行政院及所屬各機關出國報告

現代燒傷之治療及研究

服務機關：成功大學附設醫院
出國人職　　稱：主治醫師
　　姓　　名：陳琮琳
出國地區：美國
出國期間：88.8.16~89.8.17
報告日期：90.5.7

HARVARD UNIVERSITY

OFFICE OF THE SECRETARY
17 QUINCY STREET

CAMBRIDGE, MASSACHUSETTS

July 7, 2000

Dear Dr. Chen,

I beg to inform you on behalf of the University and the Dean of the Faculty of Medicine that you are appointed Research Fellow in Surgery, from July 1, 2000 to June 30, 2001, subject to the Third Statute of the University (*attached*), as amended from time to time, and to such terms, conditions and policies as may be stipulated by the Faculty of Medicine. Such terms and conditions include, but are not limited to, continuing availability of funds from sources outside the University and, in the case of faculty members who hold an appointment at an institution affiliated with Harvard Medical School or Harvard School of Dental Medicine, the continuation of the appointment at the affiliated institution.

Your obedient servant,

Secretary of the University

RE: Chung Lin Chen

HARVARD UNIVERSITY

OFFICE OF THE SECRETARY
17 QUINCY STREET

CAMBRIDGE, MASSACHUSETTS

July 14, 2000

Dear Dr. Chen,

I beg to inform you on behalf of the University and the Dean of the Faculty of Medicine that you are appointed Research Fellow in Surgery, from September 1, 1999 to June 30, 2001, subject to the Third Statute of the University (*attached*), as amended from time to time, and to such terms, conditions and policies as may be stipulated by the Faculty of Medicine. Such terms and conditions include, but are not limited to, continuing availability of funds from sources outside the University and, in the case of faculty members who hold an appointment at an institution affiliated with Harvard Medical School or Harvard School of Dental Medicine, the continuation of the appointment at the affiliated institution.

Your obedient servant,

Secretary of the University

Chung Lin Chen

This is to certify that

Chun Lin Chen, M.D.

has served as a

Research Fellow in Surgery

at the

Massachusetts General Hospital

from September 1, 1999 to August 31, 2000

President

Chief of the Surgery Service

C09002343

行政院及所屬各機關出國報告提要

出國報告名稱：現代燒傷之治療及研究

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出國計畫主辦機關/聯絡人/電話
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陳琮琳/成功大學醫學院附設醫院/外科部主治醫師/(06)2353535-5181
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現代燒傷之治療及研究

摘要：

儘管醫學進步,燒傷依然是高死亡率及高後遺症急性傷患。以目前治療燒傷及研究水準,美國仍是居領先地位。這其中又以 Shriner's Burns Hospirals 為執牛耳之重鎮。

Shriner's Burns Hospirals 治療以孩童為主,醫院經費來源主要是善心人士的捐款,醫療費用完全由國家保險制度及捐款來支出,病童家屬除可完全不用負擔費用外,尚可享有供應家屬食宿上的方便。其治療方式會因流別不同而採用自家治療的特色,但是基本上,都是提供一個舒適、隔離及無菌防護以及高效率之手術療程來治療病童。個別之

Shriner's Hospiral 一般皆有一對應之大型綜合醫院來照護大人之燒燙傷，治療之醫師皆為二家之主治醫師，因此治療方式二家醫院實為一體。至於經費管理，其他醫療從業人員則完全獨立。

就麻州總醫院及波士頓 Shriner's Burns Hospirals 而言，除提供一隔離、適溫及舒適環境於病人外，採用早期清創、早期傷口敷蓋之方式來作傷口處理。敷蓋傷口之材料有很多的選擇，如自體皮膚移植，integra 敷蓋或屍皮保護傷口；提高適溫環境(32℃)及鎮靜、止痛，甚至麻醉藥物來降低病患能量消耗及需求；利用小單位隔離及無菌操作來降低感染率，以及使用多種抗生素來預防及治療感染病菌。為了補償因麻醉藥物所產生之胃腸道消化不佳狀態，此二家醫院強調使用靜脈營養輸液提供患者所需能量及營養素。就復健而言，二者皆提供專職職能及物理治療復健師，幫助患者執行復健計畫，因此從二家醫院出院病患有較佳康復結果。在臨床治療上本人認為最重要的一點為二者皆以團隊治療為主體，尤其護理照護及治療計畫皆由團隊執行，因此病患可得充沛有計畫之療程，而不受單一主治醫師門診、開會、教學或其他研究而耽誤。

有關研究方面，幾乎從事人員有其研究方案在進行，以上二家醫院，有專門人員從事資料收集，及電腦輸入，住院病患源源不絕，因此很容易可發表臨床研究成果。至於基礎研究則有主是醫學士後或博士後研究人員專門負責，其成果也是相當傲人。

現代燒傷之治療及研究

榮幸獲教育部同意出國一年,主要進修燒傷之治療及研究,茲將心得整理如后:

麻州總醫院(MGH)及波士頓 Shriner's Burns Hospirals(SBH)為美國東北部及加拿大東岸治療嚴重燒燙傷之重鎮,知名的 Dr. Burk 即為其過去此二家燒傷中心之主持人。MGH 只照顧燒燙傷大人病患,而對面之 Shriner's Hospiral 只針對小孩子病人(二家治療方式幾乎一同)。二家醫院除醫師為互聘外,其他醫療從業人員為分別歸屬二家,財務本身也是獨立。因為在經費上 SBH 獲得美生會(一慈善團體)大力支持,其設備及人力調配比起 MGH 更為充沛,治療成果也相對較佳。因此,底下主要簡介 SBH 之現況。

此醫院為 1997 年六月啟用之九層樓建築,最高樓層為行政中心及餐廳。八樓有十三間獨立 ICU 床位,隔鄰為開刀房,如此可有效並安全運送嚴重病患於開刀房及 ICU 間。ICU 病房除獨立位外,內尚有無菌之透明內幕環繞病床外,此點除可提供病患更深一層防止感染保障外,尚可提供適宜病患體之溫度環境而不影響周圍工作人員。任何接觸病患之治療行為,皆得穿戴無菌手套伸入帳幕中操作,也就是如此其病房感染率低於百分之五。

就傷口處理而言,他們使用硝酸銀溶液每二小時浸潤一次,一天換藥一至二次方式來治療傷口。原則上儘早結痂切除,儘早自身體移植為主,若健康皮膚面積不足,則以 integra 或屍皮為傷口敷料,屍皮之製作及保存在此間醫院內有專業人員負責此皮庫中心之運作。營養則主要以靜

脈輸液為主，個人想是用其感染率低而又使用大量麻醉藥物鎮靜止痛病患遺留病患腸胃收吸不良之產物。此醫院治療主體為一團隊，不因個人差異而有不同選擇，也因其團隊治療，不因醫師個人忙碌或休假而讓療程拖延，此點為我個人對台灣醫療現況最大感嘆。

