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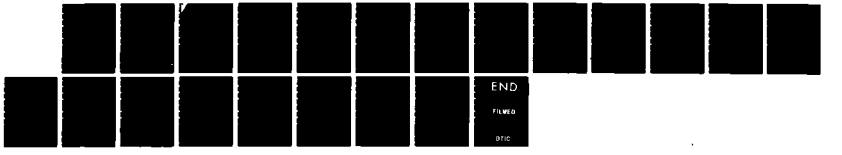

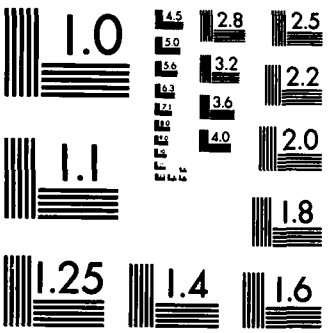

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A Program For Clinical Care in Physical Trauma

Annual Summary Report

Douglas W. Wilmore, M.D.

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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Harvard University
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A septic sheep model has been developed which is similar to extensive cellulitis secondary to penetrating injury of the leg. This model increases the release of skeletal muscle amino acids, fails to develop ketosis during starvation and injury/infection, and appears suitable to evaluate therapies that would reverse the catabolic effects of injury.

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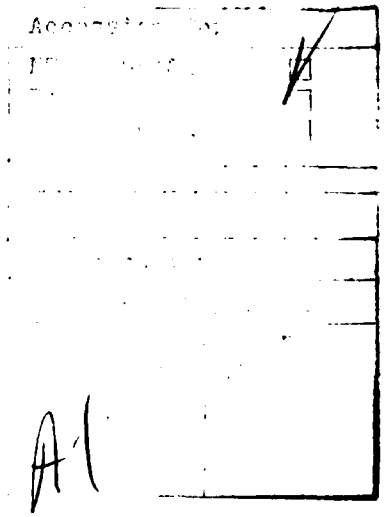
20. Abstract.

Protein turnover, determined in trauma-septic patients using stable isotopes, is increased. The increase in breakdown exceeds synthesis, and these alterations in amino acid metabolism account for the negative nitrogen balance following injury.



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SUMMARY

We have developed a septic model in the sheep that mimics extensive cellulitis secondary to penetrating injury with infection. With the development of injury and infection in the hindleg of the sheep, there is marked change in the concentrations of biochemical substrates and associated increased gluconeogenesis and skeletal muscle nitrogen release. There is also a failure to develop ketosis following starvation during this stress. This model has great benefit to evaluate therapies that would reverse the marked catabolic effects following injury and injury associated with infection.

Protein turnover was determined in normal individuals and in patients using stable isotopes. Acute febrile illnesses, severe stress and burns all increased protein turnover, and this was accompanied by increases in synthesis and catabolism. In normal individuals it was demonstrated that increasing protein intake had some effect on turnover and catabolism, but the withdrawal of the protein from the diet had a more significant effect on turnover. The marked increase in protein turnover and catabolism that is associated with trauma and trauma complicated by infection contributes to the negative nitrogen balance associated with these diseases.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

TABLE OF CONTENTS

A. DEVELOPMENT OF AN ANIMAL MODEL OF THE CATABOLIC STATE

INTRODUCTION

Because of the profound effects that injury and injury complicated by infection have on body metabolism, we sought to develop a reproducible animal model that would simulate the catabolic responses. A variety of treatments which could be utilized to modify catabolic stress could then be assessed. Accordingly, we selected the sheep to study in the awake state. To date, our work has been to develop a model of soft tissue infection which would have a predictable response and allow us to assess various treatment modalities.

METHODS AND MATERIALS

The value of using the large ruminant and the baseline date which serve to describe both biochemical and physiologic changes in the sheep was discussed in the previous application (1). We have used sheep of mixed breeds, weighing from 20-60 kg, with usual weights ranging from 30-35 kg. The animals are allowed to adjust to the stalls in the animal quarters and are fed grain and hay. Water and a salt lick are available at all times. The animals are usually studied as their own controls; but studies requiring long-term periods of catheterization (greater than four weeks) are difficult to achieve, and therefore these studies involving regional flux frequently require two groups of animals. The sheep are conditioned to the laboratory environment by bringing them into the study room everyday in a small, mobile catch pen. Generally two sheep are studied simultaneously, and the conditioned animals stand quietly or lie during the study period.

