1987.

68. DUPONT, H.L., and W.W. SPINK. Infections due to gram-negative organisms: an analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958-1966. Medicine 48:307-332, 1969.

69. ELIAS, J.A., R.C. KROL, B. FREUNDLICH, and P.M. SAMPSON. Regulation of human lung fibroblast glycosaminoglycan production by recombinant

interferons, tumor necrosis factor, and lymphotoxin. J. Clin. Invest. 81: 325-333, 1988.

70. ELLNER, J.J. Septic shock. Pediatr. Clin. North. Am. 30:365-371, 1983.

71. FEINGOLD, K.R., and C. GRUNFELD. Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat in vivo. J. Clin. Invest. 80: 184-190, 1987.

72. FELTY, A.R., and C.S. KEEFER. Bacillus coli sepsis. A clinical study of twenty-eight cases of blood stream infection by the colon bacillus. J. A. M. A. 82:1430-1433, 1924.

73. FILKINS, J.P. Phases of glucose dyshomeostasis in endotoxicosis. Circ. Shock. 5:347-355, 1978.

74. FILKINS, J.P. Endotoxin enhanced secretion of macrophage insulin-like activity. J. Reticuloendothelial. Soc. 27:507-511, 1980.

75. FILKINS, J.P. Monokines and the metabolic pathophysiology of septic shock. Fed. Proc. 44:28-32, 1985.

76. FILKINS, J.P., and B.J. BUCHANAN. In vivo versus in vitro effects of endotoxin on glycogenolysis, gluconeogenesis, and glucose utilization. Proc. Soc. Exp. Biol. Med. 155:216-218, 1977.

77. FILKINS, J.P., and R.P. CORNELL. Depression of hepatic gluconeogenesis and the hypoglycemia of endotoxin shock. Am. J. Physiol. 227:778-781, 1974.

78. FILKINS, J.P., J.M. LUBITZ, and J.J. SMITH. The effect of zymosan and glucan on the reticuloendothelial system and on resistance to traumatic shock. Angiology. 15:465-470, 1964.

79. FILKINS, J.P., and M.R. YELICH. Mechanism of hyperinsulinemia after reticuloendothelial system phagocytosis. Am. J. Physiol. 242: E115-E120, 1982.

80. FINLAND, M., W.F.,JR. JONES, and M.W. BARNES. Occurrence of serious bacterial infections since introductioon of antibacterial agents. J. A. M. A. 170:2188-2197, 1959.

81. FISCH, H., and G.E. GIFFORD. In vitro production of rabbit macrophage tun-- cell cytotoxin. Int J Cancer. 32:105-112, 1983.

82. FLICK, D.A., and G.E. GIFFORD. Pharmacokinetics of murine tumor necrosis factor. J. Immunopharmacol. 8:89-97, 1986.

83. FLICK, D.A., and G.E. GIFFORD. Production of tumor necrosis factor in unprimed mice: mechanism of endotoxin-mediated tumor necrosis. Immunobiol 171:320-328, 1986.

84. FLOHE, L., and H. GIERTZ. Endotoxins, arachidonic acid, and superoxide formation. Rev. Infect. Dis. 9:S553-S561, 1987.

85. FRANSEN, L., R. MULLER, A. MARMENOUT, J. TAVERNIER, HEYDEN.J. VAN DER, E. KAWASHIMA, A. CHOLLET, R. TIZARD, H. VAN HEUVERSWYN, A. VAN VLIET, M.-R. RUYSSCHAERT, and W. FIERS. Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. Nucleic. Acids. Res. 13:4417-4429, 1985.

86. FRATER-SCHRODER, M., W. RISAU, R. HALLMANN, P. GAUTSCHI, and P. BOHLEN. Tumor necrosis factor type alpha, a potent inhibitor of endothelial cell growth in vitro, is angiogenic in vivo. Proc. Natl. Acad. Sci. USA. 84:5277-5281, 1987.

87. GAMBLE, J.R., J.M. HARLAN, S.J. KLEBANOFF, and M.A. VADAS. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. Proc. Natl. Acad. Sci. USA. 82:8667-8671, 1985.

88. GASKILL, H.V. Continuous infusion of tumor necrosis factor: mechanisms of toxicity in the rat. J. Surg. Res. 44:664-671, 1988.

89. GHIARA, P., D. BORASCHI, L. NENCIONI, P. GHEZZI, and A. TAGLIABUE. Enhancement of in vivo immune response by tumor necrosis factor. J. Immunol. 139:3676-3679, 1987.

90. GIFFORD, G.E., and D.A. FLICK. Natural production and release of tumour necrosis factor. In: Tumour Necrosis Factor and Related Cytotoxins. Ciba Foundation Symposium 131, Chichester:John Wiley and Sons, 1987, p. 3-20.

91. GIFFORD, G.E., and M.L. LOHMANN-MATTHES. Requirement for the continual presence of lipopolysaccharide for production of tumor necrosis factor by thioglycollate-induced peritoneal murine macrophages. Int J Cancer. 38:135-137, 1986.

92. GIFFORD, G.E., and M.L. LOHMANN-MATTHES. Gamma interferon priming of mouse and human macrophages for induction of tumor necrosis factor production by bacterial lipopolysaccharide. J. Natl. Cancer. Inst. 78:121-124, 1987.

93. GODFREY, R.W., W.J. JOHNSON, and S.T. HOFFSTEIN. Recombinant tumor necrosis factor and interleukin-1 both stimulate human synovial cell arachidonic acid release and phospholipid metabolism. Biochem. Biophys. Res. Commun. 142:235-241, 1987.

94. GREEN, S., A. DOBRJANSKY, E.A. CARSWELL, R.L. KASSEL, L.J. OLD, N. FIORE, and M.K. SCHWARTZ. Partial purification of a serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA. 73:381-385, 1976.

95. GREEN, S., A. DOBRJANSKY, M.A. CHIASSON, E. CARSWELL, M.K. SCHWARTZ, and L.J. OLD. Corynebacterium parvum as the priming agent in the production of tumor necrosis factor in the mouse. J. Natl. Cancer Inst. 59:1519-1522, 1977.

96. GRODSKY, G.M., H. SANDO, S. LEVIN, J. GERICH, and J. KARAM. Synthesis and secretion of insulin in dynamic perfusion systems. Adv. Met. Disorders. 7:155-170, 1974.

97. HACKETT, R.J., L.S. DAVIS, and P.E. LIPSKY. Comparative effects of tumor necrosis factor-alpha and IL-1 beta on mitogen-induced T cell activation. J. Immunol. 140:2639-2644, 1988.

98. HAJJAR, K.A., D.P. HAJJAR, R.L. SILVERSTEIN, and R.L. NACHMAN. Tumor necrosis factor-mediated release of platelet-derived growth factor from cultured endothelial cells. J. Exp. Med. 166:235-245, 1987.

99. HANAHAN, D.J. Platelet activating factor: a biologically active phosphoglyceride. Ann. Rev. Biochem. 55:483-509, 1986.

