UNIVERSIDADE ESTADUAL DE CAMPINAS

SIMONI SÁTTOLO RIZZIOLLI

EFEITOS DO ALCOOLISMO CRÔNICO EXPERIMENTAL ASSOCIADO À REPOSIÇÃO HORMONAL SOBRE O LOBO VENTRAL DA PRÓSTATA DE RATOS (*Rattus norvegicus albinus*).

Este exemplar corresponde à redação final da tese defendida pelo(a) candidato (a) Simoni Sáttolo Rizziolli

e aprovada pela Comissão Julgadora.

Tese apresentada ao Instituto de Biologia para obtenção do Título de Mestre em Biologia Celular e Estrutural na área de Anatomia.

Orientadora: Profa. Dra. Valéria Helena Alves Cagnon Quitete

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECA DO INSTITUTO DE BIOLOGIA - UNICAMP

Rizziolli, Simoni Sáttolo

R529e Efeitos do alcoolismo crônico experimental associado à reposição hormonal sobre o lobo ventral próstata de ratos (*Rattus norvegicus*) / Simoni Sáttolo Rizziolli. -- Campinas, SP:[s.n.], 2003.

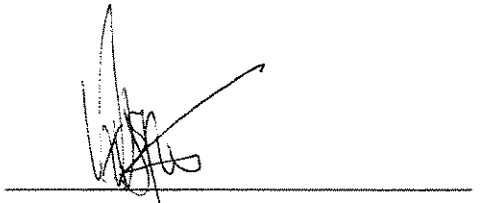
Orientadora: Valéria Helena Alves Cagnon Quitete
Dissertação (mestrado) – Universidade Estadual de Campinas.
Instituto de Biologia.

1. Alcoolismo. 2. Prostata. 3. Hormonio. I. Quitete, Valéria Helena Alves Cagnon. II. Universidade Estadual de Campinas. Instituto de Biologia. III. Título.

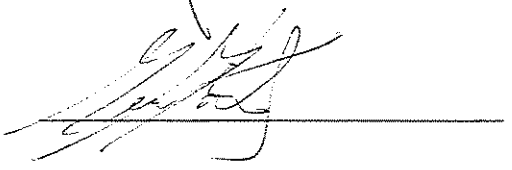
Campinas, 11 de Setembro de 2003.

Banca Examinadora:

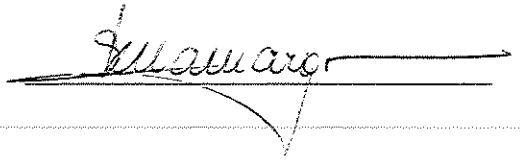
Profa. Dra. Valéria Helena Alves Cagnon Quitete



Prof. Dr. Marcelo Martinez



Profa. Dra. Isabel Cristina Cherici de Camargo



Prof. Dr. Wilson de Mello Júnior

ÀQUELE QUE ME DÁ MOTIVOS PRA VIVER...

MEU SENHOR JESUS

Dedico.

Agradecimentos especiais

A Deus, porque sem Ele nada posso fazer.

Aos meus pais, Wilson e Marilene que certamente sonharam com este momento mais do que eu e foram incansáveis para que eu o alcançasse.

Aos meus irmãos, Leandro e Renata que com pequenos gestos contribuíram pra essa conquista.

Ao meu marido Rodrigo, pelo incentivo, apoio, compreensão e "paciência".

A Dra. Valéria Helena Alves Cagnon Quitete, que foi mais do que orientadora, tornou-se amiga.

A amiga Fernanda Rossi Patrício, simplesmente por ser minha amiga.

Agradecimentos

Aos professores Dr. Marcelo Martinez, Drª. Isabel Cristina Cherici Camargo e Dr. Wilson de Mello Jr., pelas valiosas sugestões para redação final deste trabalho.

Aos Professores Dr. Hernandes Faustino de Carvalho, Dr. Antonio Carlos Bosquero e Dr. Gerson Eduardo Rocha Campos, que contribuíram para o aprimoramento das técnicas utilizadas.

Ao Professor Dr. Francisco Eduardo Martinez pelos préstimos e auxílio nas análises de dosagem hormonal.

A Dra. Iara Maria Silva de Luca, pelo auxílio nos estudos estereológicos.

Aos professores Dr. Carlos Roberto Padovani e Dr. Flávio Ferrari Aragon, pela análise estatística dos resultados.

Aos Srs. Norivaldo Celestino e Marco Auréllio Ribeiro de Paula, pelos valiosos serviços técnicos realizados.

A Sra. Marlene Lima, pela valiosa contribuição no tratamento dos animais.

As Sras. Antonia Maria Ferreira Lima e Adriane Sarti Sprogis, pela colaboração técnica prestada.

As Sras. Ana Floriana Rodrigues, Lourdes Pinheiro e Silvia Helena Burghi Kalal, pelos préstimos e colaboração.

A Sra. Lílian Alves Senne Panagio, pela atenção e colaboração.

Aos amigos Adriana Pertille, César Alexandre Fabrega de Carvalho pela impagável contibuição e auxílio.

Aos amigos do curso de pós graduação do Departamento de Anatomia: Cândida Luiza, Zarif, Viviane, Eduardo Candido, Eduardo Caldeira, Elaine Minatel, Alexandre, Rosane, Fabiano, Wagner e Ricardo pelo incentivo, apoio e auxílio.

Aos Professores Dr. José Meciano Filho, Drª. Evanisi Teresa Palomari e Drª. Maria do Carmo Alberto Rincon e aos docentes do departamento de Anatomia/IB/UNCAMP pela colaboração em minha formação.

À CAPES e FUNCAMP, pelo auxílio financeiro concedido.

"Apaixonar-se por Deus é o maior dos romances:

Procurá-lo a maior das aventuras;

E encontrá-lo a maior de todas as realizações".

SUMÁRIO

RESUMO

A influência nociva do uso crônico de álcool sobre o sistema genital masculino leva a importantes alterações incluindo o hipogonadismo e a feminilização, além da desorganização morfo-funcional das diferentes glândulas sexuais. Assim, o presente estudo teve o objetivo de analisar as alterações estruturais, histoquímicas e hormonais séricas no lobo ventral da próstata de ratos alcoolistas associados à reposição hormonal. Um total de 30 ratos (*Rattus norvegicus albinus*) foram divididos em três grupos: controle – recebeu água; alcoolista – recebeu etanol diluído à 20% e alcoolista tratado com hormônio – recebeu etanol diluído à 20% associado à administração de cipionato de testosterona (5mg/kg de peso) durante os últimos 30 dias de tratamento. Após 150 dias de tratamento os animais foram sacrificados, a próstata retirada e submetida a técnicas de microscopias eletrônicas de transmissão e varredura, histoquímica para fosfatase ácida, dosagem hormonal e análise estereológica. Os resultados demonstraram redução do volume celular total e alterações das organelas envolvidas no processo secretor. No tratamento com testosterona houve recuperação parcial do volume celular. Concluiu-se que o etanol comprometeu a morfologia celular e a reposição hormonal por si só não promoveu efetiva remodelação das organelas responsáveis pelo processo secretor.

1. INTRODUÇÃO

Generalidades do álcool

A prevalência do uso do álcool e os problemas relacionados à ele têm aumentado substâncialmente nos últimos anos. Em 1983, a Assembléia Mundial de Saúde declarou que os distúrbios decorrentes do consumo de álcool são responsáveis por grande parte dos problemas mundiais de saúde (Jernigan et al., 2000). Atualmente, o alcoolismo foi caracterizado como o terceiro maior problema de saúde pública sendo excedido apenas por doenças cardiovasculares e câncer (Giesbrecht & Rankin, 2000; Allamani et al., 2000). Baseado em estimativas norte-americanas, sete por cento de sua população consome álcool, o que não representa o número real de alcoólicos existentes, pois muitos indivíduos omitem o uso da droga. Na Inglaterra, o alcoolismo atinge cerca de 1,5 milhão de indivíduos (Palmer, 1989). Com relação à população feminina, sabe-se que as mulheres são mais susceptíveis, quando comparadas aos homens, considerando-se os efeitos deletérios do álcool sobre o fígado. A ingestão de apenas 20 gramas diárias de álcool são suficientes para causar cirrose hepática nas mulheres, já nos homens são necessárias aproximadamente 80 gramas diárias. Porém, as mulheres são menos vulneráveis que os homens aos efeitos tóxicos do álcool sobre as gônadas (Galvão Teles et al., 1986). É conhecido que, a ingestão de bebidas alcóolicas por gestantes afeta o crescimento e a sobrevivência do feto (Van Thiel & Lestter, 1979). Além disso, os recém-nascidos podem apresentar a síndrome alcoólica fetal, cujas características são: crescimento pós-natal deficiente, atraso no desenvolvimento, microcefalia, pequenas fissuras nas pálpebras, deformidades articulares, problemas cardíacos e disfunções motoras (Van Thiel & Lester, 1979).

O alcoolismo foi identificado como doença em 1786, pelo psiquiatra americano Benjamin Rush, sendo considerado de forma crônica quando tem início e continuidade por influência de fatores genéticos, psicossociais e ambientais

(Clair, 1991). O reconhecimento do alcoolismo como doença pode ser estabelecido quando três ou mais dos seguintes aspectos forem caracterizados: 1) forte desejo pela substância; 2) falta de controle em relação ao consumo, quantidade e cessação do hábito; 3) síndrome de abstinência; 4) tolerância; 5) perda progressiva de outros prazeres e hábitos e 6) continuidade do hábito, mesmo com o desenvolvimento de outros males (Madden, 1992).

Divergências ocorrem sobre o conceito do indivíduo "alcoólico" e o termo "alcoolismo". Segundo Wexberg (1950), alcoolismo é o hábito de beber incontrolavelmente, decorrente da ausência de luta contra o desejo de ingerir bebida alcoólica. Fink e Rosalki (1978) conceituaram alcoolismo como consumo excessivo de álcool levando a dependência. Sonnenreich (1971) relatou que, alcoólico é o indivíduo que consome bebida alcoólica diariamente, ou quase sem interrupções, apresentando estado de intoxicação caracterizado por distúrbios psíquicos ou somáticos. Em 1978, Wright classificou como alcoólicos crônicos os consumidores diários de álcool, independente do volume ingerido.

O álcool é freqüentemente mencionado com tendo "calorias vazias", isto é, as bebidas alcoólicas contém quantidade insuficiente de vitaminas e minerais. É conhecido que as calorias ingeridas provenientes do álcool, que excedem as necessidades calóricas diárias, são convertidas em gordura. Esse processo ocorre no fígado, através da acil-coenzima A de cadeia curta para formar acetil-coenzima A, a qual é oxidada no ciclo do ácido cítrico (Lehninger, 1991). O consumo agudo de etanol leva muitos indivíduos a hipoglicemia, pois inibe a neoglicogênese a partir do lactato e aminoácidos. Além disso, o etanol é ainda uma fonte de calorias muito dispendiosa, pois ao comparar 280 gramas de açúcar à seis latas de cerveja, os quais são equivalentes energéticos, tem-se o custo da bebida 20 vezes maior em relação ao custo do açúcar (Lehninger, 1991). Sabe-se que, o álcool (etanol, metanol ou n-propanol) possui alto conteúdo energético, produzindo cerca de 7,1 kcal/g quando oxidado. Este valor encontra-se entre os dos carboídratos e dos lipídios (4,2 e 9,5 kcal/g respectivamente). Problemas nutricionais podem ser observados em usuários habituais de álcool (Lieber,

1984b). No entanto, não há relação direta ou potencial da má nutrição na patogênese das doenças associadas ao alcoolismo (Li, 1983). A associação entre a ingestão de álcool e a má nutrição tem influência nas alterações morfológicas observadas nos tecidos, porém o álcool é o fator mais importante (Leo et al., 1983; Lieber & Shaw, 1983; Lieber, 1984a; Lieber 1984b). O álcool é rapidamente absorvido pela mucosa de todo trato gastro-intestinal, desde a boca até o reto, pelos pulmões (vapor de álcool), pela bexiga urinária e pelas cavidades peritoneal e pleural. Sua distribuição ocorre por difusão rápida e uniforme através de capilares e outras membranas biológicas, atingindo todos os tecidos e líquidos extra e intracelulares do organismo. Devido a sua grande capacidade de difusão, o álcool incorpora-se ao leite materno, placenta, líquido amniótico, humor vítreo, líquidos cerebrospinal e ascítico, bile, urina e saliva (Geokas et al., 1981; Kalant, 1983; Clair, 1991). Segundo Fish e Nelson (1942), após 30 minutos da ingestão por via oral, 46,9% do álcool ingerido atinge as gônadas. A excreção corpórea do álcool é dada pela soma das taxas de eliminação através da urina, da respiração, da saliva e do suor. Esses processos são responsáveis por até 10% do total a ser expelido pelo corpo, podendo esta taxa ser elevada através de exercícios físicos. A principal via excretora ocorre no fígado através das ações de enzimas oxidativas: álcool desidrogenase, catalase e sistema de oxidações metabólicas microssomais (Kalant, 1983).

O álcool e seus metabólitos provocam distúrbios generalizados nos sistemas nervoso central, genital masculino, circulatório e órgãos hematopoiéticos. Atuam também, diretamente no fígado, pâncreas, intestinos e glândulas endócrinas (Marks & Wright, 1978). Já foi demonstrado que, a ingestão de álcool provoca modificações metabólicas e patológicas em diferentes órgãos e tecidos resultando por exemplo, em cirrose hepática (Galvão Teles, 1986; Palmer, 1989), alterações na barreira hemato-urinária na bexiga (Mello Jr. et al., 1997), psicose (Edwards & Peters, 1994) e infertilidade masculina (Clavert et al., 1990).

O uso de modelo animal tem elucidado muitos aspectos biológicos e bioquímicos envolvidos no hábito da ingestão de bebidas alcoólicas por seres

humanos. Desde 1926, caracterizou-se que roedores escolhem voluntariamente etanol diluído em diferentes concentrações à água, sendo esta preferência influenciada por fatores ambientais, fisiológicos e psicológicos (Richter, 1926; Cícero & Badger, 1977). Desde então, roedores têm sido utilizados nos estudos experimentais sobre alcoolismo, recebendo dosagens que variam entre 5% e 40%. Estes estudos têm duração de alguns dias ou semanas no alcoolismo agudo e meses no alcoolismo crônico (Banderas et al., 1992).