Studies of fed animals are carried out in the early morning on a day when food and water are available to the animals. During the starvation studies hay and grain are withdrawn, but water and the salt lick remain. The animals are fasted for a period of up to 7 days and then re-studied. Progressive weight loss of approximately 1 kg/day is generally observed during fasting. After simple fasting studies, the animals are allowed at least 10 days of uninterrupted feedings before further studies are planned.

Soft tissue infection is created by anesthetizing the animal and then surgically creating a pocket in the muscle of one thigh. After incision, the muscle is split and a single gauze sponge contaminated with colonic bacteria is placed deep in the muscle bed. The fascia is closed tightly with chronic gut and a subcutaneous pocket is formed. A second contaminated sponge is placed in this percutaneous space, and the skin closed. Short-acting penicillin (100 million units) is administered to the animal to prevent clostridia infection. The animals develop marked swelling of the extremity over the next two days, and gram negative bacteria are formed in their bloodstream on the second or third day. The organisms cultured have been predominantly (90%) E. coli and Klepsillia species. Because the infected animal will not eat during illness, we have withdrawn food completely and compared the infected group with fasted animals. If extensive extremity swelling occurs and oral fluid intake is not adequate, salt solution will be infused intravenously or given by gavage on the first day following infection. After the first day, however, hydration is spontaneously maintained. At the end of the experiment, all animals are sacrificed and their wounds examined and cultured. Purulence has been present in both muscle and subcutaneous tissue in all cases; it is rare to see metastatic lesions in liver, kidney or other visceral organs.

In some animals we have implanted long-term indwelling catheters to aid blood sampling and regional studies. These polyvinyl catheters are placed under general anesthesia after the animal has been initially conditioned. Through a midline wound, #7 French polyvinyl catheters are inserted into the bifurcation of the aorta and inferior vena cava, and after placement the distal ends exited through a stab wound in the flank.

A Jelco heparin lock is placed on a needle affixed to each catheter, and it is filled with heparin. The exiting catheters are fixed to the animal with adhesive tape and are easily maintained for periods of up to four weeks. Similar catheters have been inserted in the hepatic, portal, and mesenteric veins, and in the renal vein. In one study, catheters were carefully placed in the lower aorta and inferior vena cava so that a dye infusion and blood sampling allowed calculation of hindquarter flux and amino acid turnover before and after infection (2). The infection in this case was placed on the front shoulder of the animal so that the hindquarter would not be affected directly but rather reflect the indirect effects of distant infection.

RESULTS

Over 30 sheep have now been studied. The animals are extremely docile and yield consistent results. They are easily maintained as chronic preparations and can be studied for periods of up to 6 weeks.

With infection, the hindlimbs swell, and core temperature rises. Infection has marked effects on the basal arterial concentration of blood substrates. The most specific effect appears to be the lack of ketosis that occurs in the starved animals that are also infected (Table I). This response is also associated with a marked fall in blood alanine. While glucose is reduced, this response is more variable between the infected and starved animals.

In catheterized animals we have measured hepatic and portal bloodflow and substrate flux using techniques described by Bergman (3). Simultaneously, a tritiated glucose is infused, and after steady state is obtained with the H^3-6-glucose, samples are obtained to determine glucose turnover by both techniques.

The results agree with those found in the literature and indicate that the prime constant infusion isotopic technique is an acceptable approach for study of glucose turnover in these animals.

In 6 animals with catheters placed in the inferior vena cava and lower aorta, paraminohippurate (PAH) was infused into the arterial catheter and A-V samples were obtained for PAH (the dilutional marker to calculate bloodflow), beta-hydroxy-butyrate, glucose, lactate, and alanine. After studying the animals in the control state, infection was placed on the front shoulder, and after three days the animals were re-studied. The data (Table II) demonstrate the marked increase in alanine release associated with this infection. This increased nitrogen flux is similar to that seen in man and most probably accounts for negative nitrogen balance and weight loss associated with infection in these animals.

Basal arterial substrates and glucose turnover have been determined in fed, three-day fasted, and three-day fasted-infected animals. The infection was placed in a hindleg on Day 0 of the study when the fast was initiated.

Glucose production increased in the infected animals when compared with the starved controls (Table III). In addition there was a rise in body temperature and a fall in blood amino acids. No major change was seen in lactate, pyruvate or ketone bodies with this short-term infection.