100. HARANAKA, K., N. SATOMI, A. SAKURAI, and O. KUNII. Role of lipid A in the production of tumor necrosis factor and differences in antitumor activity between tumor necrosis factor and lipopolysaccharide. Tohoku. J. Exp. Med. 144:385-396, 1984.

101. HAVELL, E.A. Production of tumor necrosis factor during murine listeriosis. J. Immunol. 139:4225-4231, 1987.

102. HENSEL, G., D.N. MANNEL, K. PFIZENMAIER, and M. KRONKE. Autocrine stimulation of TNF-alpha mRNA expression in HL-60 cells. Lymphokine. Res. 6:119-125, 1987.

103. HESSE, D.G., K.J. TRACEY, Y. FONG, K.R. MANOGUE, M.A.JR. PALLADINO, A. CERAMI, G.T. SHIRES, and S.F. LOWRY. Cytokine appearance in human endotoxemia and primate bacteremia. Surg. Gynecol. Obstet. 166: 147-153, 1988.

104. HILL, M.R., R.D. STITH, and R.E. MCCALLUM. Interleukin 1: a regulatory role in glucocorticoid-regulated hepatic metabolism. J. Immunol. 137:858-862, 1986.

105. HILL, R.B., B.E. DAHRLING, T.E. STARZL, and D. RIFKIND. Death after transplantation: an analysis of sixty cases. Am. J. Med. 42: 327-334, 1967.

106. HINSHAW, L.B. Concise review: the role of glucose in endotoxin shock. Circ. Shock. 3:1-10, 1976.

107. HINSHAW, L.B. Overview of endotoxin shock. In: Pathophysiology of Shock, Anemia, and Ischemia, edited by COWLEY, R.A., and B.F. TRUMP. Baltimore:Williams and Wilkins, 1982, p. 219-234.

108. HOFFMANN, M.K., H.F. OETTGEN, L.J. OLD, R.S. MITTLER, and U. HAMMERLING. Induction and immunological properties of tumor necrosis factor. J. Reticuloendothel. Soc. 23:307-319, 1978.

109. HOLTMANN, H., and D. WALLACH. Down regulation of the receptors for tumor necrosis factor by interleukin 1 and 4 beta-phorbol-12-myristate-13-acetate. J. Immunol. 139:1161-1167, 1987.

110. IMAMURA, K., D. SPRIGGS, and D. KUFE. Expression of tumor necrosis factor receptors on human monocytes and internalization of receptor bound ligand. J. Immunol. 139:2989-2992, 1987.

111. ITO, H., T. SHIRAI, S. YAMAMOTO, M. AKIRA, S. KAWAHARA, C.W. TODD, and R.B. WALLACE. Molecular cloning of the gene encoding rabbit tumor necrosis factor. DNA. 5:157-165, 1986.

112. ITO, H., S. YAMAMOTO, S. KURODA, H. SAKAMOTO, J. KAJIHARA, T. KIYOTA, H. HAYASHI, M. KATO, and M. SEKO. Molecular cloning and expression in Escherichia coli of the cDNA coding for rabbit tumor necrosis factor. DNA. 5:149-156, 1986.

113. JANSSEN, O., and D. KABELITZ. Tumor necrosis factor selectively inhibits activation of human B cells by Epstein-Barr virus. J. Immunol. 140:125-130, 1988.

114. JELINEK, D.F., and P.E. LIPSKY. Enhancement of human B cell proliferation and differentiation by tumor necrosis factor-alpha and interleukin 1. J. Immunol. 139:2970-2976, 1987.

115. JOHNSON, S.E., and C. BAGLIONI. Tumor necrosis factor receptors and cytocidal activity are down-regulated by activators of protein kinase C. J. Biol. Chem. 263:5686-5692, 1988.

116. JONES, S.B., and F.D. ROMANO. Plasma catecholamines in the conscious rat during endotoxicosis. 14:189-201, 1984.

117. JONES, S.B., and M.R. YELICH. Simultaneous elevation of plasma insulin and catecholamines during endotoxicosis in the conscious and anesthetized rat. Life. Sci. 41:1935-1943, 1987.

118. JONGENEEL, C.V., S.A. NEDOSPASOV, G. PLAETINCK, P. NAQUET, and J.C. CEROTTINI. Expression of the tumor necrosis factor locus is not necessary for the cytolytic activity of T lymphocytes. J. Immunol. 140:1916-1922, 1988.

119. KAWAKAMI, M., and A. CERAMI. Studies of endotoxin-induced decrease in lipoprotein lipase activity. J. Exp. Med. 154:631-639, 1981.

120. KAWAKAMI, M., S. ISHIBASHI, H. OGAWA, T. MURASE, F. TAKAKU, and S. SHIBATA. Cachectin/TNF as well as interleukin-1 induces prostacyclin synthesis in cultured vascular endothelial cells. Biochem. Biophys. Res. Commun. 141:482-487, 1986.

121. KAWAKAMI, M., P.H. PEKALA, M.D. LANE, and A. CERAMI. Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cells. Proc. Natl. Acad. Sci. USA. 79:912-916, 1982.

122. KEHRL, J.H., A. MILLER, and A.S. FAUCI. Effect of tumor necrosis factor alpha on mitogen-activated human B cells. J. Exp. Med. 166:786-791, 1987.

123. KELLEHER, D.L., G.J. BAGBY, and J.J. SPITZER. Metabolic and endocrine alterations following endotoxin administration in normal and diabetic rats. In: Homeostasis in injury and shock,(26th ed.), edited by BIRO, ZS., A.G.B. KOVACH, J.J. SPITZER, and H.B. STONER. Elmsford:Pergamon Press, 1980, p. 181-189.

124. KEPPLER, D., W. HAGMANN, and S. RAPP. Role of leukotrienes in endotoxin action in vivo. Rev. Infect. Dis. 9:S580-S584, 1987.

125. KETTELHUT, I.C., W. FIERS, and A.L. GOLDBERG. The toxic effects of tumor necrosis factor in vivo and their prevention by cyclooxygenase inhibitors. Proc. Natl. Acad. Sci. USA 84:4273-4277, 1987.

126. KETTELHUT, I.C., and A.L. GOLDBERG. Tumor necrosis factor can induce fever in rats without activating protein breakdown in muscle or lipolysis in adipose tissue. J. Clin. Invest. 81:1384-1389, 1988.

127. KIENER, P.A., F. MAREK, G. RODGERS, P.-F. LIN, G. WARR, and J. DESIDERIO. Induction of tumor necrosis factor, interferon-gamma, and acute lethality in mice by toxic and non-toxic forms of lipid A. J. Immunol. 141:870-874, 1988.

128. KIRSTEIN, M., and C. BAGLIONI. Tumor necrosis factor induces synthesis of two proteins in human fibroblasts. J. Biol. Chem. 261:9565-9567, 1986.