此醫院每天早上 7:00 查房，護理人力為二班制，因此所有團隊成員皆參加。在每周三下有二小時團隊會議討論病人治療計畫，各從事人員各師其務貢獻所長，這點與台灣現況醫師獨大也有所不同。

醫院七樓為有 33 床之燒傷病房床位及整形重建床位。病房外有 playground 可供病童玩耍。六樓則闢為門診診間，其診別有外科、整形外科、精神外科等，只是並非每天有門診服務。

此醫院並無設立急診處，急診病患需經 MGH 急診處置後轉入，SBH 與 MGH 間有一地下通道，方便病人相互轉送，SBH並無昂貴檢查儀器，如電腦斷層或核磁共振等設備，病患需要的話亦是經由此通道到 MGH 作檢查。

SBH 出名處除臨床成績傲人外，他們發費在研究上也相當可觀。關於這點可由研究空間佔據此大樓之二樓及四樓，並有六十幾位主要研究員或博士後研究生在此工作即可見一斑，研究範疇從 Tissue Engineer, scar management 到代謝及菌株病體變化皆有人投入。研究人員之經費來源主要來自美國衛生院(NIH)，SBH 研究經費或有興趣之藥廠提供援助。在此很容易看到產、經、學三者相互合作，彼此貢獻所長來完成尖端之研究成果。主要研究人員或研究生人事費用取自研究計畫，因此研究人員或研究生皆會賣力完成研究部分以爭取次年研究機會。此點在基礎上與我

們用部分人力或部分工時的條件要與全職的他們來比較成果，在先天上即有空談之譏。

就研究人員而言，他們每週三上午八時至九時會邀請出名之專家學者來院作特別演講，彼此分享及討論研究項目，並激發出更穩當之理論。同時他們也會每月邀請專科之教授(MIT 或 Havard)來作評語，其過程相當精彩。

個人在波士頓一年，也學著作些研究，其結果也發表在今年美國燒傷學會會議上(如附件)。

行政院及所屬各機關出國報告審核表

出國報告名稱：現代燒傷之治療及研究	
出國計畫主辦機關名稱：教育部	
出國人姓名/職稱/服務單位：(若二人或以上，則列○○○等_人)	
出國計畫主辦機關審核意見	☐1. 依限繳交出國報告 ☐2. 格式完整 ☐3. 內容充實完備 ☐4. 建議具參考價值 ☐5. 送本機關參考或研辦 ☐6. 送上級機關參考 ☐7. 退回補正，原因：☐①不符原核定出國計畫 ☐②以外文撰寫或僅以所蒐集外文資料為內容 ☐③內容空洞簡略 ☐④未依行政院所屬各機關出國報告規格辦理 ☐⑤未於資訊網登錄提要資料及傳送出國報告電子檔 ☐8. 其他處理意見：
層轉機關審核意見	☐同意主辦機關審核意見 ☐全部 ☐部分_____(填寫審核意見編號) ☐退回補正，原因：_____(填寫審核意見編號) ☐其他處理意見：

說明：

一、出國計畫主辦機關即層轉機關時，不需填寫「層轉機關審核意見」。

二、各機關可依需要自行增列審核項目內容，出國報告審核完畢本表請自行保存。

三、審核作業應於出國報告提出後二個月內完成。

HARVARD MEDICAL SCHOOL MASSACHUSETTS GENERAL HOSPITAL

Ronald G. Tompkins, M.D., Sc.D.
John F. Burke Professor of Surgery

Chief, Trauma and Burn Services
Massachusetts General Hospital

Chief of Staff
Shriners Burns Institute

July 26, 2000

Tsu-Fuh Yeh, M.D.
Superintendent
Professor of Pediatrics
National Cheng Kung University Hospital
Tainan, Taiwan, Republic of China

RE: Chung-Lin Chen, M.D.

Dear Dr. Yeh:

I am writing on behalf of Dr. Chung-Lin Chen to affirm that Dr. Chen has been a Research Fellow in Surgery at the Massachusetts General Hospital, Shriners Hospitals for Children - Boston, and the Harvard Medical School from 1 September 1999 to 31 August 2000.

We have been delighted to have Dr. Chen here at Harvard. Dr. Chen has been a clinical observer on our Burns Service both at the adult unit at the Massachusetts General Hospital and the pediatric unit at the Shriners Hospital. Dr. Chen has been a pleasure to have as an observer and we feel very confident that he has learned a great deal from this experience. We look forward to visiting him in Tainan to see how well he has implemented these techniques in his own practice.

Dr. Chen also was actively engaged in laboratory research here at the Shriners Hospital. He worked very diligently upon an NIH-supported research project involving metabolic changes after burn injury. The manuscript describing this work is entitled "Studies on Metabolic Fates of Arginine in Liver after Burn Injury" and Dr. Chen is the first author recognizing his very important contributions to this work.

We are delighted that Dr. Chen has spent this last year with us. Our evaluation of his activities here is very favorable. We look forward to his success in Tainan and hope that we might continue an excellent working relationship in the future.

Sincerely,

Ronald Tompkins

Ronald G. Tompkins, M.D., Sc.D.

MGH, GRB 1302
55 Fruit Street
Boston, Massachusetts 02114-2696

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Metabolic Fate of Arginine in Relations to Urea Cycle in Liver after Burn Injury

Chung-Lin Chen, MD,[1,2] Zhewei Fei, MD,[2] Yong-Ming Yu, MD, PhD,[2] Edward A. Carter, PhD,[2] Xiao-Ming Lu, PhD,[2] Rey-Heng Hu, MD,[2] Vernon R. Young, PhD,[3] and Ronald G. Tompkins, MD, ScD.[2]

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Chen was supported by a grant from National Cheng Kung University Hospital, Taiwan, R.O.C..

Short running head: Burn injury and liver arginine metabolism.

A quantitative evaluation of altered hepatic arginine and urea cycle metabolism by stable isotope tracers in a liver perfusion system to explore the metabolic basis for the burn injury-induced protein catabolic state.

Conclusion

The increased transport and utilization of the extrahepatic arginine contributes significantly to the accelerated urea production after burn injury; hence, enriched arginine supply may spare nitrogen loss from other amino acids and improve the nitrogen economy of the burn patients.

INTRODUCTION

Hypermetabolism and significantly elevated net nitrogen loss are hallmarks of the metabolic aberrations commonly seen in the severely burned patients. Since more than 90% of the nitrogen loss from these patients are in the form of urea,[1] exploring the regulation of urea cycle activity in health and diseased condition would shed light on the mechanisms of such accelerated urea cycle activity and potentially, to improve the metabolic care of the critically ill patients. Our previous study[2] using a rat liver perfusion model revealed that when perfused with the same composition of medium, livers from burned rats produced more urea than those from the sham burn rats. It appears that the urea cycle activity is intrinsically up-regulated in response to burn induced stress. However, the contributing factors related to this up-regulation remains to be determined. Arginine is an intermediate of the urea cycle and serves as an immediate precursor for urea synthesis. Under the in vivo condition, arginine exists in the blood circulation, it can also be synthesized *de novo* intrahepatically through a spatially well-organized urea cycle enzyme system, using the nitrogen source from other amino acids. The source of arginine for the elevated urea production from the liver and the major metabolic fate of the extrahepatic arginine within the liver under the burn injury induced protein catabolic state remains unclear. Therefore, exploring the changes in the extra- and intra-hepatic arginine transport, its intrahepatic metabolic pathways in relation to cellular function after the insult of burn injury would lead to further understanding the mechanism of surgical trauma induced whole body catabolic state, and a possible approach to improve the metabolic care of the severely burned patients.