O álcool e o sistema genital masculino

No sistema genital, uma das conseqüências do alcoolismo é o hipogonadismo e a feminilização, que ocorre tanto no homem como em animais de laboratório (Bannister & Lowosky, 1987; Tadic et al., 2000). Alguns autores acreditavam que essa disfunção sexual estava relacionada à doença crônica do fígado (Lloyd & Williams, 1948; Pincus et al., 1951; Brown et al., 1964; Van Thiel et al., 1974; Van Thiel & Lester, 1979). Atualmente, sabe-se que a disfunção hepática não está associada à debilitação da função reprodutora (Anderson et al., 1980; Tadic et al., 2000). Entretanto, as alterações relativas ao sistema genital são exacerbadas em indivíduos com cirrose alcoólica (Van Thiel et al., 1975; Wang et al., 1991). Sendo assim, acredita-se que o etanol seja uma toxina que atua de forma direta sobre as gônadas, alterando a síntese de testosterona testicular (Gary et al., 1976; Elligboe & Carole, 1979; Rivier & Vale, 1983; Bannister & Lowosky, 1987; Anderson et al., 1989; Saxena et al., 1990) e indireta provocando efeitos deletérios sobre o eixo hipotálamo-hipófise-gônadal (Van Thiel, 1983; Salonen & Hutaniemi, 1990). No epitélio glandular da próstata ventral de roedores acredita-se tanto no efeito direto como no indireto do etanol (Martinez et al., 1993; Juarranz et al., 1998).

A influência nociva do álcool sobre o sistema genital masculino de roedores foi demonstrada com variadas metodologias para a indução do alcoolismo. As principais alterações morfológicas encontradas são: lesões das células

testiculares, diminuição dos diâmetros dos túbulos seminíferos, depressão no nível de testosterona no plasma sangüíneo, redução do peso das glândulas sexuais acessórias, diminuição significativa nas alturas das células epiteliais secretoras das vesículas seminais, próstatas ventral, lateral e dorsal (Klassen & Persaud, 1978; Van Thiel et al. 1979; Semczuk & Rzeszowska 1981; Willis et al. 1983; Anderson et al. 1985; Salonen & Huhtaniemi 1990; Cagnon et al., 1996, 1998, 2001; Martinez et al., 1997; Gomes et al., 2002). Semczuk & Rzeszowska (1981) e Willis et al. (1983), após analisarem diferentes aspectos morfológicos das glândulas sexuais acessórias, concluíram que a severidade das alterações patológicas é dependente do teor e da duração da exposição ao etanol.

Morfologia da Próstata

A próstata é uma glândula sexual acessória presente em todos os mamíferos e apresenta características próprias para cada espécie (Setchell & Brooks, 1988). A próstata juntamente com as outras glândulas sexuais acessórias têm fundamental importância no processo reprodutivo (Price & Williams-Ashman, 1961; Cavazos, 1975; Mann & Lutwak-Mann, 1981). Sua secreção é formada por diversos nutrientes que compõem o líquido seminal, essencial para a nutrição e motilidade dos espermatozóides. Nos roedores, a próstata também secreta proteínas, entre as quais há grande variedade de enzimas, carboidratos e outras substâncias, incluindo o citrato. Dentre as enzimas, acredita-se que a fosfatase ácida seja exclusivamente secretada pela próstata. Contudo, ela é detectada em diferentes tipos de tecidos como fígado, testículos, baço, rins, além da próstata (Tenniswood et al., 1975). Sabe-se que esta enzima age em uma variedade de substratos como fenilfosfato, α-naftil fosfato e paranitro fenil fosfato, participando portanto do mecanismo básico para a hidrólise desses ésteres de fosfato (Blandy & Lytton, 1986). A síntese da fosfatase ácida, assim como o desenvolvimento e a manutenção da próstata é andrógeno dependente (Tenniswood et al., 1975)

sendo sua atividade funcional detectada em várias espécies incluindo o homem (Costello & Franklin, 1994).

A próstata de roedores é uma glândula complexa formada por três pares de lobos: ventral, lateral e dorsal (Langworthy, 1965; Purinton et al., 1972; Vaalasti & Hervonen, 1979; Jesik et al., 1982; Prins, 1992), os quais drenam suas secreções, através de ductos, para a uretra prostática (Jesik et al., 1982; Cavazos, 1975). O lobo ventral da próstata é constituído de ácinos revestidos por epitélio simples. Cada ácino consiste de células colunares entremeadas à células basais assentadas em nítida membrana basal (Brandes & Portella, 1960; Bourne & Danielle, 1966; Cavazos, 1975; Carvalho & Line, 1996). Ichinara e Pelliniemi (1975) sugerem que as células basais da próstata atuam na regulação do transporte de material entre o estroma do órgão e o epitélio e podem se diferenciar em células epiteliais secretoras. Ultra-estruturalmente, as células secretoras do lobo ventral da próstata apresentam núcleo basal, retículo endoplasmático granular com cisternas paralelas e achatadas, complexo de Golgi bem desenvolvido e grânulos de secreção evidentes nas regiões supranuclear e apical do citoplasma celular (Cavazos, 1975; Dahl et al, 1973). O estroma prostático é formado por fibras colágenas, elásticas, células de músculo liso e vasos sangüíneos (Farnsworth, 1999).

Próstata e hormônios

Estudos realizados em homens e em roedores, demonstraram que a morfogênese, a manutenção da atividade morfo-funcional (Lung & Cunha, 1981; Jarred et al., 2000) assim como a proliferação e diferenciação (Rajfer & Coffey, 1978; Okamoto et al., 1982; Davies & Eaton, 1991) das células das glândulas sexuais acessórias são reguladas por andrógenos testiculares. A testosterona é o principal andrógeno a induzir a diferenciação da próstata (Costello & Franklin, 1994) e da vesícula seminal (Tsuji et al., 1994). No entanto, o desenvolvimento da próstata é regulado principalmente pela di-hidrotestosterona (Tsuji et al., 1994), a

qual é resultante da conversão da testosterona através da enzima 5α-redutase (Orlowski, 1986; Cukierski et al., 1991). A remoção da fonte primária de andrógenos pela castração ou administração de hormônios antagonistas como os estrógenos ou antiandrógenos, produz alterações marcantes no funcionamento normal das glândulas sexuais acessórias (Lung & Cunha, 1981). Bhalla et al. (1983) relataram experimentalmente que os níveis de testosterona circulante são alterados pelo efeito direto do álcool sobre os testículos, comprometendo a produção de testosterona e ou por mecanismos secundários não relacionados à biosíntese de testosterona no órgão, acelerando o metabolismo da testosterona no fígado.

Além da testosterona, outros hormônios estão envolvidos no processo de crescimento e funcionamento da próstata (Jarred et al., 2000) como a prolactina, que atua sinergisticamente à testosterona, regulando a secreção de outras substâncias como o citrato e também os níveis de receptores hormonais (Van Thiel & Lester, 1979; Reiter et al., 1999).

2. JUSTIFICATIVA E OBJETIVO

A toxicidade do álcool sobre o sistema genital masculino não está totalmente esclarecida. Dúvidas persistem a respeito do efeito do álcool sobre a biologia básica das células epiteliais secretoras e do estroma da próstata ventral de roedores. Dentre os principais aspectos a serem demonstrados, pode-se destacar a avaliação das organelas envolvidas no processo secretor, a análise dos principais componentes do estroma celular e as interações epitélio-estroma. Além disso, não há correlação entre as transformações celulares detectadas no alcoolismo crônico e a ocorrência de patologias prostáticas. Assim, o objetivo do presente estudo foi analisar as alterações morfológicas, estereológicas, histoquímicas e hormonais ocorridas no lobo ventral da próstata de ratos, frente ao uso abusivo de álcool associadas à influência da reposição hormonal, além de estabelecer correlação dos achados morfológicos à ação direta e indireta do etanol sobre a glândula prostática.

3. MATERIAIS E MÉTODOS

3.1. ANIMAIS E PROCEDIMENTOS EXPERIMENTAIS

No presente trabalho foram utilizados 30 ratos machos *(Rattus norvegicus albinus)* com 50 dias de idade, obtidos no centro de Bioterismo da Unicamp/SP e mantidos no Biotério do Departamento de Anatomia do Instituto de Biologia. Os animais foram divididos em três grupos experimentais: controle, alcoolista 20% e alcoolista tratado com hormônio.

O grupo controle (10 animais) recebeu água. Os grupos alcoolista 20% (10 animais) e o grupo alcoolista tratado com hormônio (10 animais) receberam etanol diluído à 20° Gay Lussac (20% v/v) ao longo de 150 dias. Após 120 dias, do início da ingestão de etanol, no grupo alcoolista tratado com hormônio foi simultaneamente administrado 5mg de cipionato de testosterona (Deposteron-Novaquímica) por kg de peso corpóreo ao longo de 30 dias. As aplicações hormonais foram realizadas quatro vezes por semana em dias alternados, através de injeções subcutâneas. Após 150 dias de tratamento, todos os animais foram pesados em balança semi-analítica Marte AS 5500 e sacrificados. Amostras do lobo ventral da próstata foram coletadas para análises macroscópica, histoquímica e de microscopias eletrônicas de transmissão e varredura.

Nos grupos alcoolistas, antecedendo o início do experimento, foram administradas doses crescentes de etanol na escala de diluição: 5%, 10%, 15% e 20%. Estas diluições alcoólicas tiveram duração de uma semana. A administração gradativa de etanol teve como objetivo a adaptação dos animais ao modelo experimental.

O modelo de alcoolismo utilizado foi o semivoluntário, no qual o etanol é fornecido como único alimento líquido disponível para os animais. Os animais, dos três grupos experimentais, receberam a mesma dieta sólida *ad libitum* (Nuvilab CR1). As mensurações dos consumos sólido e líquido foram realizadas semanalmente. A partir destes dados foi calculada a ingestão calórica média dos

animais, nos diferentes grupos de estudo. Os cálculos foram realizados à partir da energia média metabolizável da ração (para os três grupos experimentais) e da mistura etanol+água (para os grupos alcoolistas). A ração Nuvilab CR1 tem energia média metabolizável de 2,70 kcal/g. O etanol diluído à 20% tem energia média metabolizável de 1,12 kcal/g.

3.2. MACROSCOPIA

Os animais dos grupos experimentais foram anestesiados com Francotar/Virbaxyl (1:1) na dosagem de 0,25 ml para cada 100 gramas de peso corpóreo. A seguir, realizou-se uma incisão na parede abdômino-pélvica expondo o complexo urogenital. Parte desse complexo que inclui próstata, glândula de coagulação, vesícula seminal, uretra, bexiga urinária, testículos, ureteres e ductos deferentes foi dissecado com auxílio do microscópio cirúrgico DF Vasconcelos. Posteriormente, o complexo foi fotografado, *"in situ"* com máquina Nikon F3 com objetiva macro 50mm e filme Kodacolor Gold asa 100.

3.3. DOSAGEM HORMONAL

Amostras de sangue, foram obtidas através de punção intracardíaca de cinco animais de cada grupo experimental e mantidas à 0°C por aproximadamente 30 minutos. Posteriormente, realizou-se centrifugação (Centrífuga Ependorf 5804R) à 3.000 rpm por 15 minutos. O plasma sangüíneo foi removido com auxílio de micropipetas (Pipetman – Gilson) e armazenado a –5°C. A quantificação dos níveis de testosterona no plasma sangüíneo foi obtida através de análise de radioimunoensaio –"Testosterone Direct", Immunotech.

3.4. ANÁLISE HISTOQUÍMICA PARA ENZIMA FOSFATASE ÁCIDA

Amostras do lobo ventral da próstata foram coletadas de cinco animais, de cada grupo experimental, e congeladas em nitrogênio líqüido. Posteriormente, cortes com seis micrômetros de espessura foram obtidos no criostato Mícron HM505E e incubados em meio reagente (magnésio 50 milimolar, 0,1% de alfa naftil fosfato, 0,1% de fast red e tampão acetato) à 37° por 50 minutos. A seguir, o material foi fotografado no fotomicroscópio Nikon, com objetivas planacromáticas de 20X e 100X, e filme Kodacolor 135mm, asa 100.

3.5. ANÁLISE ESTERELÓGICA EM NÍVEL DE MICROSCOPIA DE LUZ

Para o estudo morfométrico foram utilizados cinco animais de cada grupo experimental (controle, alcoolista 20% e alcoolista 20% com hormônio). As secções da próstata ventral foram provenientes das lâminas de cortes semifinos com 0,5 micrômetro de espessura, obtidos no ultramicrótomo LKB 8800 Ultratome III com navalha de vidro e corados com azul de toluidina. As lâminas obtidas foram utilizadas para quantificar os volumes nuclear e citoplasmático.

Na quantificação do volume nuclear foram mensurados os diâmetros de 20 núcleos das células epiteliais do lobo ventral da próstata de cada animal, totalizando 100 núcleos por grupo experimental. Para definição dos núcleos utilizados, priorizou-se aqueles que apresentaram o mesmo plano em toda sua extensão. As medidas foram realizadas com auxílio de ocular graduada com régua (10x) e acoplada ao microscópio de luz Olympus CBB, fixando-se as observações com a objetiva de 100X. Após as mensurações dessas estruturas, procedeu-se a calibração da ocular com uma lâmina especial (Carl Zeiss), provida de divisões de 0,01 mm (10 μm), visando transformar as unidades da ocular em micrômetros. À partir destes valores foram calculadas as médias do volume do núcleo através da seguinte fórmula:

$V = 4/3\ \pi\ (d/2)^2\ D/2$,

sendo "d" diâmetro menor e "D", diâmetro maior.