129. KLEBANOFF, S.J., M.A. VADAS, J.M. HARLAN, L.H. SPARKS, J.R. GAMBLE, J.M. AGOSTI, and A.M. WALTERSDORPH. Stimulation of neutrophils by tumor necrosis factor. J. Immunol. 136:4220-4225, 1986.

130. KOHASE, M., D. HENRIKSEN-DESTEFANO, L.T. MAY, J. VILCEK, and P.B. SEHGAL. Induction of beta 2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. Cell 45:659-666, 1986.

131. KORNBLUTH, R.S., and T.S. EDGINGTON. Tumor necrosis factor production by human monocytes is a regulated event: induction of TNF-alpha-mediated cellular cytotoxicity by endotoxin. J. Immunol. 137:2585-2591, 1986.

132. KULL, F.C., and P. CUATRECASAS. Preliminary characterization of the tumor cell cytotoxin in tumor necrosis serum. J. Immunol. 126: 1279-1283, 1981.

133. KULL, F.C., and P. CUATRECASAS. Necrosin: purification and properties of a cytotoxin derived from a murine macrophage-like cell line. Proc. Natl. Acad. Sci. USA. 81:7932-7936, 1984.

134. KULL, F.C., S. JACOBS, and P. CUATRECASAS. Cellular receptor for 125I-labeled tumor necrosis factor: specific binding, affinity labeling, and relationship to sensitivity. Proc. Natl. Acad. Sci. USA. 82:5756-5760, 1985.

135. KUNKEL, S.L., M. SPENGLER, M.A. MAY, R. SPENGLER, J. LARRICK, and D. REMICK. Prostaglandin E2 regulates macrophage-derived tumor necrosis factor gene expression. J. Biol. Chem. 263:5380-5384, 1988.

136. KUNKEL, S.L., R.C. WIGGINS, S.W. CHENSUE, and J. LARRICK. Regulation of macrophage tumor necrosis factor production by prostaglandin E2. Biochem. Biophys. Res. Commun. 137:404-410, 1986.

137. LANG, C.H., G.J. BAGBY, and J.J. SPITZER. Glucose kinetics and body temperature after lethal and nonlethal doses of endotoxin. Am. J. Physiol. 248:R471-R478, 1985.

138. LANG, C.H., C. DOBRESCU, D.M. HARGROVE, G.J. BAGBY, and J.J. SPITZER. Platelet-activating factor-induced increases in glucose kinetics. Am. J. Physiol. 254:E193-E200, 1988.

139. LAPIERRE, L.A., W. FIERS, and J.S. POBER. Three distinct classes of regulatory cytokines control endothelial cell MHC antigen expression. Interactions with immune gamma interferon differentiate the effects of tumor necrosis factor and lymphotoxin from those of leukocyte alpha and fibroblast beta interferons. J. Exp. Med. 167:794-804, 1988.

140. LARRICK, J.W., D. GRAHAM, K. TOY, L.S. LIN, G. SENYK, and B.M. FENDLY. Recombinant tumor necrosis factor causes activation of human granulocytes. Blood 69:640-644, 1987.

141. LE, J.M., and J. VILCEK. Accessory function of human fibroblasts in mitogen-stimulated interferon-gamma production by T lymphocytes. Inhibition by interleukin 1 and tumor necrosis factor. J. Immunol. 139:3330-3337, 1987.

142. LEE, M.D., A. ZENTELLA, P.H. PEKALA, and A. CERAMI. Effect of endotoxin-induced monokines on glucose metabolism in the muscle cell line L6. Proc. Natl. Acad. Sci. USA 84:2590-2594, 1987.

143. LEEUWENBERG, J F., J. VAN DAMME, G.M. JEUNHOMME, and W.A. BUURMAN. Interferon beta 1, an intermediate in the tumor necrosis factor alpha-induced increased MHC class I expression and an autocrine regulator of the constitutive MHC class I expression. J. Exp. Med. 166:1180-1185, 1987.

144. LEFER, A.M. Eicosanoids as mediators of ischemia and shock. Fed. Proc. 44:275-280, 1985.

145. LEHMANN, V., M.A. FREUDENBERG, and C. GALANOS. Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine-treated mice. J. Exp. Med. 165:657-663, 1987.

146. LEIGH, D.A. Bacteraemia in patients receiving human cadaveric renal transplants. J. Clin. Path. 24:295-299, 1971.

147. LEIST, T.P., K. FREI, S. KAM-HANSEN, R.M. ZINKERNAGEL, and A. FONTANA. Tumor necrosis factor alpha in cerebrospinal fluid during bacterial, but not viral, meningitis. Evaluation in murine model infections and in patients. J. Exp. Med. 167:1743-1748, 1988.

148. LEMBERG, A., R. WIKINSKI, E.M. IZURIETA, H. HALPERIN, A.M. PAGLIONE, P. DENEUMAN, and H. JAUREGUI. Effects of prostglandin E1 and norepinephrine on glucose and lipid metabolism in isolated perfused rat liver. Biochim. Biophys. Acta. 248:198-204, 1971.

149. LIN, J.X., and J. VILCEK. Tumor necrosis factor and interleukin-1 cause a rapid and transient stimulation of c-fos and c-myc mRNA levels in human fibroblasts. J. Biol. Chem. 262:11908-11911, 1987.

150. LOCKSLEY, R.M., F.P. HEINZEL, H.M. SHEPARD, J. AGOSTI, T.E. EESSALU, B.B. AGGARWAL, and J.M. HARLAN. Tumor necrosis factors alpha and beta differ in their capacities to generate interleukin 1 release from human endothelial cells. J. Immunol. 139:1891-1895, 1987.

151. MACKIEWICZ, A., M.K. GANAPATHI, D. SCHULTZ, and I. KUSHNER. Monokines regulate glycosylation of acute-phase proteins. J. Exp. Med. 166:253-258, 1987.

152. MANDRUP-POULSEN, T., K. BENDTZEN, C.A. DINARELLO, and J. NERUP. Human tumor necrosis factor potentiates human interleukin 1-mediated rat pancreatic beta-cell cytotoxicity. J. Immunol. 139:4077-4082, 1987.

153. MANDRUP-POULSEN, T., J. EGEBERG, J. NERUP, K. BENDTZEN, J.H. NIELSEN, and C.A. DINARELLO. Ultrastructural studies of time-course and cellular specificity of interleukin-1 mediated islet cytotoxicity. Acta. Pathol. Microbiol. Immunol. Scand. 95:55-63, 1987.

154. MANNEL, D.N. Biological aspects of tumor necrosis factor. Immunobiology. 172:283-290, 1986.

155. MANNEL, D.N., W. FALK, and M.S. MELTZER. Inhibition of nonspecific tumoricidal activity by activated macrophages with antiserum against a soluble cytotoxic factor. Infect. Immun. 33:156-164, 1981.

156. MANNEL, D.N., W. FALK, and H. NORTHOFF. Endotoxic activities of tumor necrosis factor independent of IL1 secretion by macrophages/monocytes. Lymphokine. Res. 6:151-159, 1987.