DISCUSSION

The sheep appears to be an ideal model for studying the responses to soft tissue injury and infection in the awake state. Of the 20 animals that have been infected, only two have died. We initially attempted to quantitate the extent of infection, but this has been quite difficult because of the variations in animal response to fixed innoculum. We have therefore simply chosen to deal with the animals as "infected" and have cultured both the wound and blood. The longterm catheterization allows ready vascular access and allows flux studies across the regional bed. Hemodynamic measurements have not been consistently made in these animals although blood pressure is taken via the arterial line at the time of each study, and all infected, starved animals have maintained normal blood pressure. It appears that we can now evaluate a variety of substrates, hormones, and blockers to assess their effect on this response and to determine the possible mediators in the infected state which initiate this response.

B. EFFECTS OF NUTRITIONAL INTAKE, ROUTE OF FEEDING, INJURY AND SEPSIS ON TOTAL BODY PROTEIN TURNOVER

INTRODUCTION

Nitrogen economy is adversely effected in patients with severe injury and infection. This may be due to a decrease in protein synthesis, an increase in catabolism, or both. Continuous administration of 15N-enriched glycine (15N-Gly) is used in this study to estimate whole body protein turnover (Q), synthesis (S), and catabolism (C) (4). The techniques, method of administration, and method of measurement were described in the previous submission. In addition, the diet in normal controls was changed by altering caloric intake, thus allowing the examination of the effects of acute nutritional and metabolic changes in Q, S, and C in normals.

MATERIALS AND METHODS

All subjects were maintained on intravenous or oral diets to provide at least 30 calories per kilogram per day and 1 gram protein per kilogram per day. Six male control subjects, ages ranging from 18 to 30 years, were maintained on oral diets of four equally spaced meals daily. The N15 glycine in these individuals was maintained every three hours by oral dose of 0.5 mg 15N glycine per kilogram body weight per day. Those receiving IV infusions simply had the label added to the IV infusate. All subjects reached equilibrium as evidenced by the equilibrated enrichment plateau of urea 15N excretion. After steady state was reached in volunteers at 72 hours, protein intake was doubled or calories and protein withdrawn entirely. The investigation continued for 48 hours and Q, S, and C reassessed at this time.

RESULTS

Q was markedly elevated in all patients studied (Table IV). Synthesis matched catabolism in those subjects with severe injury as a result of adequate provision of calories and dietary nitrogen. In the normal control subjects, doubling protein intake acutely increased the apparent Q by 20%, 24-48 hours after change in intake. Upon sudden cessation of protein calorie intake, a 40% decrease in Q was produced. Protein doubling caused minimal changes in synthesis rate, with a moderate decrease in catabolism observed (a decrease of 18%). Cessation of intake caused a marked decrease in synthesis by 57%, with no significant changes in catabolic rate in these short-term experiments (See Table V).

DISCUSSION

Acute trauma and febrile illnesses and severe stresses produced large increases in Q, W, and C, suggesting very rapid turnover of protein in these states. Because all patients were being fed, synthesis was maintained by the nitrogen and dietary calories. As seen from the normal volunteers, increasing protein intake has some effect on Q and C, while withdrawing protein lowers Q but has a minimal effect on C. Q and C are greatly altered by acute disease processes. The metabolic response to these diseases heightens flux and catabolism, which contributes to the negative nitrogen balance associated with these illnesses.

While it is known that both synthesis and catabolism increase in these patients, it is not known if this occurs in all tissues or is due to the increased breakdown rate of skeletal muscle matched by the increased synthesis rate of visceral organs. Further studies will need to be planned in order to answer these specific points.

TABLE I

EFFECT OF A LONG-TERM FAST WITH AND WITHOUT INFECTION ON BLOOD SUBSTRATE CONCENTRATIONS (Mean±SEM)

	Body Temperature (°F)		Blood Glucose (mg/100 ml)		β-hydroxybutyrate (mM/L)		Alanine (mM/L)	
	Controls	Infected†	Controls	Infected	Controls	Infected	Controls	Infected
N	6	7	6	7	6	7	6	7
Before Fast	102.4±0.2	102.8±0.3	50±1	44±2	0.27±0.04	0.37±0.06	0.23±0.02	0.19±0.07
Day Fast Started	102.7±0.2	103.1±0.3	50±2	47±3	0.37±0.08	0.39±0.04	0.20±0.01	0.19±0.04
[-----Day of Fast-----] 1	102.6±0.2	102.6±0.1	46±2	42±3	0.55±0.07	0.52±0.05	---	---
2	102.6±0.2	102.3±0.1	43±4	40±3	0.68±0.07	0.63±0.07	---	---
3	102.4±0.3	102.0±0.1	41±4	38±2	0.80±0.10	0.73±0.10	---	---
4	102.4±0.7	102.0±0.4	38±4	37±2	0.91±0.13	0.92±0.30	0.09±0.01	0.10±0.02
5	102.1±0.4	104.3±0.3*	37±3	31±2*	1.00±0.12	0.37±0.06*	0.09±0.02	0.07±0.02*
6	102.1±0.3	104.2±0.5*	37±3	27±2*	1.00±0.12	0.71±0.12	0.09±0.02	0.06±0.02*
7	102.7±0.3	103.5±0.3	40±3	30±2	1.01±0.08	0.92±0.16	---	---