Além disso, foram feitas medidas para determinação quantitativa das frações de volume (Vv) ocupadas pelo núcleo e pelo citoplasma das células epiteliais do lobo ventral da próstata. Essas medidas foram realizadas através de uma ocular 10X contendo um retículo de integração quadrilátero com 100 pontos acoplada ao microscópio de luz Olympus CBB e objetiva de 100X. Foi realizada a contagem dos pontos localizados sobre o núcleo e sobre o citoplasma de células de dez campos previamente definidos. A fração de volume ocupada pelo núcleo em relação ao citoplasma foi calculada utilizando-se a seguinte fórmula:

$Vv = p/P$

Onde: Vv = densidade de volume ou fração de volume (%).

p = número de pontos sobre o núcleo.

P = número total de pontos ou soma dos pontos sobre o núcleo e citoplasma nos diferentes campos.

O volume citoplasmático foi calculado a partir da relação entre Vv do citoplasma, Vv do núcleo e do volume do núcleo. A determinação do volume celular foi feita a partir da soma dos valores obtidos para os volumes nuclear e citoplasmático (Aherne 1967, 1970).

3.6. MICROSCOPIA ELETRÔNICA DE TRANSMISSÃO

Um total de cinco animais, de cada grupo experimental, foram perfundidos com glutaraldeído a 2,5%, através do ventrículo esquerdo do coração (Sprando, 1990). Antecedendo-se a perfusão, realizou-se a lavagem do sistema circulatório com solução fisiológica heparinizada. As próstatas ventrais foram coletadas e fragmentadas, até que tivessem cerca de 0,5 cm de espessura. Em seguida, os fragmentos foram submetidos ao complemento de fixação, por imersão no mesmo

fixador e pós-fixados em tetróxido de ósmio a 1% por duas horas. A desidratação foi realizada em série crescente de álcool diluído em água. Os fragmentos foram incluídos em resina plástica (Polyscience) e cortados com 0,5 micrômetro de espessura, no ultramicrótomo LKB 8800 Ultratome III com navalha de vidro e corados com azul de metileno e azul II. Os cortes corados foram utilizados para definir áreas específicas para o estudo em microscopia eletrônica de transmissão. Após a análise, os blocos foram aparados e submetidos a ultramicrotomia com navalha de diamante no ultramicrótomo Ultracult UCT 020 Leica. Os cortes obtidos foram montados em telas de cobre de 200 mesh e contrastados por acetato de uranila (Watson, 1958) e por citrato de chumbo (Reynolds, 1963), examinados e fotografados no microscópio eletrônico de transmissão LEO 906 no Laboratório de Microscopia Eletrônica do Instituto de Biologia da UNICAMP/SP.

3.7. MICROSCOPIA ELETRÔNICA DE VARREDURA

Um total de cinco animais de cada grupo experimental (os mesmos utilizados para microscopia eletrônica de transmissão), foram perfundidos com glutaraldeído a 2,5%, diluído em tampão fosfato 0,1m, pH 7,2, com posterior imersão no mesmo fixador por duas horas. A seguir, as próstatas ventrais foram lavadas em tampão fosfato de sódio por 15 minutos. Posteriormente, os materiais foram pós-fixados em tetróxido de ósmio 1% durante duas horas (Wahlqvist et al., 1996). A desidratação foi realizada em série crescente de acetona diluída em água e após a última etapa da desidratação o material foi submerso em nitrogênio líquido e foi realizada a criofratura, com o auxílio de uma haste metálica. A secagem do material foi realizada no aparelho de ponto crítico Balzers. Em seguida, foram montados em bases metálicas CPD-010 e cobertos com íons de ouro em aparelho Balzers MED-010 e examinados no microscópio eletrônico de varredura JEOL JSM 5800LV regulado para 10Kv no Laboratório de Microscopia Eletrônica do Instituto de Biologia da UNICAMP/SP.

3.8. ANÁLISE ESTEREOLÓGICA EM NÍVEL DE MICROSCOPIA ELETRÔNICA DE TRANSMISSÃO

Eletromicrografias obtidas no microscópio eletrônico de transmissão LEO 906, com aumento de 2784X e ampliação de 3,2X foram utilizadas para a quantificação de vacúolos digestivos e secretores. Um total de 10 células, por grupo experimental, foram selecionadas com base nas características de núcleo visível, lâmina basal e ápice evidentes. Sobre as eletromicrografias foi aplicado o Sistema-teste de Weibel, composto de 84 segmentos de retas com 1,35µm de comprimento (Weibel, 1979). O sistema-teste foi reproduzido em acetato e fixado sobre as eletromicrografias com auxílio de grampos de papel. Os pontos sobre a estrutura ou "hits" foram considerados quando a extremidade de um segmento se encontrava sobre a organela em estudo e foram considerados como "crosses" as intersecções destes segmentos com as organelas. O total de pontos sobre cada citoplasma celular foi obtido multiplicando-se os 84 segmentos por 2 e subtraindo-se aqueles que se encontravam sobre o núcleo e o espaço intercelular. Para cada tipo de organela foram estabelecidos os "hits" cuja soma foi subtraída do total de pontos (hits) sobre o citoplasma, obtendo-se assim, o total de pontos sobre a matriz citoplasmática. Para calcular a densidade de área, utilizou-se a seguinte fórmula:

$$S/V = \frac{4.c}{l.h}$$

Onde: S/V = densidade de área.

c = número de intersecções linha/vacúolo.

l = comprimento do segmento de linha.

h = número de pontos nas extremidades das linhas que incidem sobre os vacúolos.

A estereologia foi utilizada para quantificar a densidade de área ($\mu m^2/\mu m^3$) ocupada pelos vacúolos secretores e pelos vacúolos digestivos, nas células epiteliais da próstata ventral em cada grupo experimental. Esses cálculos foram

realizados a partir do número total de pontos sobre o citoplasma (n´), do total de pontos sobre cada tipo de grânulo (n), da soma do total de intersecções ou "crosses" (c) e do comprimento da linha do sistema-teste (L).

3.9. ANÁLISE ESTATÍSTICA

O estudo da relação dos volumes celulares, em função dos grupos experimentais, foi realizado utilizando-se a técnica da análise de variância para o modelo com um fator complementada com o teste de comparações múltiplas de Tukey (Montgomery, 1991).

Para avaliar os consumos sólido e líquido foi utilizada a técnica da análise de variância multivariada dos perfis médios dos grupos complementada com a construção dos intervalos de confiança simultâneos (Winchern & Johnson, 1992).

Todas as discussões foram realizadas considerando o nível de 5% de significância.

Artigo a ser submetido à publicação no periódico: Tissue & Cell, 2003.

"Effects of experimental chronic alcoholism associated to hormonal replacement on the ventral lobe of rat's prostate (*Rattus norvegicus albinus*)."

Simoni Sáttolo[1], Valéria Helena Alves Cagnon[*1].

[1]Department of Anatomy, Institute of Biology, State University of Campinas – UNICAMP, Campinas, São Paulo, Brazil.

[*]Correspondece To: Drª. Valéria H. A. Cagnon, Department of Anatomy, Institute of Biology, State University of Campinas , São Paulo, Brazil. Telephone: (55) 19 3788 6102. Fax: (55) 19 3289 3124. E-mail: quitete@uol.com.br .

ABSTRACT

The harmful influence of the chronic alcohol ingestion on the male reproductive system leads to important alterations including hypogonadism and feminization, besides the morphological and functional disorganization of the different sexual glands. The present study aimed to analyse the structural, histochemical and hormonal alterations to the ventral lobe of the prostate of alcoholic rats associated to hormonal replacement. A total of 30 rats (*Rattus norvegicus albinus*) was divided into three groups: control – received water; alcoholic – received ethanol diluted to 20% and hormone-treated alcoholic– received ethanol diluted to 20% associated with the administering of cypionate of testosterone (5mg/kg of weight) during the last 30 days of treatment. After 150 days of treatment, the animals were sacrificed, the prostate removed and submitted to transmission and scanning electron microscopies, histochemical analysis for acid phosphatase, hormonal dosage and stereologic analysis. The results demonstrated reduction of the total cellular volume and disorganization of the organelles involved in the secretory process. It was characterized a partial recovery of the celular volume after treatment with testosterone. It was concluded that the ethanol impaired the cellular morphology and the hormonal replacement by itself did not bring about efficient remodeling of the organeles responsible for the secretory process.

I - INTRODUCTION

The excessive consumption of alcoholic beverages and the problems resulting from it have reached alarming proportions in the world. At present, alcoholism is considered the third biggest public health problem, being surpassed only by cardiovascular diseases and cancer (Giesbrecht & Rankin, 2000; Allamani et al., 2000).

Alcohol and its metabolites provoke generalized disturbances in differents systems as central nervous, male reproductive, circulatory and hematopoietic. They also affect directly on the liver, spleen, intestines, endocrinuous glands and particulary on the accessory sexual glands (Marks & Wright, 1978; Martinez et al., 1993, 1997, 1999, 2000; Cagnon et al., 1996, 1998; Jayasinghe, 2000).

Several researchers have demonstrated that the excessive consumption of ethanol results in different morphophysiological alteration to the male reproductive system, as in humans as in laboratory animals, leading to functional defficiencies (Willis et al., 1983; Galvão-Teles, 1986; Palmer, 1989; Mello Jr. et al., 1997). The main consequences of chronic ingestion of ethanol to the male reproductive system are hypogonadism and feminization achnowledged as clinical syndrome (Bannister & Lowoski, 1987; Tadic et al., 2000). Besides these consequences, the following ones have been noticed: modification of the seminal fluid (Adams & Cícero, 1991) and reduction in the number of ejaculations in rats (Menendez-Abraham et al., 1991). Also, through different methodologies for the induction of chronic alcoholism, experiments have evidenced morphological alterations in the accessory sexual glands, such as: reduction in the weights and significant decrease in the height of the cells of the secretory epithelium of the prostate and seminal vesicle (Van Thiel et al., 1979; Semczuk & Rzeszowska, 1981, Willis et al., 1983; Anderson et al., 1985; Salonen & Huhtaniemi, 1990; Cagnon et al., 1996, 1998, 2001; Juarranz et al., 1998; Gomes et al., 2002), besides ultrastrutural alterations to the ventral prostate such as: dilation of the granular endoplasmic reticulum, rupture of the mitochondrial cristae, characterizing lesion and cellular

death (Martinez, et al., 1993) and disorganizing the Golgi complex (Cagnon et al., 2001). It is also known that rats chronically treated with ethanol show increase in the concentration of ethanol in the blood serum, in contrast with the decrease of the levels of circulating testosterone (Juarranz et al., 1998).

Ethanol is known as a toxin of direct action on the male gonads, altering the synthesis of the testicular testosterone (Gary et al., 1976; Ellingboe & Carole, 1979; Rivier & Vale, 1983; Bannister & Lowoski, 1987; Anderson et al., 1989; Saxena et al., 1990) and indirect action, bringing about imbalance to the pituitary-hypotalamic-gonadal axis (Van Thiel, 1983; Salonen & Huhtaniemi, 1990). It is also believed to exist, in rodents' ventral prostate direct as well as indirect effect of the ethanol on the glandular epithelium (Martinez et al., 1993; Juarranz et al., 1998). However, there are doubts as to the etiology of the effects observed in the prostatic gland, as being primarily resulting from the decrease of testosterone or the direct action of alcohol in the tissue. Different authors reported sexual disfuncton as being related to the chronic liver disease (Lloyd & Williams, 1948; Pincus et al., 1951; Brown et al., 1964; Van Thiel et al., 1974; Van Thiel & Lester, 1979). At present studies suggest that the alterations in the homeostasis of sexual hormones take place through the effect of ethanol by itself and not as a primary consequence of hepatic deseases or circulatory changes resulting from hepatic cirrhosis (Lindhol, et al., 1978; Anderson et al., 1980; Tadic et al., 2000). However the alterations related to the reproductive system are more considerable in individuals with alcoholic cirrhosis (Van Thiel et al., 1975; Wang et al., 1991).

The toxicity of alcohol over the male reproductive system is not wholly cleared up. Doubts remain about the intensity and irreversibility of the effects of alcohol on the basic biology of the secretory epithelial cells and of the stroma of the ventral prostate of rodents, plus the association of the cellular transformations occurred to different prostatic pathologies. So, the objective of the this study was to analyse the structural, histochemical and hormonal alterations occurred in the ventral lobe of rat's prostate following chronic alcohol ingestion associated to the influence of hormonal replacement, besides setting up a correlations of the

glandular morphology with the direct and indirect action of ethanol in the prostatic gland.

II - MATERIALS AND METHODS

1. ANIMALS AND EXPERIMENTAL PROCEEDING

A total of 30 male rats (*Rattus norvegicus albinus*) with 50 days old were divided into three experimental groups (control, alcoholic and hormone-treated alcoholic). The control group (10 animals) received water. The alcoholic group (10 animals) received ethanol diluted to 20° Gay Lussac (20% v/v). The hormone-treated alcoholic group (10 animals) received ethanol diluted to 20% (v/v) for 150 days. After 120 days of the start of ethanol ingestion, 5mg of testosterone cypionate (Deposteron-Novaquímica) per kg of body weight, was simultaneously administered for 30 days. The subcutaneous hormone injections were applied on alternated days during four weeks. All groups were fed solid Nuvilab CR1 chow *ad libitum*. The mensurations of solid and liquid consumption were effected weekly. At the end of the treatment period, the experimental animals were weighed and anesthetized with Francotar/Virbaxyl (1:1) 0,25ml/100g of body weight. Then, the samples of the prostate ventral lobe were submitted to transmission and scanning electron microscopies, histochemical and stereologic analysis.

2. HORMONAL DOSAGE

The levels of testosterone in the blood plasma were quantified by radioimmunassay (Testosterone Direct – Immunotech Kit). The blood samples were colleted and kept at 0° for 30 minutes with later centrifugation (Ependorf/5804R Centrifuge).

3. HISTOCHEMICAL ANALYSIS OF THE ACID PHOSPHATASE ENZYME

Samples of the prostate ventral lobe were colleted from five animals and frozen in liquid nitrogen. Afterwards, sections with six micrometers of thickness were obtained in the Micron HM505E cryostat and incubated in the reacting medium (magnesium 50millimolar, 0,1% alpha naphtyl phosphate, 0,1% fast red and acetate buffer), at 37° for 50 minutes. Following up, the material was photographed in Nikon photomicroscope.