157. MANNEL, D.N., M.S. MELTZER, and S.E. MERGENHAGEN. Generation and characterization of a lipopolysaccharide-induced and serum-derived cytotoxic factor for tumor cells. Infect. Immun. 28:204-211, 1980.

158. MANNEL, D.N., R.N. MOORE, and S.E. MERGENHAGEN. Macrophages as a source of tumoricidal activity (tumor-necrotizing factor). Infect. Immun. 30:523-530, 1980.

159. MANNEL, D.N., D.L. ROSENSTREICH, and S.E. MERGENHAGEN. Mechanism of lipopolysaccharide-induced tumor necrosis: requirement for lipopolysaccharide-sensitive lymphoreticular cells. Infect. Immun. 24:573-576, 1979.

160. MARMENOUT, A., L. FRANSEN, J. TAVERNIER, HEYDEN.J. VAN DER, R. TIZARD, E. KAWASHIMA, A. SHAW, M.J. JOHNSON, D. SEMON, R. MULLER, M.-R. RUYSSCHAERT, A. VAN VLIET, and W. FIERS. Molecular cloning and expression of human tumor necrosis factor and comparison with mouse tumor necrosis factor. Eur. J. Biochem. 152:515-522, 1985.

161. MATHISON, J.C., E. WOLFSON, and R.J. ULEVITCH. Participation of tumor necrosis factor in the mediation of gram-negative bacterial lipopolysaccharide-induced injury in rabbits. J. Clin. Invest. 81:1925-1937, 1988.

162. MATTHEWS, N. Tumour-necrosis factor from the rabbit. II. Production by monocytes. Br. J. Cancer 38:310-315, 1978.

163. MATTHEWS, N. Tumour-necrosis factor from the rabbit. V. Synthesis in vitro by mononuclear phagocytes from various tissues of normal and BCG- injected rabbits. Br. J. Cancer. 44:418-424, 1981.

164. MATTHEWS, N. Production of an anti-tumour cytotoxin by human monocytes. Immunology 44:135-142, 1981.

165. MATTHEWS, N. Production of an anti-tumour cytotoxin by human monocytes: comparison of endotoxin, interferons, and other agents as inducers. Br. J. Cancer 45:615-617, 1982.

166. MATTHEWS, N., M.L. NEALE, S.K. JACKSON, and J.M. STARK. Tumour cell killing by tumour necrosis factor: inhibition by anaerobic conditions, free-radical scavengers and inhibitors of arachidonate metabolism. Immunology. 62:153-155, 1987.

167. MATTHEWS, N., H.C. RYLEY, and M.L. NEALE. Tumour-necrosis factor from the rabbit. IV. Purification and chemical characterization. Br. J. Cancer. 42:416-422, 1980.

168. MATTHEWS, N., and J.F. WATKINS. Tumour-necrosis factor from the rabbit. I. Mode of action, specificity, and physicochemical properties. Br. J. Cancer. 38:302-309, 1978.

169. MCCABE, W.R., and G.G. JACKSON. Gram-negative bacteremia. I. Etiology and ecology. Arch. Intern. Med. 110:847-855, 1962.

170. MCCABE, W.R., B.E. KREGER, and M. JOHNS. Type-specific and cross-reactive antibodies in gram-negative bacteremia. N. Engl. J. Med. 287:261-267, 1972.

171. MCCALLUM, R.E. Hepatocyte-Kupffer cell interactions in the inhibition of hepatic gluconeogenesis by bacterial endotoxin. In: Pathophysiological Effects of Endotoxins at the Cellular Level, edited by MAJDE, J.A., and R.J. PERSON. New York:Alan R. Liss, Inc., 1981, p. 99-113.

172. MCGOWAN, J.E., M.W. BARNES, and M. FINLAND. Bacteremia at Boston City Hospital: occurrence and mortality during 12 selected years (1935-1972), with special reference to hospital-acquired cases. J. Infect. Dis. 132:316-335, 1975.

173. MESZAROS, K., C.H. LANG, G.J. BAGBY, and J.J. SPITZER. Tumor necrosis factor increases in vivo glucose utilization of macrophage-rich tissues. Biochem. Biophys. Res. Commun. 149:1-6, 1987.

174. MICHIE, H.R., K.R. MANOGUE, D.R. SPRIGGS, A. REVHAUG, S. ODWYER, C.A. DINARELLO, A. CERAMI, S.M. WOLFF, and D.W. WILMORE. Detection of circulating tumor necrosis factor after endotoxin administration. N. Engl. J. Med. 318:1481-1486, 1988.

175. MICHIE, H.R., D.R. SPRIGGS, K.R. MANOGUE, M.L. SHERMAN, A. REVHAUG, S.T. O'DWYER, K. ARTHUR, C.A. DINARELLO, A. CERAMI, S.M. WOLFF, D.W. KUFE, and D.W. WILMORE. Tumor necrosis factor and endotoxin induce similar metabolic responses in human beings. Surgery 104:280-286, 1988.

176. MING, W.J., L. BERSANI, and A. MANTOVANI. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. J. Immunol. 138:1469-1474, 1987.

177. MIZOCK, B. Septic shock. A metabolic perspective. Arch. Intern. Med. 144:579-585, 1984.

178. MOLDAWER, L.L., C. ANDERSSON, J. GELIN, and K.G. LUNDHOLM. Regulation of food intake and hepatic protein synthesis by recombinant-derived cytokines. Am. J. Physiol. 254:G450-G456, 1988.

179. MOLDAWER, L.L., J. GELIN, T. SCHERSTEN, and K.G. LUNDHOLM. Circulating interleukin 1 and tumor necrosis factor during inflammation. Am. J. Physiol. 253:R922-R928, 1987.

180. MORRISON, D.C., and J.L. RYAN. Endotoxins and disease mechanisms. Ann. Rev. Med. 38:417-432, 1987.

181. MORTENSEN, R.F., J. SHAPIRO, B.F. LIN, S. DOUCHES, and R. NETA. Interaction of recombinant IL-1 and recombinant tumor necrosis factor in the induction of mouse acute phase proteins. J. Immunol. 140:2260-2266, 1988.

182. NAKAMURA, H., Y. SETO, S. MOTOYOSHI, T. KADOKAWA, and N. SUNAHARA. Recombinant human tumor necrosis factor causes long-lasting and prostaglandin-mediated fever, with little tolerance, in rabbits. J. Pharmacol. Exp. Ther. 245:336-341, 1988.

183. NARHI, L.O., and T. ARAKAWA. Dissociation of recombinant tumor necrosis factor-alpha studied by gel permeation chromatography. Biochem. Biophys. Res. Commun. 147:740-746, 1987.

184. NAUTS, H.C., G.A. FOWLER, and F.H. BOGATKO. A review of the influence of bacterial infection and of bacterial products (Coley's Toxins) on malignant tumors in man. Acta. Med. Scand. Suppl. 276:1-103, 1953.