The transport of arginine into the liver has been studied using the hepatocyte membrane vesicle preparations. Pacitti AJ et al[3] reported a significantly activated y^+ system mainly for arginine transport and observed an increased transport kinetics of arginine into the hepatic membrane vesicles from rats with septicemia, indicating an increased utilization of the extrahepatic arginine by the liver in

septic condition. However, the intrahepatic metabolic fate of arginine cannot be elucidated by the vesicle preparations. The present study used an in situ liver perfuison system combined with the use of L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$]arginine tracer. The liver perfusion system allows us to dissect the various in vivo factors which may influence trauma induced alteration in liver metabolism. Furthermore, by tracing the stable isotope labeling, the rate of arginine transport, and its major intrahepatic metabolism in relation to the activated urea cycle after burn injury were also evaluated. Our studies revealed an increased consumption of preformed arginine from extrahepatic source for urea production after burn injury and its potential implication in the metabolic care to the burn patients.

MATERIALS AND METHODS

Burn and Sham Burn Animal Model.

The study was conducted in sixteen male Sprague-Dawley rats (Charles River Laboratories, Boston, MA) weighing 220-280 gram. They were housed in the Animal Farm of the Massachusetts General Hospital under 12-hour light-dark cycle at least two days before the study. The animals were cared for in accordance with the Public Health Service Policy, the Guide for the Care and Use of Laboratory Animals, and Subcommittee on Research Animal Care, Massachusetts General Hospital. Tap water and standard rat chow were provided *ad libitum*. They were randomly divided into two burn and sham burn groups (n = 8 each). For the burn group, a 25% total burn surface area (BSA) full thickness burn injury was induced according to the procedures described before.[2, 4] Briefly, the animals were anesthetized with intra-peritoneal injection of a mixture of Ketamine (60 mg/Kg) and xylazine (1.3 mg/Kg). After shaving the hairs, the dorsal part was merged into boiling water for 10 seconds along a pre-drawn based on by a computer-generated template, with 25% of the calculated total body surface area. This was followed by immediate intra-peritoneal injection of saline 3.0ml. kg body weight^{-1}. % BSA^{-1} for fluid resuscitation. The animals were observed during

their recovery from anesthesia. The sham burn animals were treated in the same manner except that the dorsal sites were merged into 37 °C water bath for 10 seconds.

Perfusate and Isotope Preparations.

Two perfusion solutions were prepared. Solution A, amino acid perfusate was a modification of Eagle's Minimal Essential Medium conventionally used for Hepatocyte Culture (M0268; Sigma Chemical Co., St. Louis, MO), by adding 3% dialyzed (Hemoflow F8; Fresenius USA, Lexington, MA) inactivated bovine serum albumin (Fraction V, Sigma Chemical Co, St. MO) and an amino acid mixture with the end amino acid profile and concentrations (including glutamate, serine, alanine, proline, asparagine, asparate and glycine) being close to 2 x those in the portal vein.[5, 6] The perfusate was then filtered through a 0.20 μ NALGENE® disposable filter unit (Nalge Company, Rochester, NY, USA) for sterilization and preserved in sterile bags (300 ml of each) at -20°C until use. On the perfusation day, stable isotope-labeled tracer L-[$^{15}N_2$-guanidino, 5,5-$^{2}H_2$]arginine·HCl (MassTrace, Woburn, MA) was added to perfusate to reach a targeted enrichment of 17.5% molar ratio. Solution B was basically a Krebs-Henseleit bicarbonate solution[7] containing 3% dialyzed (Hemoflow F8; Fresenius USA, Lexington, MA) inactivated bovine serum albumin (Fraction V, Sigma Chemical Co, St. Luis, MO). It was also sterilized and stored at –20 °C prior to use.

The liver perfusion system (Figure 1) was similar to that used previously with slight modification.[2, 8] The whole system was placed in a closed thermodynamic chamber at 37 °C. It includes two separate containers, filled with either Solutions A (146.5 ml) or B (400 ml). Before starting the perfusion, each solution was circulated in this system for oxygenation and was adjusted the pH value to 7.4.

The flow rate was determined based on the body weight and the wet weight of the liver, estimated using the equation: $LV_{wet} = 0.034$ (body weight, Kg)$^{0.89}$.[9] The exact rate was measured during the perfusion by timed collecting and weighing of the output.

The perfusion studies are carried out on the post-burn (or sham burn) day 4. The cannulation of the portal vein was performed according to the procedures described previously.[2] After laparatomy under Ketamine and Xylazine anesthesia, a #16 INTRACATH (Deseret Co., Sandy, Utah) was inserted into the portal vein and secured with 3 "0" silk tie. The perfusion solution B was immediately directed into the indwelled portal vein catheter with a maximum ischemia period less than 1 minute. An incision was made on interior vena cava at a site below it branching point of the right renal vein, and a loose tie was placed around the inferior vena cava above renal vein. The perfusion of Solution B continued for washing out the residue blood in the liver. Simultaneously, a # 14 INTRACATH was placed into the superior vena cava and secured, the pre-set loose tie on the inferior vena cava was tightened immediately to redirect the washout *via* the superior vena cava catheter. After 20 minute perfusion of Solution B, the perfusate was switched to Solution A through a 3-way valve, and the output from the superior vena cava was directed into the reservoir containing solution B, the recycling perfusion procedure was thus started and continued for 120 minutes. During the perfusion period, the liver was covered with a piece of translucent plastic sheet to keep its moisture and temperature.

A perfusate sample (2 ml) were taken from the Solution A container before the addition of L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$]arginine tracer for the determination of baseline arginine enrichment. After mixing with the tracer, additional perfusate samples were taken at 0 (before the starting of the infusion) and then 10, 20, 40, 60, 90 and 120 minutes after starting the infusion. These samples were preserved in –20 °C freezer until analysis. In addition, three pairs of samples were also taken separately from the inflow and outflow lines of the liver at 10, 60 and 120 min for

determining of their oxygen content using a blood gas analyzer (238pH; CIBA Corning Diagnostic Ltd, Cambridge, MA). The volume of the remaining perfusate was recorded after perfusion.

Sample Analysis.