4. STEREOLOGIC ANALYSIS BY LIGHT MICROSCOPY

The cellular cytoplasmic and nuclear volumes were measured through samples of prostatic tissue included in plastic resin (Polyscience) and stained with toluidine blue. To determine the cytoplasmic and nuclear volumes, an ocular (X10) with a 100 grid coupled to a X100 objective (Carl Zeiss) was used. Points on the nucleus and on the cytoplasm were counted in 10 areas by each animal and the cytoplasmic and nuclear fractions were obtained. These data and the nuclear volume were used to estimate the cytoplasmic volume of each animal. The cellular volume was calculated by the addition of the nuclear and cytoplasmic volumes (Aherne, 1967, 1970). To determine the density of the area of the secretory and digestive vacuoles the stereologic methods and the formulae described by Weibel (Aherne, 1967, 1970; Weibel, 1979) were applied on 10 cells by experimental group.

5. TRANSMISSION AND SCANNING ELECTRON MICROSCOPIES

Transmission Electron Microscopy (TEM) – A total of five animals from each experimental group were perfused (Sprando, 1990) with 2,5% glutaraldeyde in 0,1M (pH 7,2) phosphate buffer. The ventral prostate was collected and routinely processed for TEM (Watson, 1958; Reynolds, 1963). Selected sections were then

examined and photographed with LEO 906 transmission electron microscope.

Scanning Electron Microscopy (SEM) – The collecting procedures were similar to the TEM. Following that, samples of ventral prostate were postfixed in osmium tetroxide 1% for two hours (Walqvist, 1996) with afterwards dehydration in acetone. In the last stage of dehydration, the samples were submitted to cryofracture. Then, the material was dried in Balzers critical point device and covered with gold in a Balzers MED-010 device for later examination in Jeol JSM 5800LV scanning electron microscope.

6. STATISTICAL ANALYSIS

The study of the comparison among groups of variables: variation of body weight (g), daily liquid consumption (ml), daily solid consumption (g), volumes of the nucleus (μm^3) and of the cytoplasm (μm^3), took place through the technique of analysis of variance (Wichern & Johnson, 1992) and the Tukey multiple comparisons test (Montgomery, 1991), considering the level of 5% of significance.

III - RESULTS

1. BODY WEIGHT AND CALORIC ANALYSIS

In the control group, the increase in body weight and the consumption of solid were significantly higher when related to the alcoholic and hormone-treated alcoholic groups which were similar (Table 1). The consumption of liquid was also higher in the control group, compared to the alcoholic one. However, the values of the hormone-treated alcoholic group remained different, in an intermediate position between the control and alcoholic groups (Table 1). The average daily values of the total of kilocalories ingested was higher in the control group in relation to the alcoholic groups. These values were 77,65; 59,75 and 67,20 kcal for the control, alcoholic and hormone-treated alcoholic groups, respectively.

2. HORMONAL DOSAGE

In the control group, the average levels of serum testosterone was 1,93ng/ml, higher than 0,96 ng/ml of the alcoholic group. In the hormone-treated alcoholic group, the average level of testosterone was 8,58 ng/ml, showing considerable increase in the total hormonal concentration, relative to the amounts of the other two groups.

3. HISTOCHEMICAL ANALYSIS OF THE ACID PHOSPHATASE ENZYME

The histochemical results demonstrated that all experimental groups showed positive reactivity to acid phosphatase enzyme, in the three cellular regions: apical, supranuclear and basal. In the control group, the reaction presented itself slightly intensified in the apical region. The alcoholic group showed intense reactivity in the supranuclear region. However, it was lower than the hormone-treated alcoholic and control groups. The hormone-treated alcoholic group showed positive reactivity, intensified in the supranuclear region when compared to the remaining groups (Table 2, Figures 1a, b, c).

4. STEREOLOGIC ANALYSIS BY LIGHT MICROSCOPY

In the control group, the nuclear, cytoplasmic and total volumes were higher than the values of remaining experimental groups (Table 3 and Figure 2a). This difference, however, is statistically supported only in relation to the alcoholic group, characterizing cellular atrophy (Table 3 and Figures 2a, b). The average values of the cellular fractions occupied by the nucleous and the cytoplasm of the secretory epithelial cells showed similarity between the control group and hormone-treated alcoholic group (Table 3 and Figures 2a, c). In the alcoholic group, significant differences were verified when compared to the remaining groups, confirming

specially the cellular atrophy by the cytoplasmic volume reduction (Table 3 and Figures 2a, b, c).

5. TRANSMISSION AND SCANNING ELECTRON MICROSCOPIES

TEM – In the **control group**, the ultrastructure of the ventral prostate showed simple secretor epithelium with high columnar cells (Table 3 and Figure 3a) sandwiched with occasional basal cells on intact basal lamina (Figure 3a). The cellular spherical nuclei were in the basal region with evident nucleolus, nuclear membrane and chromatin distributed in the peripheral region (Figure 3a). In the basal cytoplasmic and perinuclear regions, granular endoplasmic reticulum was observed with parallel and flattened cisterns (Figures 3a, b, d), besides occasional digestive vacuoles with area density of 0,35$\mu m^2/\mu m^3$. The Golgi complex was in the supranuclear region with flattened cisterns (Figures 3b, e). In the apical part of the cytoplasm, secretory vacuoles were noticed with secretion of flocculent aspect and area density of 0,43$\mu m^2/\mu m^3$ (Figure 3a, b). The mitochondria were distributed evenly in the cytoplasm (Figures 3b, c, d, e). The microvilli were short and distributed through out the cellular surface (Figures 3a, b, e). The stroma had evidenced muscular cells, blood vessels, collagen and nervous fibers. In the **alcoholic group**, the main glandular alterations shown were the decrease of total cellular volume characterizing cellular atrophy and tissue disorganization of the organelles responsible for the secretory process (Table 3 and Figure 4a). The primary alterations verified were the irregularity of the nuclear shape with intensive invagination of the envelope (Figures 4a, d), compact nucleolus and prominent chromatin in the nuclear peripheral portion and around the nucleolus (Figure 4d). Also noticeable were the granular endoplasmic reticulum and the Golgi complex with dilation of the cisterns, located in the basal, perinuclear and supranuclear regions, respectively (Figures 4a, b). Secondly, were noticed accumulation of digestive vacuoles with area density of 0,5$\mu m^2/\mu m^3$ and lipid droplets in the basal and supranuclear regions of the cytoplasm (Figures 4c, e). In the apical cytoplasm,

occasional secretory vacuoles were evidenced, in several sizes, with secretion of flocculent aspect with area density of 0,31$\mu m^2/\mu m^3$ (Figure 4e). The mitochondria showed rupture of the mitochondrial cristae with uniform distribution in the cytoplasm (Figures 4b, f). The microvilli showed to be discontinous and sparse lining the apical cellular surface (Figures 4a, d, e). The intercellular spacing was evident (Figure 4d). In the stroma were noticeable smooth muscle cells, blood vessels, collagen and nervous fibers similar to the control animals. In the **hormone-treated alcoholic group**, the secretory epithelial cells showed increase in the total cellular volume relative to alcoholic animals (Table 3 and Figure 5a). Ultrastructurally, important alterations were detected again, as nuclei with considerable envelope invagination similar to alcoholic animals (Figure 5a). The granular endoplasmic reticulum and the Golgi complex showed dilation of the cisterns in the supranuclear region (Figures 5a, b, c, d). However, the dilation of the cisterns of the Golgi complex was not so evident as in the alcoholic group. The existence of secretory vacuoles with area density of 0,35$\mu m^2/\mu m^3$ and secretion with flocculent characteristic was shown in the apical region of the cytoplasm (Figure 5c), as well as lipid droplets in the perinuclear region (Figures 5b, d). The microvilli showed to be sparse and discontinuous as in the alcoholic group (Figures 5a, c). In the apical and basal regions of the cytoplasm, digestive vacuoles were evidenced with area density of 0,42$\mu m^2/\mu m^3$ (Figures 5b, c, e). The mitochondria had ruptures in their cristae throughout the whole cytoplasm (Figures 5b, c, e). The intercellular spacing was reduced when compared to alcoholic animals (Figure 5e). The stroma showed smooth muscle cells, blood vessels, collagenous and nervous fibers.

SEM – In the **control group**, the ventral prostate had as characteristics the folding of the acini (Figure 6a). The secretory epithelial cells had surface with polygonal shape and defined intercellular limits (Figure 6b). Short microvilli had a homogeneous distribution throughout cellular surface (Figure 6b). In the **alcoholic group**, the acini did not present folding (Figure 6c). The surface of the secretory cells had irregular shape without definition of the intercellular limits (Figure 6d).

Short microvilli were noticed in the apical surfaces of the cells (Figure 6d). In the **hormone-treated alcohlic group**, folds in the acinar surface were observed (Figure 6e). The irregular shape of the cellular surface, as well as the intercellular spacing was evidenced (Figure 6f). Short and discontinuous microvilli were verified lining the apical surface of the cells (Figure 6f).

IV - DISCUSSION

The results of the present study demonstrated a body weight gain in all experimental animals along the treatment period, which was significantly smaller in the alcoholic groups. Mean daily food ingestion was 28.7 g for the control group and 13.3 g for the alcoholic groups. In the alcoholic groups percent calorie intake from the liquid diet was about 43%. Other studies also reported a lower weight gain in animals receiving alcohol at dilutions ranging from 3 to 30% v/v (Symons & Marks, 1975; Cícero & Badger, 1977; Klassen & Persaud, 1978). Furthermore, Ratcliffe (1972) observed that alcoholic animals, in addition to smaller weight gain, showed a slower growth compared to control animals. However, some authors did not find significant differences in final body weight between animals treated with alcohol (3 to 6% v/v) and the isocaloric control groups (Willis et al., 1983; Anderson et al., 1989; Salonen & Huhtaniemi, 1990). It should be mentioned that isocaloric control groups refer to animals that received a sugar (sucrose) containing diet at calorie concentrations equivalent to the ethanol dose administered in the experiments. According to Lieber (1984a), alcohol is a drug rich in energy and in many societies alcoholic beverages are considered to be part of the basic diet. However, high doses of alcoholic beverages have a damaging effect on nutritional status both in humans and in laboratory animals, resulting in primary or secondary malnutrition. Primary malnutrition is characterized by the ability of alcohol to deviate high-energy nutrients from the diet, while secondary malnutrition is related to difficulties in the digestion and absorption of nutrients due to gastrointestinal problems associated with alcoholism. In addition, the above author considers the

use of rodents inadequate in experiments in which alcohol is the only liquid food available, since he believes that these animals are unable to ingest ethanol amounts equivalent to those consumed by an alcohol-dependent human, which can reach about 50% of the total calorie intake with no signs of malnutrition. According to the Institute for Laboratory Animal Research (1995), mean daily ingestion of approximately 15 g ration is sufficient for nutritional maintenance in adult rats. In 1975, Campana and colleagues, in studies on rodents, characterized the protein malnutrition based on the occurrence of behavioral disturbances, diarrhea, edema, weight loss and alterations in fur distribution. Thus, it was concluded that rodents represent a viable experimental model for the induction of chronic alcoholism and that the alterations observed are the result of the action of alcohol itself and not of possible protein malnutrition.

In the present study, the alcoholic group showed drastic morphological alterations such as atrophy of secretory epithelial cells as evidenced by a decrease in cytoplasmic volume. In contrast, in the hormone-treated alcoholic group, the decline in cell volume, although present, was not significant compared to the control group. However, the volumetric values were significantly higher than those observed for animals of the alcoholic group. In addition, animals of the two alcoholic groups presented marked tissue transformations involving organelles responsible for the glandular secretory process, featuring disorganization of the cell membrane systems. Although hormone-treated alcoholic animals showed 09-fold higher plasma testosterone levels than the alcoholic group, no cell morphology compatible with that of control animals was observed. Only digestive and secretory vacuoles showed signs of remodeling indicating a correlation with control animals in terms of cell integrity. The present results also demonstrated a positive expression of acid phosphatase in the three cell regions (supranuclear, apical and basal) studied in the different experimental groups, with higher expression being observed in the supranuclear regions of the two alcoholic groups. However, the intensity of the phosphatase reaction was lower in the alcoholic group compared to the hormone-treated alcoholic group. In control animals, expression of the enzyme

was marked in the apical region. The method used for histochemical analysis of acid phosphatase did not permit the separate identification of the two forms of the enzyme (secretory and lysosomal). However, the localization of the cell organelles involved in the secretory mechanism suggests the expression of the secretory form, particularly, in the apical and supranuclear regions.