185. NAWROTH, P.P., I. BANK, D. HANDLEY, J. CASSIMERIS, L. CHESS, and D. STERN. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J. Exp. Med. 163:1363-1375, 1986.

186. NAWROTH, P.P., and D.M. STERN. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J. Exp. Med. 163:740-745, 1986.

187. NAYLOR, J.M., and D.S. KRONFELD. In vivo studies of hypoglycemia and lactic acidosis in endotoxic shock. Am. J. Physiol. 248:E309-E316, 1985.

188. NISSEN-MEYER, J., and J. HAMMERSTROM. Physicochemical characterization of cytostatic factors released from human monocytes. Infect. Immun. 38:67-73, 1982.

189. O'MALLEY, W.E., B. ACHINSTEIN, and M.J. SHEAR. Action of bacterial polysaccharide on tumors. II. Damage of sarcoma 37 by serum of mice treated with Serratia marcescens polysaccharide, and induced tolerance. J. Natl. Cancer. Inst. 29:1169-1175, 1962.

190. OKUSAWA, S., J.A. GELFAND, T. IKEJIMA, [illegible].J. CONNOLLY, and C.A. DINARELLO. Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclo-oxygenase inhibition. J. Clin. Invest. 81:1162-1172, 1988.

191. OLIFF, A., D. DEFEO-JONES, M. BOYER, D. MARTINEZ, D. KIEFER, G. VUOCOLO, A. WOLFE, and S.H. SOCHER. Tumors secreting human TNF/cachectin induce cachexia in mice. Cell 50:555-563, 1987.

192. ONOZAKI, K., H. URAWA, T. TAMATANI, Y. IWAMURA, T. HASHIMOTO, T. BABA, H. SUZUKI, M. YAMADA, S. YAMAMOTO, J.J. OPPENHEIM, and K. MATSUSHIMA. Synergistic interactions of interleukin 1, interferon-beta, and tumor necrosis factor in terminally differentiating a mouse myeloid leukemic cell line (M1). Evidence that interferon-beta is an autocrine differentiating factor. J. Immunol. 140:112-119, 1988.

193. PALOMBELLA, V.J., D.J. YAMASHIRO, F.R. MAXFIELD, S.J. DECKER, and J. VILCEK. Tumor necrosis factor increases the number of epidermal growth factor receptors on human fibroblasts. J. Biol. Chem. 262:1950-1954, 1987.

194. PAPE, M.E., and K.H. KIM. Effect of tumor necrosis factor on acetyl-coenzyme A carboxylase gene expression and preadipocyte differentiation. Mol. Endo. 2:395-403, 1988.

195. PATTON, J.S., P.M. PETERS, J. MCCABE, D. CRASE, S. HANSEN, A.B. CHEN, and D. LIGGITT. Development of partial tolerance to the gastrointestinal effects of high doses of recombinant tumor necrosis factor-alpha in rodents. J. Clin. Invest. 80:1587-1596, 1987.

196. PATTON, J.S., H.M. SHEPARD, H. WILKING, G. LEWIS, B.B. AGGARWAL, T.E. EESSALU, L.A. GAVIN, and C. GRUNFELD. Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. Proc. Natl. Acad. Sci. USA. 83:8313-8317, 1986.

197. PENNICA, D., J.S. HAYFLICK, T.S. BRINGMAN, M.A. PALLADINO, and D.V. GOEDDEL. Cloning and expression in Escherichia coli of the cDNA for murine tumor necrosis factor. Proc. Natl. Acad. Sci. USA. 82: 6060-6064, 1985.

198. PENNICA, D., G.E. NEDWIN, J.S. HAYFLICK, P.H. SEEBURG, R. DERYNCK, M.A. PALLADINO, W.J. KOHR, B.B. AGGARWAL, and D.V. GOEDDEL. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 312:724-729, 1984.

199. PERLMUTTER, D.H., C.A. DINARELLO, P.I. PUNSAL, and H.R. COLTEN. Cachectin/tumor necrosis factor regulates hepatic acute-phase gene expression. J. Clin. Invest. 78:1349-1354, 1986.

200. PESSINA, G.P., A. PACINI, V. BOCCI, E. MAIOLI, and A. NALDINI. Studies on tumor necrosis factor (TNF): II. Metabolic fate and distribution of human recombinant TNF. Lymphokine. Res. 6:35-44, 1987.

201. PETERS, P.M., J.R. ORTALDO, M.R. SHALABY, L.P. SVEDERSKY, G.E. NEDWIN, T.S. BRINGMAN, P.E. HASS, B.B. AGGARWAL, R.B. HERBERMAN, D.V. GOEDDEL, and M.A.JR. PALLADINO. Natural killer-sensitive targets stimulate production of TNF-alpha but not TNF-beta (lymphotoxin) by highly purified human peripheral blood large granular lymphocytes. J. Immunol. 137:2592-2598, 1986.

202. PHILIP, R., and L.B. EPSTEIN. Tumour necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, gamma-interferon and interleukin-1. Nature 323:86-89, 1986.

203. POBER, J.S., M.P. BEVILACQUA, D.L. MENDRICK, L.A. LAPIERRE, W. FIERS, and M.A. GIMBRONE. Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J. Immunol. 136:1680-1687, 1986.

204. POBER, J.S., M.A. GIMBRONE, L.A. LAPIERRE, D.L. MENDRICK, W. FIERS, R. ROTHLEIN, and T.A. SPRINGER. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J. Immunol. 137:1893-1896, 1986.

205. POHLMAN, T.H., K.A. STANNESS, P.G. BEATTY, H.D. OCHS, and J.M. HARLAN. An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin 1, and tumor necrosis factor-alpha increases neutrophil adherence by a CDw18-dependent mechanism. J. Immunol. 136:4548-4553, 1986.

206. PUKEL, C., H. BAQUERIZO, and A. RABINOVITCH. Destruction of rat islet cell monolayers by cytokines. Synergistic interactions of interferon-gamma, tumor necrosis factor, lymphotoxin, and interleukin 1. Diabetes. 37:133-136, 1988.

207. RANGES, G.E., I.S. FIGARI, T. ESPEVIK, and M.A. PALLADINO. Inhibition of cytotoxic T cell development by transforming growth factor beta and reversal by recombinant tumor necrosis factor alpha. J. Exp. Med. 166:991-998, 1987.

208. RANGES, G.E., A. ZLOTNIK, T. ESPEVIK, C.A. DINARELLO, A. CERAMI, and M.A. PALLADINO. Tumor necrosis factor alpha/cachectin is a growth factor for thymocytes. Synergistic interactions with other cytokines. J. Exp. Med. 167:1472-1478, 1988.

209. REIS, L.F., J.M. LE, T. HIRANO, T. KISHIMOTO, and J. VILCEK. Antiviral action of tumor necrosis factor in human fibroblasts is not mediated by B cell stimulatory factor 2/IFN-beta 2, and is inhibited by specific antibodies to IFN-beta. J. Immunol. 140:1566-1570, 1988.