The concentration of glucose in the perfusate samples was measured on a Glucose Analyzer 2 (Beckman Instrument Inc., Fullerton, CA). The concentration of urea was analyzed using a Sigma Diagnostic Kit (Sigma Diagnostics #640-A; Sigma Chemical Co., St. Louis, MO).

The isotopic enrichments of the labeled arginine in perfusate samples were determined using the gas chromatograph- mass spectrometry technique as described before [10, 11, 12, 13]. The arginine concentration in the perfusate was simultaneously determined using the isotope dilution method. In brief, 20 µl of L-[U-$^{13}N_6$, U-$^{15}N_4$] Arginine·HCl(U-$^{13}N_6$,98%+; U-$^{15}N_4$,96-98%, concentration: 20.39µM) was added to 200 µl of each perfusate sample as internal standard. The amino acid were extracted using ion exchange column and prepared as methyl ester trifluoroacetyl derivative comparable to the one reported by Nissim et al.[14] This was analyzed using on-column injection with a HP 5980 series II gas chrmatograph coupled to a HP 5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA). Selective ion monitoring (SIM) of arginine was conducted on the [M-20]$^-$ ion, using negative chemical ionization with methane as the reagent gas. The ion corresponds to a loss of HF from the molecular ion. Arginine was measured by SIM m/z 456, m/z 458, m/z 460 and m/z 466 for natural (M + 0), L-[5,5, $^2H_2$] arginine (M+2), L-[$^{15}N_2$-guanidino, 5,5-$^2H_2$] arginine (M+4) and of L- [U-$^{13}N_6$, U-$^{15}N_4$] arginine (M+10). The enrichments were corrected by multiple linear regression analysis on the data from standard samples containing known amount of the arginine isotopomers (natural, M+2 and M+4).[15] The concentration was measured also against another standard curve based on a known amount of natural arginine against the same amount of internal standard (M+10) arginine, by the

same notion we used previously for quantifying plasma ketoisocaproate concentrations.[16]

The isotope abundance of [$^{15}N_2$]urea was also measured in the perfusate on the tetra-butyldimethylsilyl (t-BDMS) derivative, using the electron – impact mode. SIM was performed at m/z 231 [M-57] for natural urea and at m/z 233 for [$^{15}N_2$] urea.

Calculation and statistical analysis.

The oxygen consumption of the perfused liver is calculated by the difference in oxygen content between the inflow and outflow and the perfusion rate, expressed as micromoles per gram dry liver per minute.

Oxygen content of perfusate (CO_2) = PO_2 (mmHg) × 0.003 (mLO_2. dL^{-1}. $mmHg^{-1}$) = mL O_2 dL^{-1} perfusate[17]

Oxygen consumption (μmol. g wet $liver^{-1}$.$min.^{-1}$) = (inflow CO_2 – outflow CO_2) × 10 dL. L^{-1} × flow rate (mL. g wet $liver^{-1}$) ÷ 22.4 (mmol. mL^{-1}) × 10^3 (μmol. $mmol^{-1}$)

The rate of oxygen consumption and other metabolic parameters are averaged to per g of dry liver weight.

The rates of glucose, [$^{15}N_2$]urea and urea production was calculated based on the linear regression slopes for the change of the total glucose contents in the perfusion medium at different time points of the study (corrected for the volume loss due to the perfusate sampling).

The rate of arginine uptake or transport into the liver was calculated from the disappearance rate of the total arginine and L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$]arginine in the perfusate, estimated by the linear regression of total amount of arginine and L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$] arginine remaining in the perfusate at each time point of sampling during the perfusion period. Because the enrichment of L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$]arginine in the perfusion medium remained constant during

the study period, this represented the unidirectional uptake rate of arginine into the liver.

The fraction of the transported arginine utilized for urea production (F_{A-U}) was calculated using the equation:

$$F_{A-U} = \frac{\text{Rate of } [^{15}N_2]\text{urea production}}{\text{Rate of } [^{15}N_2\text{-guanidino, 5,5, } ^{2}H_2] \text{ arginine transport}}$$

Data, expressed as the mean ± SE, were analyzed with Student's *t* test between the burn (n=8) and sham burn (n=8) groups. Values of $p < 0.05$ were considered significant.

RESULTS

The Rate of Oxygen Consumption and Glucose Production from the Liver

The liver dry weight from the sham burn rats was 2.30 ± 0.07g and that from the burn rats was 2.20 ± 0.12g ($p > 0.05$). The oxygen consumption of burn group was approximately 2 times higher than the sham burn (4.1 ± 0.4 *vs.* 2.1 ± 0.4 μmol.g dry liver^{-1}.min.$^{-1}$) during the first 10 minutes of the infusion ($p<0.05$). It remained quite constant during the whole perfusion period with a slight decrease (3.6 ± 0.4 and 3.6 ± 0.3 μmol.g dry liver^{-1}.min.$^{-1}$ at 60 min. and 120 min. separately), but was not statistically different from the initial values measured during the initial 10 minutes. The oxygen consumption in the sham burn group showed a constant increment to3.1 ± 0.4 and 3.8 ± 0.4 μmol.g dry liver^{-1}.min.$^{-1}$ at 60 min. and 120 min, the latter two values were not statistically different from the burn group ($p>0.05$ on each time of both groups). These values suggested the viability of the livers throughout the perfusion periods.

During the perfusion period, the concentration of glucose in the perfusion media showed a steady linear increase in all the livers as our previous publisher.[2] The glucose productions in sham burn and burn groups are not statistical significant (30.7 ± 4.9 and 38.3 ± 4.0 μmol.g dry liver^{-1}.h^{-1}, separately).

Arginine Transport and M+2 Arginine Production

The initial concentration of arginine in the perfusate was 701.2 ±12.7 μM in the sham burn group and 708.1 ± 14.0 μM in the burn group ($p > 0.1$ by paired Student t-test). They are comparable to the targeted concentration of 723.3 μM. The initial concentrations of L-[$^{15}N_2$ guanidino, 5,5, $^{2}H_2$]arginine in the perfusate were126.2 ± 3.7 μM and 120.7 ± 4.7 μM in the sham burn and burn groups ($p>0.05$). During the recycling perfusion, there was a steady linear decline in the concentrations of both total arginine and the concentration of L-[$^{15}N_2$ guanidino, 5,5, $^{2}H_2$]arginine in the perfusate, during the entire time period, the enrichment of the labeled arginine remains at a constant level within each group of animals. The enrichment of L-[$^{15}N_2$ guanidino, 5,5, $^{2}H_2$]arginine (molar ratio excess) remained constant at the beginning 0.17 ± 0.01 and 0.18 ± 0.01 and the end 0.18 ± 0.01 and 0.18 ± 0.03 for burn and sham burn group respectively. The time (min.) required for the decline of total L-[$^{15}N_2$ guanidino, 5,5, $^{2}H_2$]arginine to 10% of its original content was 30.0 ± 8.0 min. for the burn and 87.5 ± 16.5 for the sham burn group, respectively ($p<0.05$). From the decline slopes, it was estimated that of total arginine transport into the liver proceeded at 3.4 ± 1.0 μmol.g dry liver^{-1}.min.$^{-1}$, in the burn animals and 1.0 ± 0.3 μmol.g dry liver^{-1}.min.$^{-1}$ in the sham burn group ($p< 0.05$), and the transport of L-[$^{15}N_2$ guanidino, 5,5, $^{2}H_2$]arginine proceeded at 0.5 ± 0.1 and 0.1 ± 0.0 μmol.g dry liver^{-1}.min.$^{-1}$ ($p<0.05$), indicating a significantly enhanced extra-hepatic to intra-hepatic transport of arginine after burn injury.