It is known that alcohol and its main metabolite, acetaldehyde, act in a direct manner on the male gonads by altering the synthesis of testicular testosterone (Gary et al., 1976; Van Thiel & Lester, 1979), and also in an indirect manner, causing imbalance in the hypothalamus-pituitary-gonadal axis (Van Thiel et al., 1979; Geokas, et al., 1981; Van Thiel, 1983; Salonen & Huhtanieni, 1990). According to Martinez et al. (1993) and Juarranz et al.(1998), both a direct and an indirect tissue effect of ethanol is believed to be present in the ventral prostate of rodents. Studies using different amounts of alcohol (6 to 30% v/v) and times of exposure (60 to 260 days) have reported the damaging effects of alcohol on the main accessory sex glands of rodents. These effects include a reduction in cell volume in the secretory epithelium of the ventral and lateral lobes of the rat prostate and coagulation gland (Martinez et al., 1993; Cagnon et al., 1996, 1998), disorganization of granular endoplasmic reticulum cisternae in the epithelial cells of the ventral and lateral lobes of the rat prostate and coagulation gland and of the seminal vesicle of *Calomys callosus* (Martinez et al., 1993, 2001; Cagnon et al., 1996, 1998) and disorganization of the Golgi complex in the secretory epithelium of the seminal vesicle and ventral lobe of the mouse prostate and rat coagulation gland (Cagnon et al., 1996, 2001; Gomes et al., 2002), rupture of the mitochondrial cristae in epithelial cells of the rat ventral prostate and coagulation gland (Martinez et al., 1993; Cagnon et al., 1996), irregularities in the nuclear envelope of the seminal vesicle epithelium of *Calomys callosus* and of the ventral and dorsal lobes of the rat prostate (Martinez et al., 1993, 2001; Cagnon et al., 2001; Garcia et al., 1999), and microvillus discontinuity in the secretory epithelium of the ventral and lateral lobes of the rat prostate and of the ventral lobe of mice (Martinez et al., 1993; Cagnon et al., 1998, 2001). In addition, the occurrence of apoptotic cells in

the seminal vesicle of mice has been reported in experiments using prolonged periods of alcohol exposure and alcohol concentrations ranging from 25 to 35% v/v (Gomes et al., 2002), as well as a reduction in plasma and testicular testosterone concentration (Van Thiel et al., 1975; 1979; Salonen & Huhtaniemi, 1990). However, existing studies have failed to show a correlation between the behavior of acid phosphatase secreted by the prostate and chronic alcohol ingestion. On the other hand, experiments aiming at the morphofunctional analysis of the prostate under androgen suppression have demonstrated a hormone dependence of acid phosphatase synthesis in the prostate. These studies showed an increase in enzyme expression in prostate epithelial cells in the presence of androgens and a decline during testosterone deficiency (Tenniswood et al., 1975, 1978; Orlowski & Clark, 1986, 1990). Thus, the effects of chronic experimental alcoholism observed in the present study agree with previous reports. It was concluded that ethanol has a negative effect on morphology, as well as a suppressive effect on the expression of acid phosphatase in the ventral lobe of the rat prostate, leading to consequent functional damage and impairment of fertility of the animals. However, the secretory process did not cease completely in the two alcoholic groups as demonstrated by the presence of secretion in the glandular lumen, of vacuoles containing secretory material and of positive expression of acid phosphatase enzyme.

Furthermore, it is important to note that the specialized literature has investigated the effects of discontinuation of prolonged ethanol treatment on the prostate of chronically alcoholic rodents (Anderson et al., 1985; Martinez, et al., 1997; Cagnon et al., 1998). These studies have employed various concentrations of ethanol ingested by the animals (5 to 30% v/v), different periods of exposure to the drug (35 to 240 days), and different periods of abstinence (60 to 70 days). The results revealed not only cell restoration but also effective recovery of the physiological conditions of the prostate (Anderson et al., 1985; Martinez, et al., 1997; Cagnon et al., 1998). Thus, on the basis of the present experimental model

and of literature data, it was suggested that the damage caused by chronic ethanol use in the prostate cellular structure cannot be considered irreversible.

No studies investigating chronic ethanol ingestion and the simultaneous administration of androgen hormones are available in the literature. On the other hand, studies on castrated animals, with castration representing one of the most widely used methods for the understanding of the effects of testosterone on the maintenance of cell integrity and functioning of the accessory sex glands, have demonstrated atrophy of these glands (Martins & Valle, 1938, 1939; Wakade et al., 1975; Sjostrand & Swedin, 1976), collapse of the granular endoplasmic reticulum cisternae, a reduction in the number of mitochondria and the presence of electron-dense bodies in the supranuclear region of the prostate cells (Harkin, 1963). In addition, a loss in secretory activity and a reduction in Golgi complex cisternae and in the number of secretory granules in the ventral lobe of the prostate (Cavazos, 1975), as well as changes in excitability, sensitivity and contractibility of these glands (Picarelli & Valle, 1969; Kurkiewicz et al., 1977; Markus et al., 1981) have been reported. According to Murakoshi et al. (1992), maintenance of the height and folding of the secretory epithelium of the ventral prostate was observed in rats submitted to castration accompanied by the simultaneous administration of 1 mg/day testosterone for 6 weeks. Also, Harkin (1963), administering seven daily injections of testosterone propionate to castrated rats and subsequently, Thompson & Heidger (1978), submitting castrated animals to treatment with 5 mg testosterone propionate for 3 days and 2 mg for 7 days, demonstrated restructuring of the granular endoplasmic reticulum cisternae in secretory epithelial cells of the ventral lobe of the prostate. The latter authors also reported recovery of the Golgi complex and of the secretory granules. Murakoshi et al. (1997), analyzing castrated rats receiving 1 mg/animal of testosterone propionate for 3 and 7 days, observed a reduction in the number of apoptotic cells in the ventral prostate. The same was demonstrated by Wright et al. (1996) who administered increasing doses of testosterone (0.05, 0.1, 0.3, 0.5 and 0.7 mg) for 21 days through a subcutaneous implant. Taken together, the present results indicate that hormone

replacement minimized the harmful effects of ethanol ingestion, suggesting signs of hormone activity on the prostate. However, hormone replacement alone was not sufficient to maintain the tissue hormone balance since most morphological alterations resulting from the action of alcohol continued to be observed. In this respect, the few signs of tissue remodeling identified here do not permit the demonstration of complete suppression of the indirect effect of ethanol on the prostate. In addition, one may suggest that even after hormone replacement the negative effects of ethanol resulting from both its indirect and direct action continue to be present.

Finally, it is known that dihydrotestosterone, the dominant androgen in the prostate, has a higher affinity for nuclear membrane receptors than testosterone (Rittmaster et al., 1995). In addition, it has been shown that chemical inhibitors such as finasteride cause a reduction in the activity of 5α-reductase, compromising the conversion of testosterone to dihydrotestosterone and that the amount of androgen receptors is directly proportional to the levels of the androgen (Mainwaring & Mangan, 1973). Based on these considerations, ethanol might act in a manner similar to that of a 5α-reductase inhibitor, impairing the conversion of testosterone to dihydrotestosterone, as well as reducing the expression of androgen receptors or altering their structure, thus damaging the hormone-receptor interaction. However, doubts remain regarding the action of ethanol on the mechanism of hormone action on prostate cells, which require further studies.

V - BIBLIOGRAPHY

Adams, M.L., Cícero T.J. 1991. Effects of alcohol on beta-endorphin and reproductive hormones in the male rat. *Alcohol Clin. Exp. Res.*, 15(4), 685-692.

Aherne, W. 1967. Methods of counting discrete tissue components in microscopical sections. *J. Roj. Micr. Soc.*, 87, 493-508.

Aherne, W. 1970. Quantitative methods in histology. *J. Med. Lab. Techn.*, 27, 160-170.

Allamani, A, Forni, E., Ammannati, P., Sani, L.B., Centurioni, A. 2000. Alcohol carousel and children´s school drawings as part of a community educational strategy. *Subst. Use Misuse,* 35(1-2), 125-139.

Anderson Jr., R.A., Willis, B.R., Oswald, C., Reddy, J.M., Beyler, S.A., Zaneveld, L.J.D. 1980. Hormonal imbalance and alterations in testicular morphology induced by chronic ingestion of ethanol. *Biochem. Pharmacol.,* 29, 1409-1419.

Anderson Jr., R.A, Willis, B.R., Oswald, C., Zaneveld, L.J.D. 1985. Partial reversal of ethanol-induced male reproductive pathology following abstinence. *Alcohol & Alcoholism,* 20(3), 273-286.

Anderson Jr, R.A., Philips, J.F., Zaneveld, L.J.D. 1989. Chronic ethanol ingestion during puberty alters the transient increase in testicular 5α-reductase in the Swiss-Webster mouse. *Journal of Andrology*, 10, (1), 28-36.

Bannister, P., Lowosky, M.S. 1987. Ethanol and hipogonadism. *Alcohol. Alcohol,* 22, 213-217.

Brown, J.B., Ctrean, G.P., Finsberg, J. 1964. Oestrogen metabolism and excretion in liver disease. *GUT.,* 5, 56-59.

Cagnon, V.H.A., Garcia, P.J., Martinez, M., Padovani, C.R. 1996. Ultrastructural study of the coagulating gland of wistar rats submitted to experimental chronic alcohol ingestion. *The Prostate* , 28, 341-346.

Cagnon, V.H.A., Garcia, P.J., Guazzelli Filho, J., Martinez, F.E. 1998. Ultrastructural study of the lateral lobe of the prostate of Wistar rats submitted to experimental chronic alcohol ingestion. *J. Submicrosc. Cytol. Pathol.,* 30, (1), 77-84.

Cagnon, V.H.A., Tomazini, F.M., Garcia, P.J., Martinez, F.E., Martinez, E., Padovani. C.R. 2001. Structure and ultrastructure of the ventral prostate of isogenic mice (C57B1/6J) submitted to chronic alcohol ingestion. *Tissue & Cell,* 33(4), 354-360.

Campana, A.O., Burini, R.C., Outa, A.Y., Camargo, J.L.V. 1975. Experimental protein deficiency in adult rats. *Rev. Bras. de Pesquisas Méd. Biol.*, 8 (3-4), 221-226.

Cavazos, F. 1975. Fine structure and functional correlates of male accessory sex glands of rodents. In: Washington Roy O. Greep, Edwin B. Astwood Handbook of Physiology. American Physiological Society, 353-381.

Cicero, T.J., Badger, T.M. 1977. Effects of alcohol on the hypotalamic-pituitary-gonadal axis in the male rat. *The journal of pharmacology and experimental therapeutics*, 201 (2), 427-433.

Ellingboe, J., Carole, C.V. 1979. Ethanol inhibits testosterone biosynthesis by direct action on leidg cells. *Res. Commun. Chem. Pathol. Pharmacol.,* 24, 87-102.

Galvão-Teles, A., Monteiro, E., Gavaler J.S., Van Thiel D.H. 1986. Gonadal consequences of alcohol abuse: Lessons from the liver. Special Article by the American Association for the Study of Liver Diseases. *Hepatology*, 6(1), 135-140.

Garcia, P.J., Cagnon, V.H.A, Mello Jr., W., Martinez, M., Martinez, F.E. 1999. Ultrastructural observations of the dorasl lobe of the prostate of rats (*Rattus norvegicus*) submitted to experimental chronic alcohol ingestion. *Rev. Chil. Anat.,* 17 (2), 153-159.

Gary, G.G., Actman, K., Southrgn, L. 1976. Effect of alcohol (ethanol) administration on Sex hormone metabolism in normal men. *N. Engl. J. Med.,* 295, 793-797.

Geokas, M.C., Lieber, C.S. French, S., Halsted, C.H. 1981. Ethanol, the Liver and the Gastrintestinal Tract. *Annals of Internal Medicine*, 95(2), 198-211.

Giesbrecht, N., Rankin, J. 2000. Reducing alcohol problems through community action research projects: contexts, strategies, implications and challanges. *Subst. Use Misuse,* 35(1-2), 31-53.

Gomes, I.C., Cagnon, V.H.A., Carvalho, C.A.F., De Luca, I.M.S. 2002. Stereology and ultrastructure of the seminal vesicle of C57/BL/6J mice following chronic alcohol ingestion. *Tissue & Cell*, 34(3), 177-186.

Harkin, J.C. 1963. Prostatic ultrastructure. *Natl. Cancer Inst. Monograph*, 12, 85-97.

Institute for laboratory animal research. 1995. Nutrient requerements of laboratory animals. 4ªed. 11-15.

Jayasinghe, S. 2000. Alcohol consumption and risk of coronary heart disease. *Jama,* 283(6), 745-746.

Juarranz, M.G., Guijarro, L.G., Bodega, G., Prieto, J.C. 1998. G-Protein regulation of adenylate cyclase activity in rat prostatic membranes after chronic ethanol ingestion. *Prostate*, 36, 226-234.

Klassen, R.W., Persaud, T.V.N. 1978. Influence of alcohol on the reproductive system of the male rat. *International Journal of Fertility*, 23 (3), 176-184.

Kurkiewicz, A., Langeloh, A., Guedes, A.D. 1977. Time-response curves for barium and noradrenaline in vas deferens of castrated rat. *Eur. J. Pharmacol.*, 45(2), 145-151.

Lester R., 1979. Effect of chronic alcohol abuse on sexual function. Clin. Endocrinol. Metab., 8, 499-510.

Lieber, C.S. 1984. Alcohol-nutrition interaction. *Alcohol*, 1, 151-157.

Lloyd, C.W., Williams, R.H. 1948. Endocrine changes associated with Laennec's cirrhosis of the liver. American J. Med., v. 4, p. 315-330. apud: Van Thiel, D. H.,

Mainwaring, W.I., Mangan, F.R. 1973. A study of the androgen receptors in a variety of androgenous-sesitive tissues. *J. Endocrinol.*, 59(1), 121-139.

Marks, V., Wright, J. 1978. Metabolic effects of alcohol. *Clin. Endocrinol. Metab.,* 7, 245-466.

Markus, R.P., Gonçalo, M.C., Lapa, A.J. 1981. Castration atrophy and pharmacological reactivity of the rat coagulating glans. *Brazilian J. Med. Biol. Res.*, 14, 181-185.

Martinez, F.E. Garcia, P.J., Padovani, C.R. Cagnon, V.H.A., Martinez, M. 1993. Ultrastructural study of the ventral lobe of the prostate of rats submitted to experimental chronic alcoholism. *Prostate.*, 22, 1-8.

Martinez, F.E., Garcia, P.J., Padovani, C.R. Cagnon, V.H.A., Martinez, M. 1997. Morfhometric ultrastructural study of the seminal vesicle of rats submitted to experimental chronic alcoholism. *J. Submicrosc. Cytol. Pathol., 29* (4), 537-542.

Martinez, F.E. Martinez, M., Padovani, C.R., Bustos-Obregon, E. 2000. Morphology of testis and epididymis in an ethanol-drinking rat strain (UchA and UchB). *J. Submicrosc. Cytol. Pathol.*, 32(2), 175-184.

Martinez, M., Martinez, F.E., Garcia, P.J., Cagnon, V.H.A., Mello Jr, W., Padovani, C.R. 1999. Morphometric analysis of the endometrial epithelium of rats submmited to chronic experimental alcoholism. *J. Submicrosc. Cytol. Pathol.*, 31(4), 469-475.