210. REMICK, D.G., R.G. KUNKEL, J.W. LARRICK, and S.L. KUNKEL. Acute in vivo effects of human recombinant tumor necrosis factor. Lab. Invest. 56:583-590, 1987.

211. REMICK, D.G., J. LARRICK, and S.L. KUNKEL. Tumor necrosis factor-induced alterations in circulating leukocyte populations. Biochem. Biophys. Res. Commun. 141:818-824, 1986.

212. ROFE, A.M., R.A. CONYERS, R. BAIS, J.R. GAMBLE, and M.A. VADAS. The effects of recombinant tumour necrosis factor (cachectin) on

metabolism in isolated rat adipocyte, hepatocyte and muscle preparations. Biochem. J. 247:789-792, 1987.

213. ROH, M.S., K.A. DRAZENOVICH, J.J. BARBOSE, C.A. DINARELLO, and C.F. COBB. Direct stimulation of the adrenal cortex by interleukin-1. Surgery 102:140-146, 1987.

214. ROTHSTEIN, J.L., and H. SCHREIBER. Synergy between tumor necrosis factor and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice. Proc. Natl. Acad. Sci. USA. 85:607-611, 1988.

215. ROUZER, C.A., and A. CERAMI. Hypertriglyceridemia associated with Trypanosoma brucei infection in rabbits: role of defective triglyceride removal. Mol. Biochem. Parasitol. 2:31-38, 1980.

216. RUFF, M.R., and G.E. GIFFORD. Purification and physicochemical characterization of rabbit tumor necrosis factor. J. Immunol. 125:1671-1677, 1980.

217. RUGGIERO, V., J. TAVERNIER, W. FIERS, and C. BAGLIONI. Induction of the synthesis of tumor necrosis factor receptors by interferon-gamma. J. Immunol. 136:2445-2450, 1986.

218. RUSH, J.S., and C.J. WAECHTER. Inhibitors of protein kinase C block activation of B lymphocytes by bacterial lipopolysaccharide. Biochem. Biophy. Res. Com. 145:1315-1320, 1987.

219. SACCO-GIBSON, N., and J.P. FILKINS. Glucoregulatory effects of interleukin-1: implications to the carbohydrate dyshomeostasis of septic shock. In: Progress in Clinical and Biological Research. vol. 264: Perspectives in Shock Research, edited by BOND, R.F., H.R. ADAMS, and I.H. CHAUDRY. New York:A.R. Liss, 1988, p. 355-360.

220. SACCO-GIBSON, N., and J.P. FILKINS. Macrophages, monokines, and the metabolic pathophysiology of septic shock. In: Molecular and Cellular Mechansims of Septic Shock. vol 286. Progress in Clinical and Biological Research, edited by ROTH, B.L., T. NIELSEN, and A. MCKEE. New York:A.R. Liss, Inc., 1988, p. 203-216.

221. SARIBAN, E., K. IMAMURA, R. LUEBBERS, and D. KUFE. Transcriptional and posttranscriptional regulation of tumor necrosis factor gene expression in human monocytes. J. Clin. Invest. 81:1506-1510, 1988.

222. SATOH, M., H. INAGAWA, H. MINAGAWA, T. KAJIKAWA, H. OSHIMA, S. ABE, M. YAMAZAKI, and D. MIZUNO. Endogenous production of TNF in mice long after BCG sensitization. J. Biol. Response. Mod. 5:117-123, 1986.

223. SATOMI, N., K. HARANAKA, and O. KUNII. Research on the production site of tumor necrosis factor (TNF). Japan. J. Exp. Med. 51:317-322, 1981.

224. SCHEURICH, P., M. KRONKE, C. SCHLUTER, U. UCER, and K. PFIZENMAIER. Noncytocidal mechanisms of action of tumor necrosis factor-alpha on human tumor cells: enhancement of HLA gene expression synergistic with interferon-gamma. Immunobiology. 172:291-300, 1986.

225. SCHEURICH, P., B. THOMA, U. UCER, and K. PFIZENMAIER. Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)-alpha: induction of TNF receptors on human T cells and TNF-alpha-mediated enhancement of T cell responses. J. Immunol. 138:1786-1790, 1987.

226. SCHEURICH, P., U. UCER, M. KRONKE, and K. PFIZENMAIER. Quantification and characterization of high-affinity membrane receptors for tumor necrosis factor on human leukemic cell lines [published erratum appears in Int J Cancer 1986 Dec 15;38(6):929]. Int J Cancer. 38:127-133, 1986.

227. SCUDERI, P., K.E. STERLING, K.S. LAM, P.R. FINLEY, K.J. RYAN, C.G. RAY, E. PETERSEN, D.J. SLYMEN, and S.E. SALMON. Raised serum levels of tumour necrosis factor in parasitic infections. Lancet 2: 1364-1365, 1986.

228. SEMB, H., J. PETERSON, J. TAVERNIER, and T. OLIVECRONA. Multiple effects of tumor necrosis factor on lipoprotein lipase in vivo. J. Biol. Chem. 262:8390-8394, 1987.

229. SEOW, W.K., Y.H. THONG, and A. FERRANTE. Macrophage-neutrophil interactions: contrasting effects of the monokines interleukin-1 and tumour necrosis factor (cachectin) on human neutrophil adherence. Immunology. 62:357-361, 1987.

230. SHALABY, M.R., B.B. AGGARWAL, E. RINDERKNECHT, L.P. SVEDERSKY, B.S. FINKLE, and M.A. PALLADINO. Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factors. J. Immunol. 135:2069-2073, 1985.

231. SHALABY, M.R., M.A. PALLADINO, S.E. HIRABAYASHI, T.E. EESSALU, G.D. LEWIS, H.M. SHEPARD, and B.B. AGGARWAL. Receptor binding and activation of polymorphonuclear neutrophils by tumor necrosis factor-alpha. J. Leukocyte. Biol. 41:196-204, 1987.

232. SHEAR, M.J., and A. PERRAULT. Chemical treatment of tumors. IX. Reactions of mice with primary subcutaneous tumors to injection of a hemorrhage-producing bacterial polysaccharide. J. Natl. Cancer Inst. 4:461-476, 1943.

233. SHEAR, M.J., F.C. TURNER, A. PERRAULT, and T. SHOVELTON. Chemical treatment of tumors. V. Isolation of the hemorrhage-producing fraction from Serratia marcescens (Bacillus prodigiosus) culture filtrate. J. Natl. Cancer Inst. 4:81-97, 1943.

234. SHIRAI, T., H. YAMAGUCHI, H. ITO, C.W. TODD, and R.B. WALLACE. Cloning and expression in Escherichia coli of the gene for human tumour necrosis factor. Nature 313:803-806, 1985.

235. SHORT, B.L., M. GARDINER, R.I. WALKER, S.R. JONES, and J.R. FLETHCHER. Indomethacin improves survival in gram-negative sepsis. Adv. Shock. Res. 6:27-36, 1981.