A slight release of M+2 arginine into the perfusion media, presumably the formation of L-[5,5, $^{2}H_2$]arginine, was observed at a rate of 0.08 ± 0.02 and 0.02± 0.01μmol.g dry liver^{-1}.min.$^{-1}$, respectively, for the burn and sham burn groups

($p<0.05$), they accounted for less than 1% of the total arginine transport into the liver. This suggested that almost all the arginine formed within the urea cycle remains within the cycle in both healthy and burned condition.

Hepatic Urea Production and Its Relationship with Arginine Metabolism

When perfused by the same medium, the total amount of hepatic urea produced from the burned animals during the perfusion period was 233.8 ± 9.7μmol.g dry liver^{-1}, which is significantly higher than the value of 179.2 ± 14.3μmol.g dry liver^{-1} obtained from the sham burned animals ($p< 0.01$ unpaired t-test).(Fig.3) During the first stage of perfusion when arginine was available in the perfusion medium, the rate of urea production was 3.4 ± 1.0μmol.g dry liver^{-1}.min.$^{-1}$ in the burn and 1.0 ± 0.3μmol.g dry liver^{-1}.min.$^{-1}$ in the sham burn group ($p< 0.01$), consistent with a higher urea cycle activity in burned animals. Meanwhile, the rate of net [$^{15}N_2$]urea production from the perfused liver also showed a higher level for the burned animals (0.27 ± 0.09μmol.g dry liver^{-1}.min.$^{-1}$) than that from the sham burn animals (0.05 ± 0.02μmol.g dry liver^{-1}.min.$^{-1}$, $p< 0.01$), indicating a higher rate of the extrahepatic arginine-derived urea production. Combined with the data presented earlier on the transport rates of L-[$^{15}N_2$.guanidino, 5,5, $^{2}H_2$]arginine in these two groups, we further calculated the fraction of the transported arginine to urea production which are 34 ± 9 % in the burn and 14 ± 4 % in the sham burn animals, suggesting a higher fraction of the transported arginine nitrogen fueled into urea. After the arginine in the extrahepatic arginine was consumed, i.e. after the initial 30.0 ± 8.0 min. in the burn group and 87.5 ± 16.5min. in the sham burn group, the rate of urea production showed a decline in both groups (1.57 ± 0.19μmol.g dry liver^{-1}.min.$^{-1}$ in burn and 0.35 ± 0.19μmol.g dry liver^{-1}.min.$^{-1}$ in sham burn), both were significantly lower, respectively than their previous values ($p< 0.05$ by paired t-test), however the urea production from the burned animals was still higher than the sham burned ones. It appeared that even after the

exogenous arginine was consumed, urea cycle was still operating in a higher level ($p< 0.05$ by unpaired t-test) in the burn as compared to the sham burn group.

In addition to urea production, the remaining fraction of the intrahepatically transported arginine was utilized by the "non-urea production" pathways, which was proceeded at the rate of 1.9 ± 0.5 μmol.g dry liver^{-1}.min^{-1} in the burn and 0.8 ± 0.2 μmol.g dry liver^{-1}.min^{-1} in the sham burn groups ($p< 0.05$; in Table 1). The major "non-urea production" pathways for arginine utilization include the formation of nitric oxide,[18] creatine,[19] other guanido-containing substances,[20] and its utilization for protein synthesis, of which, the quantitatively most important pathway was protein synthesis.[10] Therefore, it appeared that the increased transport of exogenous arginine also contributed significantly to the rate of protein synthesis in the liver, presumably for the facilitated synthesis of acute phase proteins in response to the insult of burn injury.

DISSCUSSION

The liver plays an important role in the regulation of the metabolic response to severe injury and trauma. The activated gluconeogenesis and urea production which are among the major metabolic aberrations seen after burn injury, occurs exclusively in the liver. The present study is a continuation of our previous study[2], with the purpose to further explore the factors that affect the altered hepatic nitrogen and energy metabolism after burn injury. Most of the results from the previous study are in general reproduced in the present study, namely, when perfused with the same solution livers from the burned rats demonstrated a similar rate of glucose output but higher levels of oxygen consumption and urea production than those from the sham burn animals. They confirm the hypothesis that some intrinsic factor(s) within the liver turns on the machinery of urea cycle after burn injury. Since arginine is the immediate precursor for urea synthesis, understanding arginine metabolism in the liver would potentially help identify the

major regulatory factors related to the observed alteration in urea metabolism after burn injury.

The major feature of the present study is to apply stable isotope labeled L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$]arginine tracer in the liver perfusate which allowed us to explore in more detail the alteration in the major metabolic process of exogenous arginine in the liver. The major sequelae of regulatory sites in hepatic arginine metabolism include its uni-directional transport into the liver, its subsequent major metabolic pathways within the liver, and the relative contribution of the extrahepatic versus intrahepatic arginine to the accelerated urea production and other metabolic pathways.

One of our major findings is that burn injury significantly stimulated the rate of arginine transport into the liver. This finding from the present liver perfusion model is in agreement with the previous reports from the hepatocyte plasma membrane vesicle preparation system,[21] on an increased hepatic arginine transport and the up-regulation of the membrane bound arginine transport system y^+ in sepsis[3] and after burn injury.[22] Because intracellular arginine concentration is nearly undetectable due to the high intracellular arginase activity, the transport of extrahepatic arginine into hepatocytes is a major modulator of the intrahepatic arginine availability and its metabolic utilization. By tracing the fate of the labeled arginine moieties in the metabolites, our study further revealed the quantitative information on the subsequent major metabolic pathways in the liver. It was found that the increased arginine transport after burn injury was coupled with its increased utilization of arginine for both urea production and protein synthesis. We can further speculate that the increased intrahepatic arginine availability is also related to the production of nitric oxide and polyamine in the burn patients when the compensatory mechanisms for hemodynamic regulation and tissue repair are highly activated. Almost all these important metabolic pathways are highly stimulated in severe injury and sepsis induced stress conditions.[23]

Therefore. it can be identified that up-regulation of extrahepatic – intrahepatic arginine transport system is one of the "intrinsic" factors which lead to the accelerated urea production after burn injury.