Martinez, M., Mattos, E.C.T., Mello Jr., W., Cagnon, V.H.A., Martinez, F.E. 2001. Morphology of the seminal vesicle of *Calomys callosus* submitted to experimental chronic alcoholism. *J. Submicrosc. Cytol. Pathol.*, 33(4), 453-461.

Martins, T.H., Valle, J.R. 1938. Contractilité et pharmacologie comparée *in vitro* dês canaux déférents de *Macacus rhesus* normaux castrés au injectés d'hormones sexuelles. *Comptes Rendus des Séances de la Société de Biologie et de Ses Filiales.* Paris. 129, 1122-1125.

Martins, T.H., Valle, J.R. 1939. Endocrine control of the motility of the male accessory genital organs. *Endocrinology*, 25, 80-90.

Mello Jr, W., Garcia, P.J., Cagnon, V.H.A., Martinez, M. 1997. Morphologic changes in the vesical transition epithelium of alcoholic rats. *J. Submicros. Cytol. Pathol.*, 29 (3), 393-399.

Menendez-Abraham, E., Velasco, A.P., Marin B. 1991. Effect of alcohol treatment on sexual behaviour of male rats. *Indian J. Med. Res.*, 94, 320-326.

Montgomery, D.C. 1991. Design and analysis of experiments. 3th Ed. Jonh Wiley, New York. 649.

Murakoshi, M., Tagawa, M., Inada, R., Suzuki, M. 1992. Effects of testosterone, and testosterone plus estrogen, in the castrated rat ventral prostate – Histopatological and immunocytochemical studies. *Tokai J. Exp. Med.*, 17 (3-4), 133-137.

Murakoshi, M., Ikeda, R., Tagawa, M., Suzuki, M., Takekoshi, S., Watanabe, K. 1997. Expression of glutathione-peroxidase (GSH-PO) in the rat ventral prostate – effect of castration and administration of testosterone. *Tokai j. Exp. Clin. Med.*, 22(3), 111-118.

Orlowski, J., Bird, C.E., Clark, A.F. 1988. Androgen metabolism and regulation of rat ventral prostate growth and acid phosphatase during sexual maturation. *J. Endocrinol.*, 116, 81-90.

Orlowski, J., Clark, F. 1986. Androgen metabolism and actions in rat ventral prostate epithelial and stromal cell cultures. *Biochem. Cell. Biol.*, v. 64(6), p. 583-593.

Orlowski, J., Clark, A.F. 1990. Differential androgen modulation of acid phosphatase isozimes in primary cultures of rat ventral prostate epithelial and stromal cells. *Endocrinology*, 127(4), 2009-2016.

Palmer, T.N. 1989. The biochemistry of alcohol and alcohol abuse. *Scci. Prog.*, 73, 1-15.

Picarelli, Z.P., Valle J.R. 1969. Hormonal regulation of rat seminal vesicle sensitivity to adrenaline, noradrenaline, acetylcholine and acetyl-beta – methylcholine. *Braz. J. Pharmacol.*, 35(3), 468-475.

Pincus, I.J., Rakoff, A.E., Cohn, E.M. 1951. Hormonal studies in patients with chronic liver disease. *Gastroenterology,* 19, 735-54.

Ratcliffe, F. 1972. The effect of chronic ethanol administration on the growth of rats. *Arch. Int. Pharmacodyn.*, 197, 19-30.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biology*, 17, 208.

Rittmaster, R.S., Manning, A.P., Wright, A.S., Thomas, L.N., Whitefield, S., Norman, R.W., Lazier, C.B., Rowden, G. 1995. Evidence for atophy and apoptosis in the ventral prostate of rats given the 5α reductase inhibitor finasteride. *Endocrinology*, 136 (2), 741-748.

Rivier, C., Vale, W. 1983. Influence of ethanol on reproductive junctions of the adult male as juction of body weight. *Alcohol. Clin. Exp. Res.*, 7, 210-211.

Salonen, I.; Huhtaniemi, I. Effects of chronic ethanol diet on pituitary-testicular function of the rat. *Biology of Reproduction*, 42, 55-62, 1990.

Saxena, S., Meehan, D., Coney, P., Wimalasena, J. 1990. Ethanol has direct inhibitory effects an steroidogenesis in human granulosa cells: specifc inhibition of LH action. *Alcohol. Clin. Exp. Res.,* 14, 522-527.

Semczuk, M., Rzeszowska G. 1981. Chronic alcohol intoxication and the morphological state of seminal vesicles and prostate gland in a white rat. *Materia Medica Polona*, 2, 130-135.

Sjostrand, N.O., Swedin, G. 1976. Influence of age, growth, castration and testosterone treatment on the noradrenaline levels of the ductus deferens and the auxiliary male reproductive glans of the rat. *Acta Physiologica Scandinavica*, 98, 323-338.

Sprando, R.L. 1990. Perfusion of rat testis through the heart using heparin. In: Russel I.D., Ettlin A.P.S., Cleeg E. D., eds. Histological and histopatological evaluation of the testis. Clearwater: Cache River Press. p. 277-280.

Symons, A.M., Marks V. 1975. The effects of alcohol on weight gain and the hipothalamic-pituitary-gonadotrophin axis in the maturing male rat. *Biochemical Pharmacology*, 24, 955-958.

Tadic, S.D., Elm, M.S., Subbotin, V.M., Eagon P.K. 2000. Hypogonadism precedes liver feminization in chronic alcohol-fed male rats. *Hepatology*, 31(5), 1135-1140.

Tenniswood, M.; Bird, C.E.; Clarck, A.F. 1975. Acid phosphatases: androgen dependent markers of rat prostate. *Can. J. Biochem.*, 54, 350-357.

Tenniswood, M., Abrahams, P.P., Bird, C.E., Clark, A.F. 1978. Effects of castration and androgen replacement on acid phosphatase activity in the adult rat prostate gland. *J. Endocrinol.*, 77, 302-308.

Thompson, S.A.; Heidger Jr., P.M. 1978. Synergistic effects of prolactin and testosterone in the restoration of rat prostatic epithelium following castration. *Anat. Rec.*, 191, 31-46.

Van Thiel, D.H. 1983. Ethanol; its adverse effects upon the hypothalamic-pituitary-gonadal axis. *J. Lab. Clin. Med.,* 101, 21.

Van Thiel, D.H.; Lester, R. 1979. The effect of chronic alcohol abuse on sexual function *Clinics in Endocrinolgy and Metabolism,* 8 (3), 499-510.

Van Thiel, D.H.; Lester, R.; Sherins, R.J. 1974. Hypogonadism in alcoholic liver disease: evidence for a double defect. Gastroenterology, 67, 1188-1199.

Van Thiel ,D.H. Gavaler, J.S., Lester, R., Goodman, D. 1975. Alcohol-induced testicular atrophy. An experimental model for hypogonadism occuring in chronic alcoholic men: *Gastroenterology,* 69, 326-332.

Van Thiel, D.H., Gavaler, J.S., Cobb, C.F., Sherins, R.J., Lester, R. 1979. Alcohol-induced testicular atrophy in the adult male rat. *Endocrinology*, 105(4), 888-895.

Wakade, A.R.; Garcia, A.G.; Kiperkar S.W. 1975. Effect of castration on the smooth muscle cells of the internal sex organs of the rat: influence of the smooth muscle on the sympathetic neurons innervating the vas deferens, seminal vesicle and coagulating glans. *Journal of Pharmacology and Experimental Therapeutics*, 193, 424-434.

Walqvist, R.; Dahl, E.; Tveter, K.J. 1996. Scanning electron microscopy of the acessory sex glands of the adult male rat. *Scanning Microscopy*, 10(4), 1143-1154.

Wang, Y.J., Lee, D.S.W.V., Tsay, Y.Y.,Lo, K.J. 1991. Gonadal disfunction and changes in sex hormones in postnecrotic cirrotic men: a matched study with alcoholic cirrotic men. *Hepato-Gastroenterol.* 38, 531-534.

Watson, M.L. 1958. Staining of tissues sections for eletron-microscopy with heavy metals. *J. Biophys. Biochem. Cytol.*, 4, 475.

Weibel, E.R. 1979. Stereological principles for morphometry in electron microscopy cytology. *Int. Rev. Cytol.*, 26, 235-302.

Wichern, D.W., Johanson R.A. 1992. Applied multivariate statistical analysis. 3rd ed. In: N.J. Prentice Hall. P. 642.

Willis, B.R., Anderson Jr., R.A., Oswald, C., Zaneveld, L.J.D. 1983. Ethanol-induced male reproductive tract pathology as a function of ethanol dose and duration of exposure. *Journal of Pharmacology and Experimental Therapeutics*, 225 (9), 470-478.

Wright, A.S., Thomas, L.N., Douglas, R.C., Lazier, C.B., Rittmaster, R.S. 1996. Relative potency of testosterone and dihidrotestosterone in preventing atrophy and apoptosis in the prostate of the castrated rat. *J. Clin. Invest.*, 98(11), 2558-2563.

Figure legends

Figure 1. Photomicrographs showing the expression of acid phosphatase in the different groups. Bar 100μm.

a. Control group – acini with intense positive labeling in the apical cellular region (arrow).
b. Alcoholic group – acini showing weak positive enzyme expression concentrated in the supranuclear region (arrow).
c. Hormone treated alcoholic group – acini with major enzymatic activity especially in the supranuclear region (arrow).

Figure 2. Photomicrographs of the secretory epithelium of the ventral lobe of rats' prostate from the three experimental groups: control, alcoholic and alcoholic with hormone (toluidine blue, bar 100μm).

a. Control: High columnar secretory epithelial cells sandwiched with occasional basal cells. Nucleus (n) located in the basal region of the cytoplasm and evident nucleolus. Supranuclear region clear (dotted area), probably corresponding to the Golgi complex, stroma with thin smooth muscle layer and collagen fibers.
b. Alcoholic: Secretory epithelial cells clearly atrophied. Basal nucleus (n) with irregular shape. Stroma with folding of fibrilary elements.
c. Hormone-treated alcoholic: High columnar epithelial cells, basal nucleus (n) with irregular shape and evident nucleolus. Clear supranuclear region (dotted area) showing probable Golgi complex region. Stroma showed fibers collagen, elastic, nerve and smooth muscle cells.

Figure 3. Electronmicrographs of the cells of the secretory epithelium of the ventral lobe of the prostate of rats from the control group.

a. High columnar epithelial cells. Lumen (Lu) with secretion with flocculent aspect. Short microvilli (Mv). Secretory vacuoles (Sv) with material of flocculent aspect. Parallel cisterns of the granular endoplasmic reticulum (GER) in the perinuclear cytoplasm. Basal nucleus (N) with regular shape. Basal cell nucleus (short arrow). Bar 1μm.

b. Supranuclear region. Lumen (Lu). Small microvilli (Mv). Mitochondria (M). Golgi complex with flattened cisterns (g). Granular endoplasmic reticulum (GER) with parallel and flattened cisterns. Secretory vacuoles (Sv) with secretion of flocculent aspect. Bar 1μm.

c. Basal region: Nucleus (N). Mitochondria (M). Intact basal lamina (short arrow). Stroma with collagen fibers and smooth muscle cell. Bar 1μm.

d. Detail of basal region. Nucleus (N). Mitochondria (M). Granular endoplasmic reticulum (GER) with parallel and flattened cisterns. Evident basal lamina (short arrow). Bar 1μm.

e. Detail of the apical region. Lumen (Lu). Microvilli (Mv). Mitochondria (M). Golgi complex (g) with flattened cisterns. Bar 1μm.

Figure 4. Electronmicrographs of the cells of ventral lobe secretory epithelium of rats' prostate of the alcoholic group.

a. Atrophied epithelial cells sandwiched with occasional basal cells (Bc). Lumen (Lu). Discontinuity of microvilli (Mv). Dilated cisterns of granular endoplasmic reticulum (GER). Nucleus (N) of irregular shape. Bar 1μm.

b. Supranuclear region. Lumen (Lu). Microvilli (Mv). Mitochondria (M) with rupture of the cristae. Dilation of the cisterns of the Golgi complex (g). Nucleus (N). Bar 1μm.

c. Basal region. Nucleus (N) of irregular shape. Digestive vacuoles (Dv) in different stages of maturation. Lipid droplets (Ld). Evident basal lamina (short arrow). Bar 1μm.

d. Atrophied columnar epithelial cells. Sparse and discontinuous microvilli (Mv). Evident spacing among adjacent cells (arrow). Nuclei (N) with irregular shape. Nucleolus (nu). Bar 1μm.

e. Apical region. Lumen (Lu). Discontinuous microvilli (Mv). Presence of secretory vacuoles (Sv) and digestive vacuoles (Dv) in different stages of maturation. Bar 1μm.

f. Detail of basal region. Nucleus (N) with evident irregularity of nuclear envelope. Mitochondria (M) with rupture cristae. Evident basal lamina (short arrow). Bar 1μm.

Figure 5. Electronmicrographs of the cells of the secretory epithelium of the ventral prostate of animals of the hormone-treated alcoholic group.

a. High columnar epithelial cells. Lumen (Lu). Discontinuous, sparse and short microvilli (Mv). Dilated cisterns of the granular endoplasmic reticulum (GER). Irregular contour nucleus (N). Bar 1μm.

b. Supranuclear region. Mitochondria (M) with rupture of the cristae. Dilated cisterns of the granular endoplasmic reticulum (GER). Dilated Golgi complex (g). Digestive vacuoles (Dv). Nucleus (N) with irregular contour. Lipid droplets (Ld). Bar 1μm.

c. Apical region. Lumen (Lu). Discontinuous microvilli (Mv). Mitochondria (M) with ruptured cristae. Dilated Golgi complex (g) cisterns. Digestive vacuoles (Dv) in different stages of maturation. Secretory vacuoles (Sv). Bar 1μm.

d. Detail of perinuclear region. Irregular contour nucleous (N). Lipid droplets (Ld). Dilation of the granular endoplasmic reticulum cisterns (GER). Bar 1μm.

e. Basal region. Irregular contour nucleus (N). Mitochondria (M) with ruptured cristae. Digestive vacuoles (Dv). Intercellular spacing (arrow). Integral basal lamina (short arrow). Stroma with collagen fibers and smooth muscle cell. Bar 1μm.