236. SIPE, J.D., S.N. VOGEL, S. DOUCHES, and R. NETA. Tumor necrosis factor/cachectin is a less potent inducer of serum amyloid A synthesis than interleukin 1. Lymphokine. Res. 6:93-101, 1987.

237. SMITH, R.A., and C. BAGLIONI. The active form of tumor necrosis factor is a trimer. J. Biol. Chem. 262:6951-6954, 1987.

238. SMITH, R.A., M. KIRSTEIN, W. FIERS, and C. BAGLIONI. Species specificity of human and murine tumor necrosis factor. A comparative study of tumor necrosis factor receptors. J. Biol. Chem. 261:14871-14874, 1986.

239. SPIES, T., C.C. MORTON, S.A. NEDOSPASOV, W. FIERS, D. PIOUS, and J.L. STROMINGER. Genes for the tumor necrosis factors alpha and beta are linked to the human major histocompatibility complex. Proc. Natl. Acad. Sci. USA. 83:8699-8702, 1986.

240. SPRIGGS, D.R., K. IMAMURA, C. RODRIGUEZ, E. SARIBAN, and D.W. KUFE. Tumor necrosis factor expression in human epithelial tumor cell lines. J. Clin. Invest. 81:455-460, 1988.

241. STEFFEN, M., O.G. OTTMANN, and M.A. MOORE. Simultaneous production of tumor necrosis factor-alpha and lymphotoxin by normal T cells after induction with IL-2 and anti-T3. J. Immunol. 140:2621-2624, 1988.

242. STOLPEN, A.H., E.C. GUINAN, W. FIERS, and J.S. POBER. Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. Am. J. Pathol. 123:16-24, 1986.

243. STONER, H.B. Review article: metabolism after trauma and in sepsis. Circ. Shock. 19:75-87, 1986.

244. SUGARMAN, B.J., B.B. AGGARWAL, P.E. HASS, I.S. FIGARI, M.A.JR. PALLADINO, and H.M. SHEPARD. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230:943-945, 1985.

245. SUN, X.M., and W. HSUEH. Bowel necrosis induced by tumor necrosis factor in rats is mediated by platelet-activating factor. J. Clin. Invest. 81:1328-1331, 1988.

246. SUNG, S.S., J.M. BJORNDAHL, C.Y. WANG, H.T. KAO, and S.M. FU. Production of tumor necrosis factor/cachectin by human T cell lines and peripheral blood T lymphocytes stimulated by phorbol myristate acetate and anti-CD3 antibody. J. Exp. Med. 167:937-953, 1988.

247. TALMADGE, J.E., O. BOWERSOX, H. TRIBBLE, S.H. LEE, H.M. SHEPARD, and D. LIGGITT. Toxicity of tumor necrosis factor is synergistic with gamma-interferon and can be reduced with cyclooxygenase inhibitors. Am. J. Pathol. 128:410-425, 1987.

248. TORTI, F.M., B. DIECKMANN, B. BEUTLER, A. CERAMI, and G.M. RINGOLD. A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia. Science 229:867-869, 1985.

249. TRACEY, K.J., B. BEUTLER, S.F. LOWRY, J. MERRYWEATHER, S. WOLPE, I.W. MILSARK, R.J. HARIRI, T.J.3D. FAHEY, A. ZENTELLA, J.D. ALBERT, G.T. SHIRES, and A. CERAMI. Shock and tissue injury induced by recombinant human cachectin. Science 234:470-474, 1986.

250. TRACEY, K.J., Y. FONG, D.G. HESSE, K.R. MANOGUE, A.T. LEE, G.C. KUO, S.F. LOWRY, and A. CERAMI. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 330:662-664, 1987.

251. TRACEY, K.J., S.F. LOWRY, B. BEUTLER, A. CERAMI, J.D. ALBERT, and G.T. SHIRES. Cachectin/tumor necrosis factor mediates changes of skeletal muscle plasma membrane potential. J. Exp. Med. 164: 1368-1373, 1986.

252. TRACEY, K.J., S.F. LOWRY, B. BEUTLER, A. CERAMI, J.D. ALBERT, and G.T. SHIRES. Cachectin/tumor necrosis factor participates in reduction of skeletal muscle membrane potential: evidence for bioactivity in plasma during critical illness. Surg. Forum 37:13-15, 1986.

253. TRACEY, K.J., S.F. LOWRY, T.J.3D. FAHEY, J.D. ALBERT, Y. FONG, D. HESSE, B. BEUTLER, K.R. MANOGUE, S. CALVANO, H. WEI, A. CERAMI, and G.T. SHIRES. Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. Surg. Gynecol. Obstet. 164:415-422, 1987.

254. TRACEY, K.J., H. WEI, K.R. MANOGUE, Y. FONG, D.G. HESSE, H.T. NGUYEN, G.C. KUO, B. BEUTLER, R.S. COTRAN, A. CERAMI, and S.F. LOWRY. Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. J. Exp. Med. 167:1211-12[illegible], 1988.

256. TSUJIMOTO, M., R. FEINMAN, M. KOHASE, and J. VILCEK. Characterization and affinity crosslinking of receptors for tumor necrosis factor on human cells. Arch. Biochem. Biophys. 249:563-568, 1986.

257. TSUJIMOTO, M., R. FEINMAN, and J. VILCEK. Differential effects of type I IFN and IFN-gamma on the binding of tumor necrosis factor to receptors in two human cell lines. J. Immunol. 137:2272-2276, 1986.

258. TSUJIMOTO, M., Y.K. YIP, and J. VILCEK. Tumor necrosis factor: specific binding and internalization in sensitive and resistant cells. Proc. Natl. Acad. Sci. USA. 82:7626-7630, 1985.

259. TSUJIMOTO, M., Y.K. YIP, and J. VILCEK. Interferon-gamma enhances expression of cellular receptors for tumor necrosis factor. J. Immunol. 136:2441-2444, 1986.

260. TSUJIMOTO, M., S. YOKOTA, J. VILCEK, and G. WEISSMANN. Tumor necrosis factor provokes superoxide anion generation from neutrophils. Biochem. Biophys. Res. Commun. 137:1094-1100, 1986.

261. ULICH, T.R., J. DEL CASTILLO, M. KEYS, G.A. GRANGER, and R.X. NI. Kinetics and mechanisms of recombinant human interleukin 1 and tumor necrosis factor-alpha-induced changes in circulating numbers of neutrophils and lymphocytes. J. Immunol. 139:3406-3415, 1987.

262. UNGLAUB, R., B. MAXEINER, B. THOMA, K. PFIZENMAIER, and P. SCHEURICH. Downregulation of tumor necrosis factor (TNF) sensitivity modulation of TNF binding capacity by protein kinase C activators. J. Exp. Med. 166:1788-1797, 1987.

263. URBASCHEK, R., and B. URBASCHEK. Tumor necrosis factor and interleukin 1 as mediators of endotoxin-induced beneficial effects. Rev. Infect. Dis. 9:S607-S615, 1987.