Our stable isotope tracer study also demonstrated an increased dependency of the extrahepatic arginine for the intrahepatic arginine metabolism after burn injury. This is evidenced by a) an increased rate of arginine transport into the liver; and b) a higher proportion of the exogenously transported arginine versus the intrahepatically *de novo* synthesized arginine being utilized for urea production. The dependence of the exogenously pre-formed arginine for intrahepatic urea production is also supported by the observation that the rate of total urea production was relatively reduced after arginine in the perfusion medium had been consumed. Therefore, burn injury significantly increased the extraction of the extrahepatic, preformed arginine for urea synthesis. A reduced portion of intrahepatically *de novo* synthesized arginine for urea production after burn injury may reflect a compensatory mechanism which spares the source of urea nitrogen from other amino acids when the urea cycle spins at higher rates. Increased extrahepatic arginine availability may therefore reduce the use of other source of nitrogen for urea synthesis.

The present study also revealed a very limited amount of arginine release from the liver, which is derived from either intrahepatic proteolysis, or its de novo synthesis within the urea cycle, in both burn and sham burn animals. This is evidenced by 1) the enrichment of the labeled arginine in the perfusion media remained constant during the whole perfusion period; 2) the release of L-[5,5,-$^{2}H_{2}$]arginine (M+2 arginine) from the liver is very limited. Since the major extrahepatic site for arginine synthesis is the kidney,[24] an estimate of the net renal arginine output from the present group of sham burn animals (weighing 260 ± 5 g), based on the data reported by Dhanakoti et al,[25] (60.5 ± 20.7 nmol. min^{-1}. 100 g body wt^{-1}) revealed that it only accounts for about one fourth of the extrahepatic

arginine supported urea production rate. It appears that even in the healthy rats, exogenous arginine supply is required to maintain its balance. Since burn injury significantly increased the demand of extrahepatic arginine for urea synthesis, and also, previous studies revealed that the conversion rate of plasma citrulline to plasma arginine was not increased in burn patients as compared to healthy subjects,[11] it is reasonably to infer that an enriched arginine supply is required after burn injury.

In conclusion, the present study provides a detailed quantitative information on the relationship between the extrahepatic and intrahepatic arginine pool in association with the accelerated urea cycle activities after burn injury. The increased hepatic argininie transport and subsequently, the intensified utilization of the extrahepatic arginine for urea production and hepatic protein synthesis after burn injury supports the notion that enriched arginine supply to burn patients would be beneficial to the improvement of their nutritional status.

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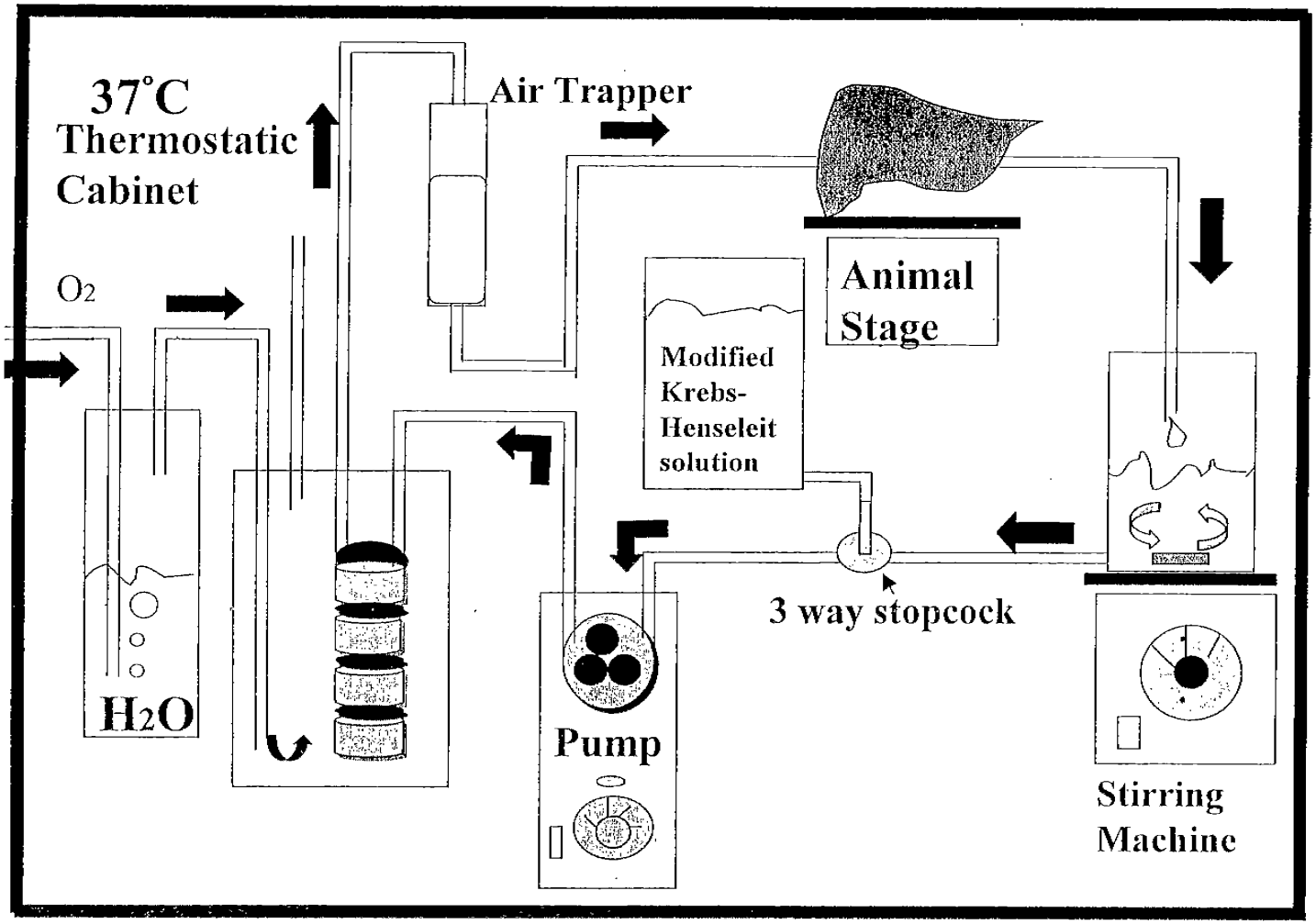
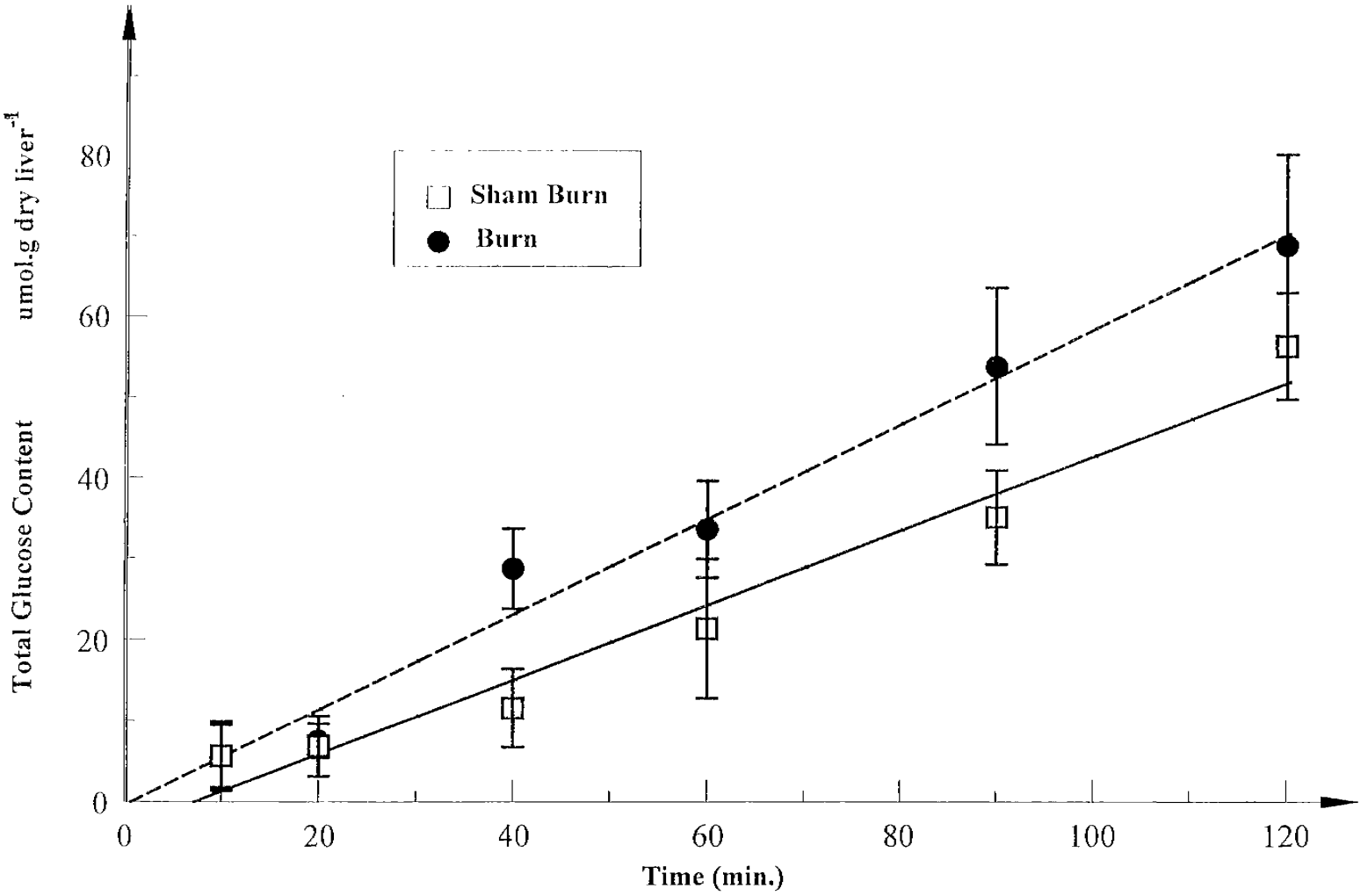