Figure 6. Images of scanning electron microscopy of the cells of the secretory epithelium of the ventral prostate of rats of the three experimental groups (control, alcoholic and hormone-treated alcoholic).

a. Control: Epithelium evidenced by folding of the mucosa. Bar 10μm.

b. Control: Cellular surface with polygonal shape and short microvilli. Evident intercellular limits. Bar 1μm.

c. Alcoholic: Cellular epithelium without acinar surface foldings. Bar 10μm.

d. Alcoholic: Cellular surface with irregular shape and sparse microvilli. Undefined cellular limits and intercellular spacing. Bar 1μm.

e. Hormone treated alcoholic: Folding of the epithelial mucosa. Bar 10μm.

f. Hormone treated alcoholic: Cellular surface with irregular shape and presence of microvilli. Intercellular spacing. Bar 1μm.

Figure 1

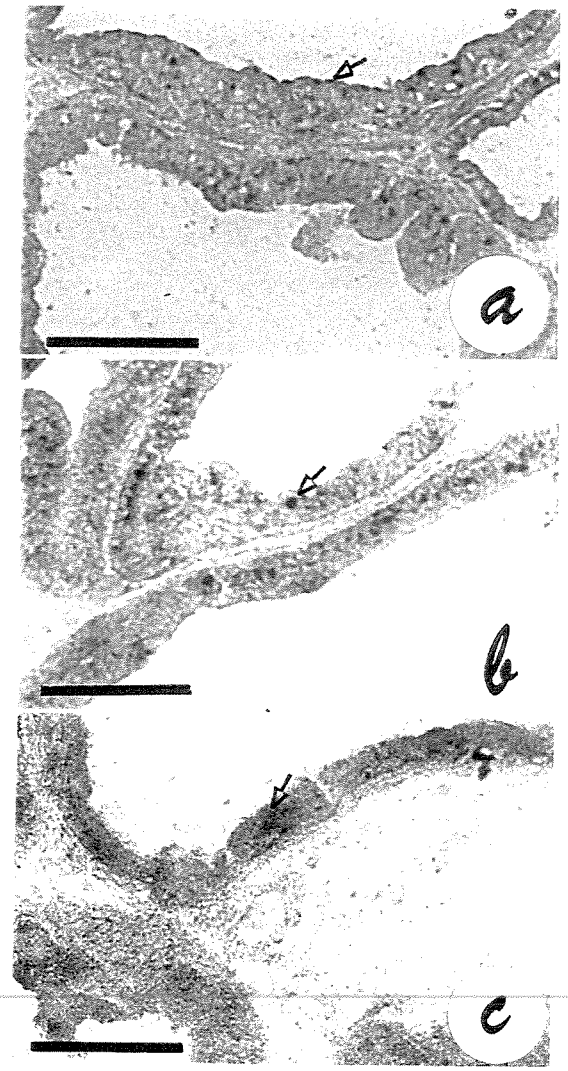
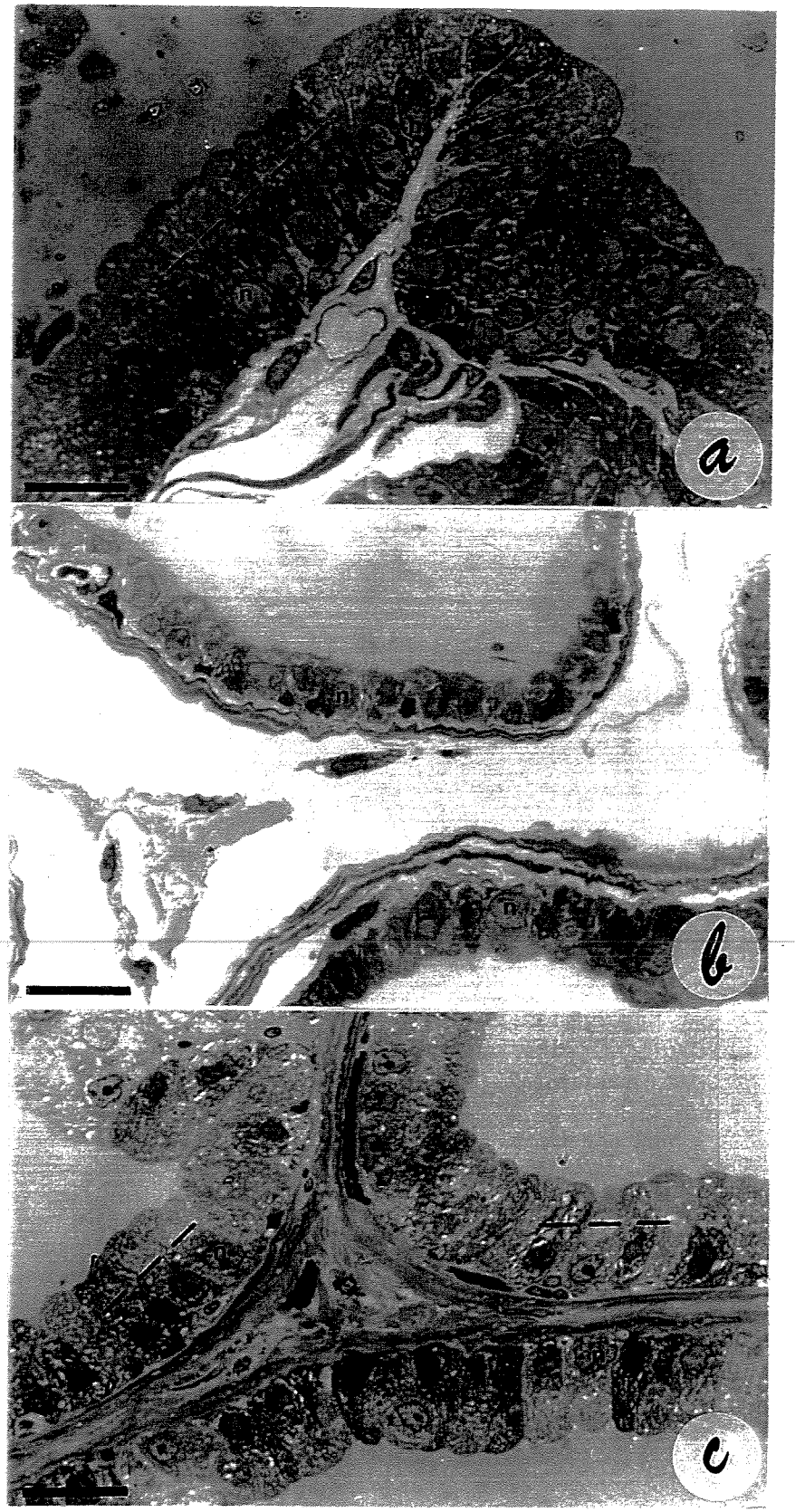
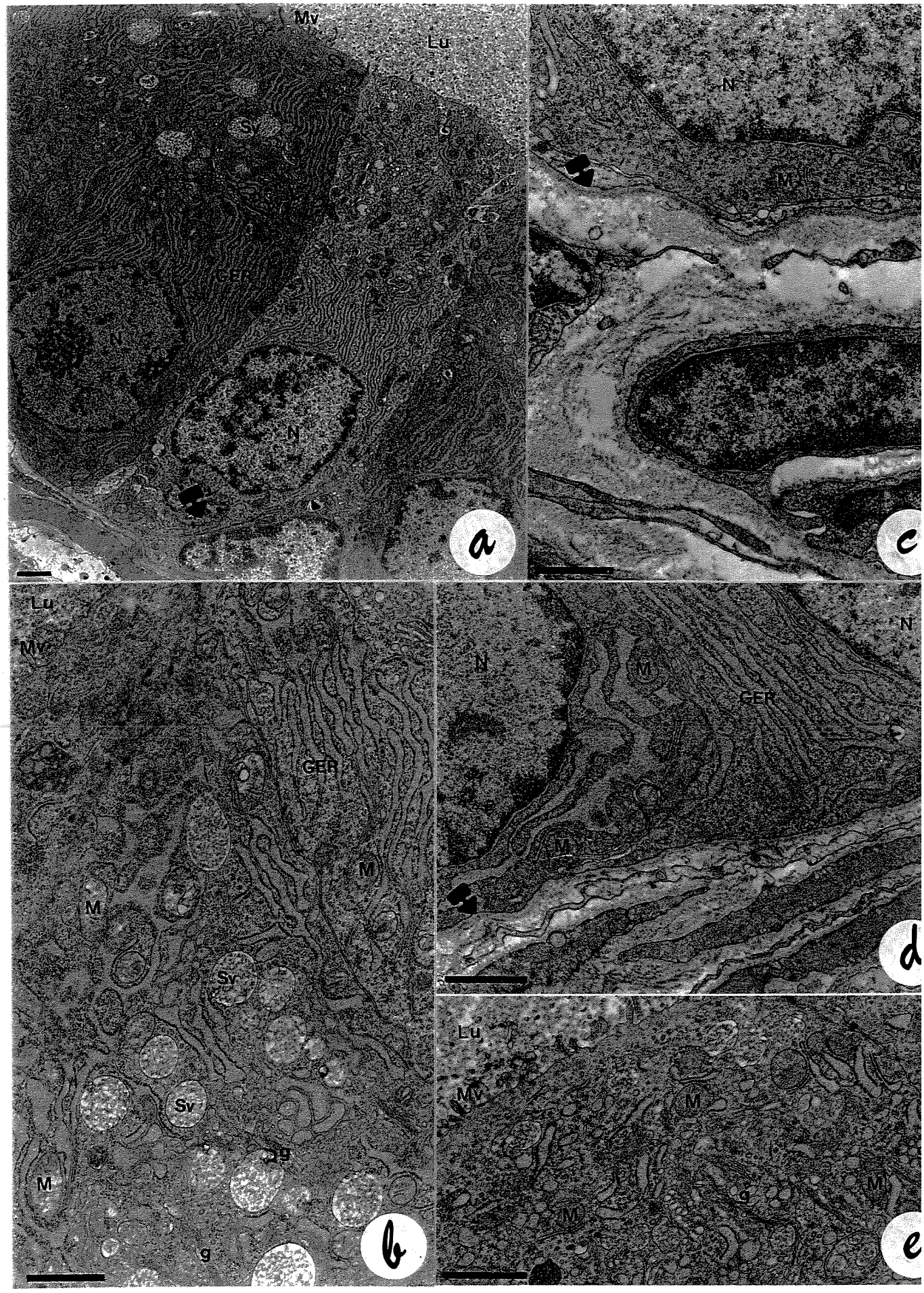
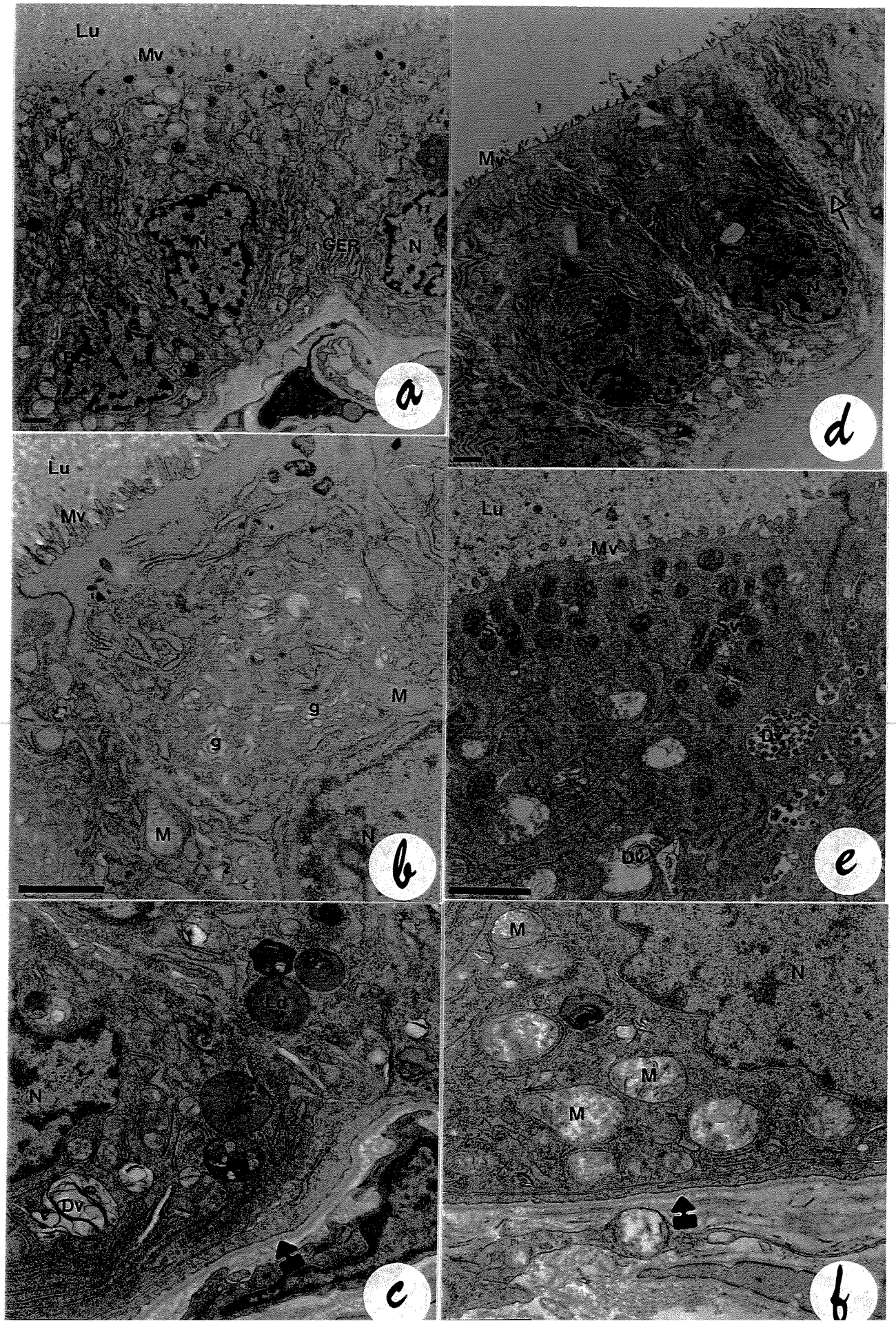