264. VILCEK, J., P.W. GRAY, E. RINDERKNECHT, and C.G. SEVASTOPOULOS. Interferon gamma: a lymphokine for all seasons. Lymphokines 11:1-32, 1985.

265. VILCEK, J., V.J. PALOMBELLA, D. HENRIKSEN DESTEFANO, C. SWENSON, R. FEINMAN, M. HIRAI, and M. TSUJIMOTO. Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. J. Exp. Med. 163:632-643, 1986.

266. WAAGE, A. Production and clearance of tumor necrosis factor in rats exposed to endotoxin and dexamethasone. Clin. Immunol. Immunopathol. 45:348-355, 1987.

267. WAAGE, A., and O. BAKKE. Glucocorticoids suppress the production of tumour necrosis factor by lipopolysaccharide-stimulated human monocytes. Immunology. 63:299-302, 1988.

268. WAAGE, A., and T. ESPEVIK. Interleukin 1 potentiates the lethal effect of tumor necrosis factor alpha/cachectin in mice. J. Exp. Med. 167:1987-1992, 1988.

269. WAAGE, A., A. HALSTENSEN, and T. ESPEVIK. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 1:355-357, 1987.

270. WAISBREN, B.A. Bacteremia due to gram-negative bacilli other than the Salmonella. A clinical and therapeutic study. Arch. Intern. Med. 88:467-488, 1951.

271. WALLACE, J.L., G. STEEL, B.J.R. WHITTLE, V. LAGENTE, and B. VARGAFTIG. Evidence for platelet-activating factor as a mediator of endotoxin-induced gastrointestinal damage in the rat. Gastroenterology 93:765-773, 1987.

272. WALLACH, D., H. HOLTMANN, H. ENGELMANN, and Y. NOPHAR. Sensitization and desensitization to lethal effects of tumor necrosis factor and IL-1. J. Immunol. 140:2994-2999, 1988.

273. WANG, A.M., A.A. CREASEY, M.B. LADNER, L.S. LIN, J. STRICKLER, J.N. VAN ARSDELL, R. YAMAMOTO, and D.F. MARK. Molecular cloning of the complementary DNA for human tumor necrosis factor. Science 228:149-154, 1985.

274. WANKOWICZ, Z., P. MEGYERI, and A. ISSEKUTZ. Synergy between tumour necrosis factor alpha and interleukin-1 in the induction of polymorphonuclear leukocyte migration during inflammation. J. Leukocyte. Biol. 43:349-356, 1988.

275. WARREN, R.S., D.B. DONNER, H.F.JR. STARNES, and M.F. BRENNAN. Modulation of endogenous hormone action by recombinant human tumor necrosis factor. Proc. Natl. Acad. Sci. USA. 84:8619-8622, 1987.

276. WARREN, R.S., H.F.JR. STARNES, J.L. GABRILOVE, H.F. OETTGEN, and M.F. BRENNAN. The acute metabolic effects of tumor necrosis factor administration in humans. Arch. Surg. 122:1396-1400, 1987.

277. WATANABE, N., Y. NIITSU, H. NEDA, H. SONE, N. YAMAUCHI, M. MAEDA, and I. URUSHIZAKI. Cytocidal mechanism of TNF: effects of lysosomal enzyme and hydroxyl radical inhibitors on cytotoxicity. Immunopharmacol. Immunotoxicol. 10:109-116, 1988.

278. WEDGWOOD, J.F., L. HATAM, and V.R. BONAGURA. Effect of interferon-gamma and tumor necrosis factor on the expression of class I and class II major histocompatibility molecules by cultured human umbilical vein endothelial cells. Cell Immunol. 111:1-9, 1988.

279. WIGHTMAN, P.D., and C.R.H. RAETZ. The activation of protein kinase C by biologically active lipid moieties of lipopolysaccharide. J. Biol. Chem. 259:10048-10052, 1984.

280. WILLIAMSON, B.D., E.A. CARSWELL, B.Y. RUBIN, J.S. PRENDERGAST, and L.J. OLD. Human tumor necrosis factor produced by human B-cell

lines: synergistic cytotoxic interaction with human interferon. Proc. Natl. Acad. Sci. USA 80:5397-5401, 1983.

281. WINSTON, D.J., R.P. GALE, D.V. MEYER, and L.S. YOUNG. Infectious complications of human bone marrow transplantation. Medicine. 58:1-31, 1979.

282. WOLFE, R.R., D. ELAHI, and J.J. SPITZER. Glucose and lactate kinetics after endotoxin administration in dogs. Am. J. Physiol. 232:E180-E185, 1977.

283. YELICH, M.R., and J.P. FILKINS. Mechanism of hyperinsulinemia in endotoxicosis. Am. J. Physiol. 239:E156-E161, 1980.

284. YELICH, M.R., L. WITEK-JANUSEK, and J.P. FILKINS. Glucose dyshomeostasis in endotoxicosis: direct versus monokine-mediated mechanisms of endotoxin action. In: Immunobiology and Immunopharmacology of Bacterial Endotoxins, edited by SZENTIVANYI, A., and H. FRIEDMAN. New York:Plenum Publishing Corporation, 1986, p. 111-132.

285. YOKOTA, S., T.D. GEPPERT, and P.E. LIPSKY. Enhancement of antigen- and mitogen-induced human T lymphocyte proliferation by tumor necrosis factor-alpha. J. Immunol. 140:531-536, 1988.

286. YOUNG, L.S. Gram-negative sepsis. In: Principles and Practice of Infectious Diseases, edited by MANDELL, G.L., R.G. DOUGLAS, and J.E. BENNETT. New York:Wiley, 1985, p. 452-475.

287. ZAHL, P.A., S.H. HUTNER, S. SPITZ, K. SUGIURA, and F.S. COOPER. The action of bacterial toxins on tumors. I. Relationship of the tumor-hemorrhagic agent to the endotoxin antigens of gram-negative bacteria. Am. J. Hyg. 36:224-242, 1942.

288. ZIMMERMAN, J.J., and K.A. DIETRICH. Current perspectives on septic shock. Pediatr. Clin. North. Am. 34:131-163, 1987.

289. ZUCALI, J.R., H.E. BROXMEYER, M.A. GROSS, and C.A. DINARELLO. Recombinant human tumor necrosis factors alpha and beta stimulate fibroblasts to produce hemopoietic growth factors in vitro. J. Immunol. 140:840-844, 1988.

APPROVAL SHEET

The dissertation submitted by Mae J. Ciancio has been read and approved by the following committee:

Dr. James P. Filkins, Director
Professor and Chairman, Physiology, Loyola

Dr. Mohammed M. Sayeed
Professor, Physiology, Loyola

Dr. Stephen B. Jones
Associate Professor, Physiology, Loyola

Dr. Herbert L. Mathews
Associate Professor, Microbiology, Loyola

Dr. Anthony Cerami
Professor and Dean of Graduate and Post Graduate Studies
Rockefeller University

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

4-7-89
Date

James P. Filkins
Director's Signature