Figure 1. Perfusion System in Thermostatic Cabinet



Glucose Production in the Perfused Livers

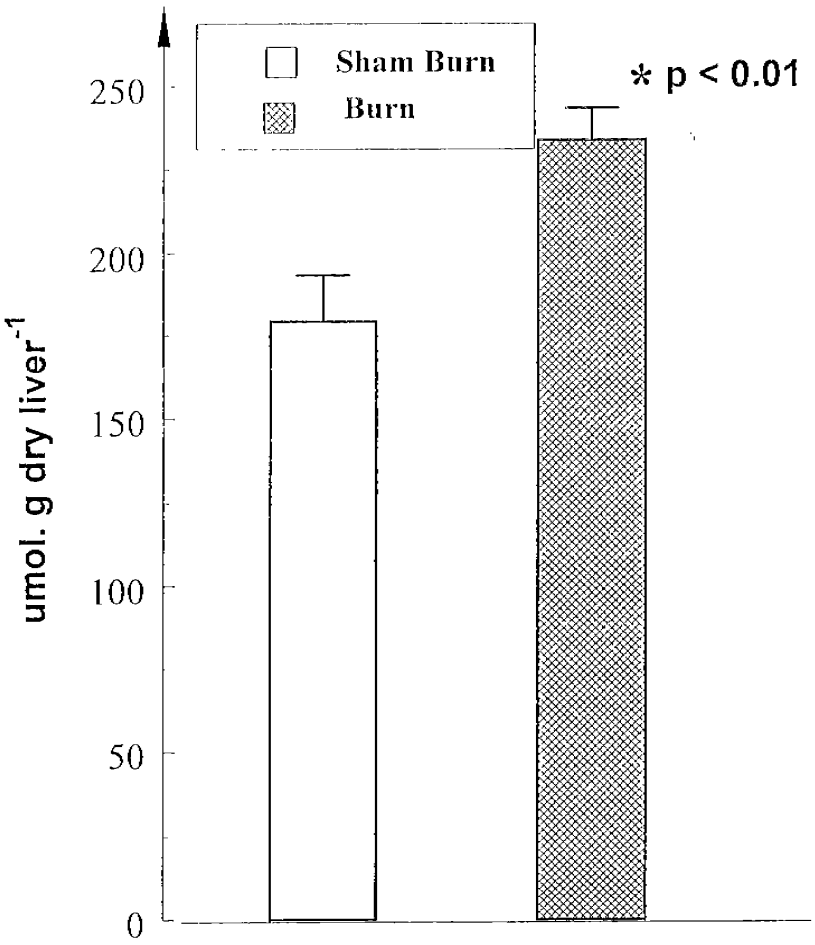
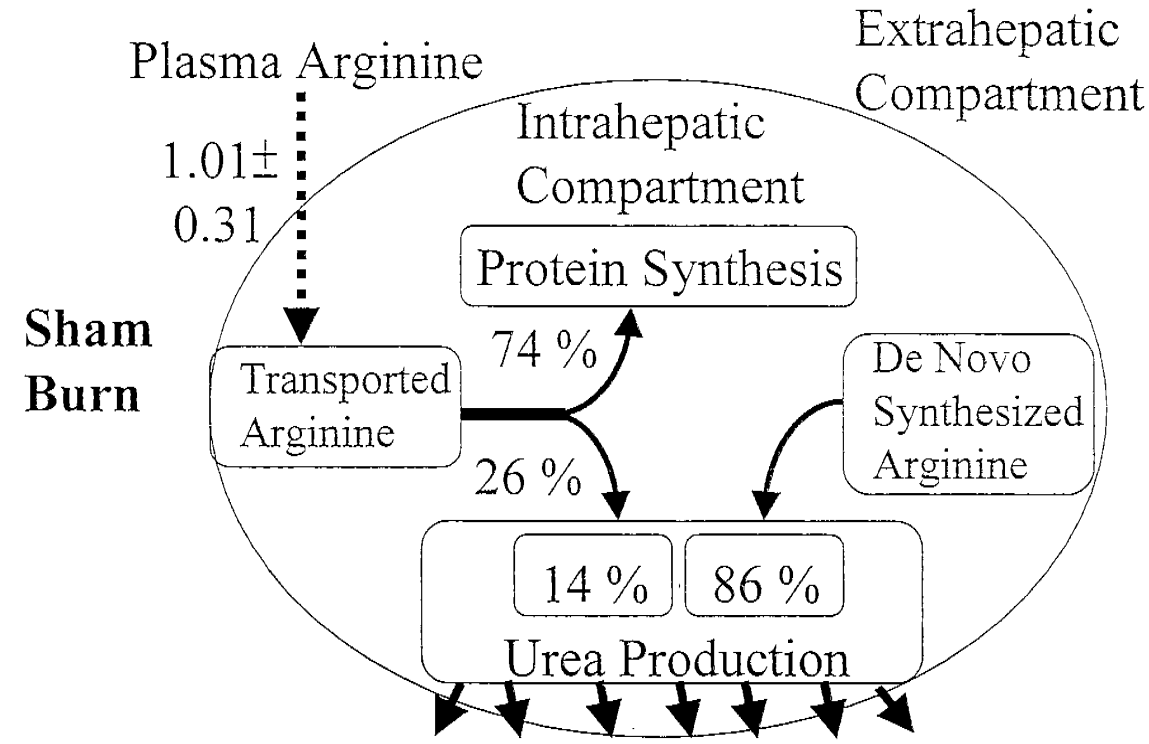