† Abscess in leg created on Day 4.

* $p < 0.05$ when compared to fasting alone

TABLE II

HINDQUARTER ALANINE KINETICS IN SHEEP
BEFORE AND AFTER INFECTION
(N=10, MEAN±S.E.)

	BLOODFLOW L/MIN	A-V DIFFERENCE μM/L	ALANINE TURNOVER μM/MIN
CONTROL	0.751±0.082	25±5	18±4
INFECTED	1.039±0.108	31±5*	37±10*

* $p < 0.05$ by paired t-test.

TABLE III

GROUP CHARACTERISTICS, ARTERIAL SUBSTRATE CONCENTRATIONS AND GLUCOSE TURNOVER IN FED, FASTED AND FASTED-INFECTED SHEEP

	FED	FASTED	FASTED AND INFECTED
N	21	9	13
WEIGHT (kg)	31.1±2.1	36.8±3.7	28.4±2.0
TEMP (°C)	39.4±0.1	39.5±0.2	40.5±0.1*
WHOLE BLOOD GLUCOSE (mg/100 ml)	59±2	50±3*	44±2*
LACTATE (mM/L)	0.62±0.09	0.67±0.31	0.77±0.14
PYRUVATE (mM/L)	0.09±.01	0.06±0.01*	0.10±0.01**
β-HYDROXYBUTYRATE (mM/L)	0.33±0.05	0.51±0.15	0.45±0.05
ACETOACETATE (mM/L)	0.03±0.01	0.07±0.01*	0.06±0.01*
ALANINE (mM/L)	0.17±0.01	0.13±0.02	0.08±0.01**
GLUCOSE TURNOVER (mg/kg · min)	1.83±0.13	1.15±0.11*	1.72±0.20**

* Different than fed. $p < 0.05$.

** Different than fasted. $p < 0.05$

TABLE IV

PROTEIN TURNOVER, SYNTHESIS AND CATABOLISM IN PATIENTS

PATIENT	AGE YRS	WEIGHT KG	DIAGNOSIS	Q MG N/KG-DAY	Q	S G N/DAY	C
NORMAL	25	70.0		506	34.2	23.9	22.2
G.P.	52	75.8	54% TBS* BURN 17 PBD+	872	66.1	45.9	34.6
G.P.		71.0	30 PBD	748	53.1	32.9	22.6
A.B.	40	78.8	70% TBS BURN 5 PBD	660	52.0	32.8	51.9
A.B.		64.0	38 PBD	979	62.7	50.2	34.6
D.P.	26	64.0	ABDOMINAL SEPSIS	862	55.2	30.1	26.2
D.P.		51.5	SEPSIS, GI BLEED, POSTOPERATIVE	822	42.3	27.5	13.0
S.C.	28	66.5	TESTICULAR CANCER STAGE III, PRE-CHEMOTHERAPY	677	45.0	27.7	24.5
S.C.		66.5	TESTICULAR CANCER POST-CHEMOTHERAPY	659	43.8	22.8	22.2

* TBS - Total Body Surface.

** PBD - Post Burn Day.

TABLE V

EFFECT OF ACUTELY ALTERED PROTEIN AND CALORIE INTAKE ON APPARENT PROTEIN TURNOVER, SYNTHESIS AND CATABOLISM

	30 Kcal/Kg-Day 1 Gm Protein/Kg-Day	30 Kcal/Kg-Day 2 Gm Protein/Kg-Day	0 Kcal 0 Protein
N	N = 8	N = 8	N = 4
Q mg N/Kg-day	505±7±70	607.6±91	303.6±24
Q gm N/day	34.2±8.5	41.0±6.9	21.9±4.5
S gm N/day	23.9±6.5	25.2±6.3	10.3±4.9
C gm N/day	22.2±6.2	18.2±7.6	21.9±4.5

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