Sham Burn
Burn
* p < 0.01
250
200
150
100
50
0
umol. g dry liver-1

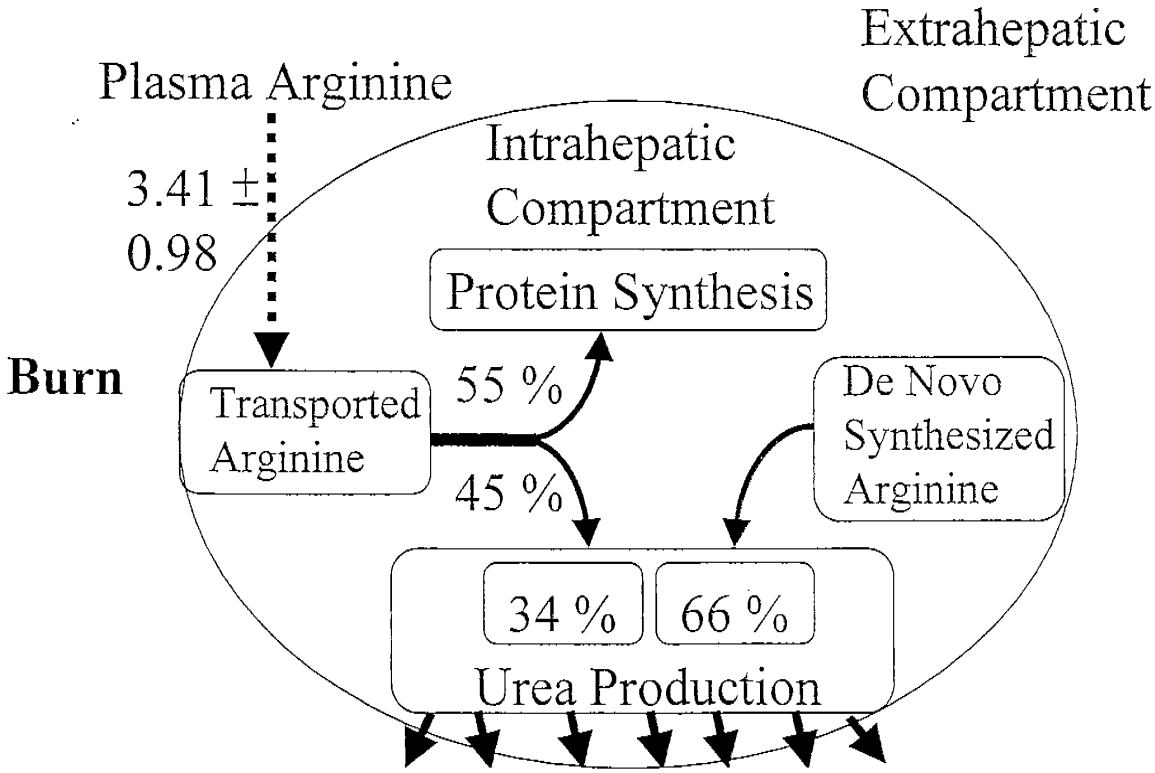
Table 1 Rate of Urea Production, Arginine Transport into the Liver and the Source of Urea Nitrogen in the Liver

	Sham Burn (n = 8)	Burn (n = 8)
Total Urea Production	2.93 ± 0.23*	4.53 ± 0.90 □
Urea Derived from Intrahepatic Arginine	1.67 ± 0.15	2.98 ± 0.46 □
Total Arginine Transport	1.01 ± 0.31	3.41 ± 0.99 □
Used for Ureagenesis	0.26 ± 0.08	1.55 ± 0.44 □
Used for Protein Synthesis	0.75 ± 0.23	1.86 ± 0.54 □
Ratio Urea Formation from Extrahepatic / Intraheptic Arginine (%)	16	52 □

* Data are mean ± SE, in μmol.g dry liver^{-1}. min.$^{-1}$ except specified.

□ $P < 0.05$ by unpaired student "t" test.


Plasma Arginine
1.01±
0.31
Extrahepatic
Compartment
Intrahepatic
Compartment
Protein Synthesis
Sham
Burn
Transported
Arginine
74 %
26 %
De Novo
Synthesized
Arginine
14 %
86 %
Urea Production


Plasma Arginine
3.41 ±
0.98
Extrahepatic
Compartment
Intrahepatic
Compartment
Protein Synthesis
Burn
Transported
Arginine
55 %
45 %
De Novo
Synthesized
Arginine
34 %
66 %
Urea Production

Legends

1. Figure 1. Perfusion system in a thermostatic cabinet.
2. Figure 2. Comparison of mean ± SE glucose production by isolated liver from sham (□, n=8) and burn (●, n=8) groups. No significant difference on each timed point from both groups, as analyzed by unpaired student "t" test.
3. Figure 3. Comparison of total urea production from perfused liver for two hours between sham (□ , n=8) and burn (⊠, n=8) group. * $p<0.05$ by unpaired student "t" test.
4. Table 1. Rate of urea production, arginine transport into liver and the source of urea nitrogen in the liver. Data are mean ± SE, in μmol. g dry^{-1}. $min.^{-1}$ except specified. □ $P< 0.05$ by unpaired student "t" test.