Figure 2




Mv
Lu
Sv
GER
N
M
a
c
Lu
Mv
GER
M
Sv
g
b
N
M
GER
d
Lu
Mv
M
g
e


Lu
Mv
N
GER
N
a
Mv
N
d
Lu
Mv
g
M
g
M
N
b
Lu
Mv
Sv
Dv
Dv
e
M
N
M
M
Lu
N
Dv
c
f

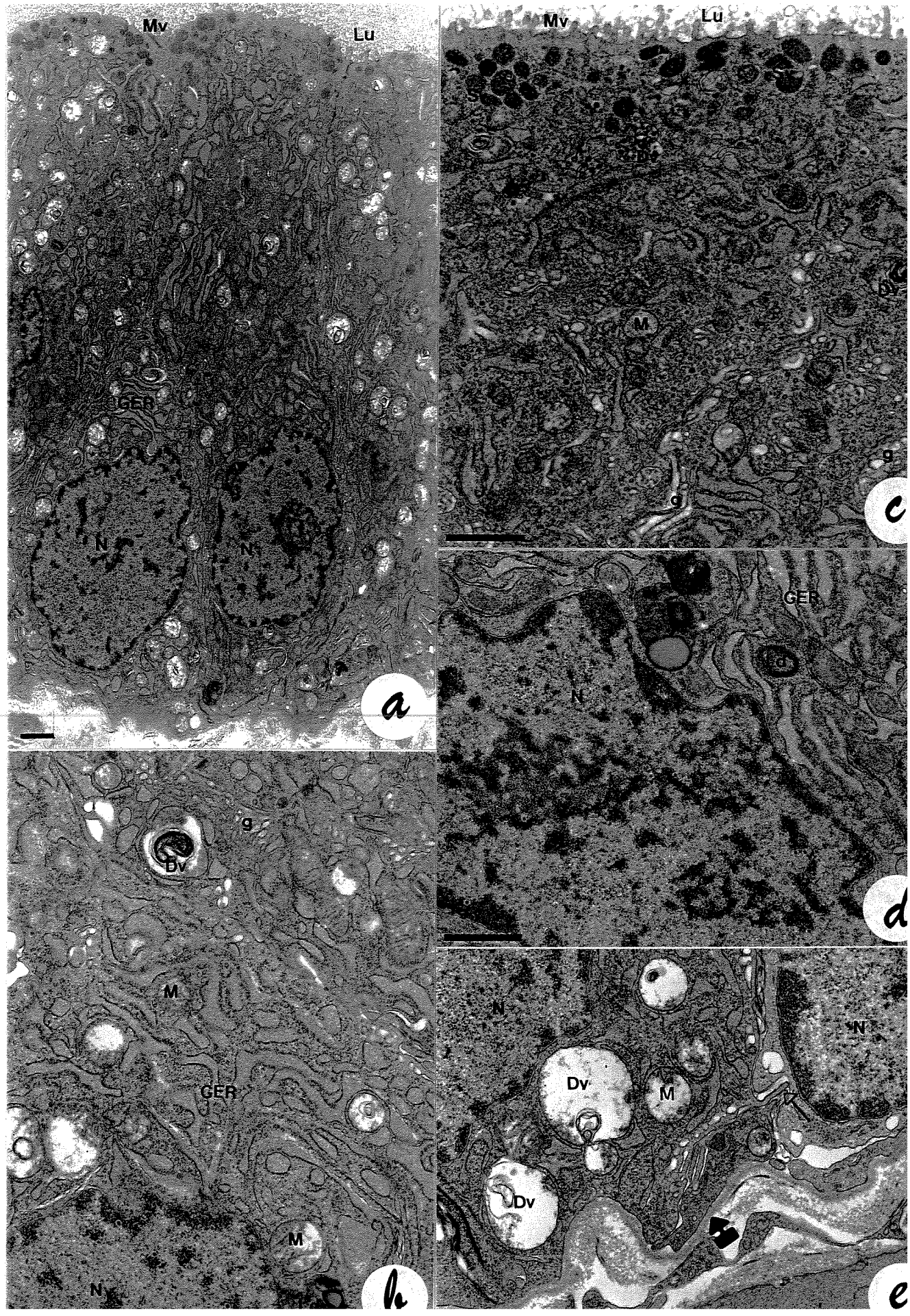
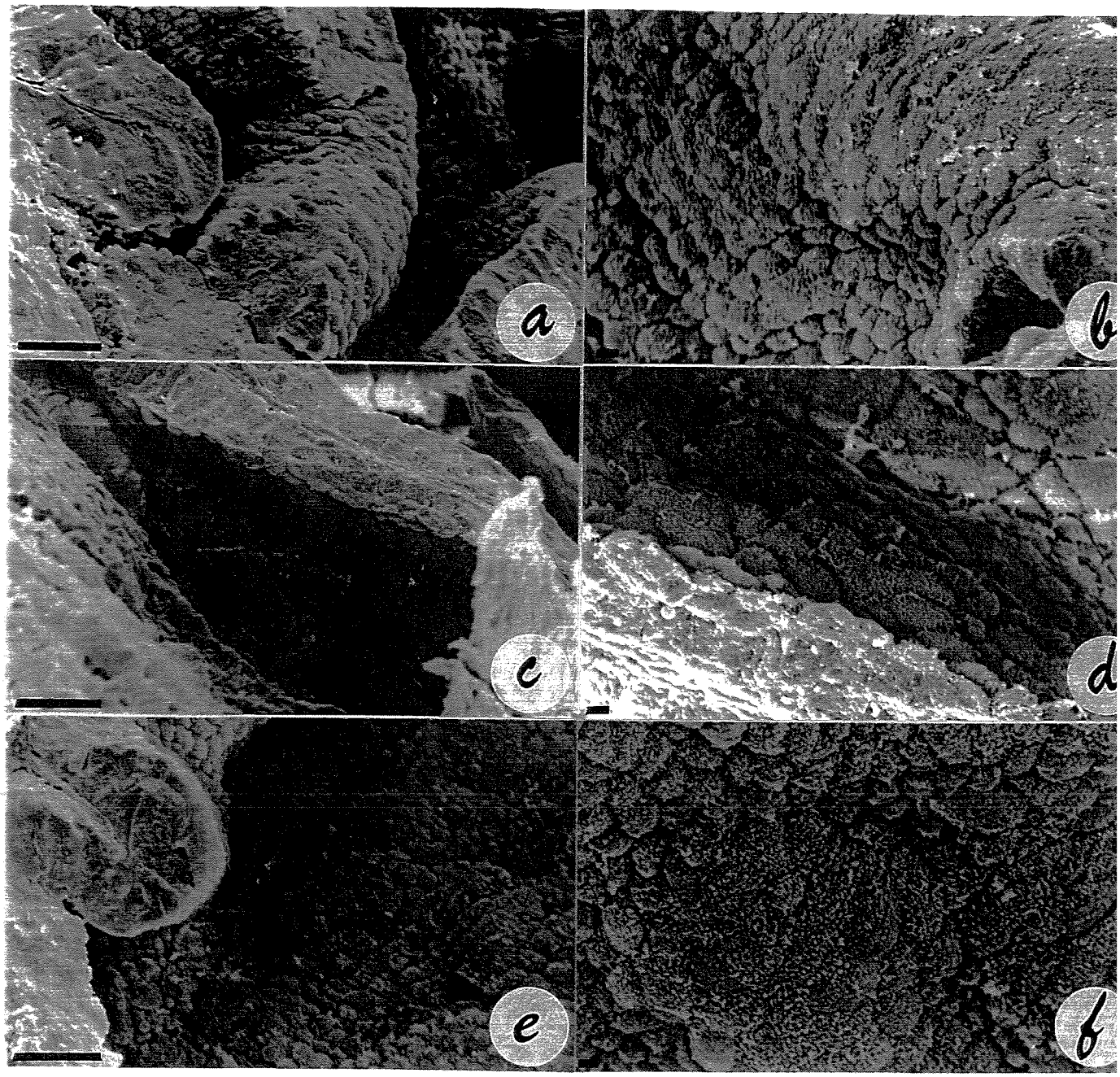

Mv
Lu
GER
N
N
a
Mv
Lu
M
g
g
c
GER
d
N
d
g
Dv
M
GER
M
N
b
N
N
Dv
M
Dv
e

Figure 6



Tables

Table 1. Means and standard deviation of the increase of body weight – ΔP (g) and daily solid consumption – CR (g) and liquid – CL (ml) of the animals of the three experimental groups: control (C), alcoholic (A) and hormone-treated alcoholic (AH).

	GROUPS		
Variables	C	A	AH
ΔP	240±33a	166±16b	141±27,b
CR	28,7±2,28a	13,4±0,88b	13,2±0,76b
CL	41,5±2,9a	20,9±4,74b	28,1±2,52c

a, b and c: Means followed by the same letter did not differ according to the Tukey multiple range text.

Table 2. Analysis of the expression of the acid phosphatase enzyme, demonstrated through the intensity of the reactions in the different cellular regions in the control (C), alcoholic (A) and hormon-treated alcoholic (AH) groups.

GROUPS	Apical region	Supranuclear region	Basal region
C	+++	++	+
A	+	++	+
AH	++	+++	+

(+) positive reaction.

(++) intense positive reaction.

(+++)very intense positive reaction.

Table 3. Means and standard deviation of the nucleus volumes (Vol.Ncl.), of the cytoplasm (Vol.Cit.), total (Vol.Tt.) - μm^3, percentage of the cell occupied by the nucleus (%Ncl.) and by the cytoplasm (%Cit.) - % in the epithelial cells of the experimental groups: control (C), alcoholic (A) and hormone-treated alcoholic (AH).

	GROUPS				
Variables	**C**	**A**	**AH**	**CV(%)**	**MSD(5%)**
Vol. Ncl.	51,4±3,6a	32,3±4,3b	48,4±2,7a	8,12	6,49
Vol. Cit.	257±20a	97±29b	205±13a	22,2	39,2
Vol. Tt .	314±31a	136±32b	253±14a	19,42	49,2
% Ncl.	16,6±0,42b	29,0±4,7a	19,1±1,1b	16,24	5,05
% Cit.	83,3±0,42a	71,0±4,7b	80,9±1,1a	4,25	5,05

a, b and c: Means followed by the same letter did not differ according to the Tukey multiple range text.

MSD: Minimum significant difference.

CV: Coefficient of variation.

5. CONSIDERAÇÕES E CONCLUSÕES FINAIS

1. Os resultados do presente estudo demonstraram que todos os animais experimentais tiveram acréscimo de peso corpóreo ao longo do tratamento, sendo este significativamente menor nos dois grupos alcoolistas. O consumo sólido médio foi de 13,3g para os animais dos dois grupos alcoolistas e 28,7g para o grupo controle. Os percentuais calóricos médios provenientes da ingesta líquida representaram 43% do consumo total para os dois grupos alcoolistas. Em nenhum dos animais experimentais, evidenciou-se sinais de desnutrição protéica caracterizada por distúrbio de comportamento, diarréia, edema, perda de peso e alterações na distribuição de pêlos. Portanto, concluiu-se que é viável o uso de roedores em modelos experimentais na indução do alcoolismo crônico e que as alterações encontradas no lobo ventral da próstata de ratos dos grupos alcoolista e alcoolista com hormônio são provenientes da ação do álcool por si só e não de um processo de desnutrição protéica.

2. A análise estereológica demonstrou que os animais do grupo alcoolista sofreram drásticas alterações morfológicas como redução das células epiteliais secretoras, especialmente, evidenciada pela diminuição do volume citoplasmático. No grupo alcoolista tratado com hormônio tal redução ocorreu, porém não foi significativa quando comparada aos valores do grupo controle. Dessa forma, pode-se concluir que o álcool agiu negativamente sobre o epitélio secretor do lobo ventral da próstata, causando atrofia celular e que a reposição hormonal levou à recuperação parcial do epitélio prostático.

3. Ultra-estruturalmente, nos animais alcoolistas e alcoolistas tratados com hormônio observou-se transformações teciduais envolvendo as organelas responsáveis pelo processo secretor da glândula prostática, indicando prejuízo funcional e provável comprometimento da fertilidade desses animais. Embora, o grupo alcoolista tratado com hormônio tenha recebido dosagem suprafisiológica

de testosterona, sua morfologia tecidual não foi compatível aos animais controles, demonstrando apenas recuperação parcial dos vacúolos digestivos e secretores. Tais achados permitem concluir que, nos dois grupos alcoolistas o processo secretor não cessou completamente e que a reposição hormonal minimizou os efeitos nocivos do etanol sobre o epitélio secretor do lobo ventral da próstata.

4. A expressão da enzima fosfatase ácida foi positiva nas três regiões celulares (apical, supranuclear e basal) nos diferentes grupos experimentais. Porém, a intensidade dessa reação apresentou-se menor no grupo alcoolista quando comparada aos grupos alcoolista tratado com hormônio e controle. Assim sendo, pode-se concluir que o álcool levou a supressão da expressão fosfatásica ácida e que a testosterona promoveu efeito positivo sobre a mesma, sugerindo sinais de atividade hormonal sobre a glândula prostática.

5. Considerando-se as alterações ultra-estruturais, estereológicas e histoquímicas observadas nos grupos alcoolista e alcoolista tratado com hormônio, pode-se propor que a reposição hormonal por si só não foi suficiente para manutenção do equilíbrio tecidual hormonal, sendo evidenciada a continuidade da maioria das alterações morfológicas provenientes da ação do álcool. Dessa forma, os poucos sinais de remodelação tecidual caracterizados nos atuais achados não permitiram sugerir completa supressão do efeito indireto do etanol sobre a glândula prostática. Ainda, pode-se inferir que mesmo após a reposição hormonal mantiveram-se os efeitos negativos do etanol provenientes tanto de sua ação indireta como direta.

6. REFERÊNCIAS BIBLIOGRÁFICAS

ADAMS, M.L.; CICERO T.J. Effects of alcohol on beta-endorphin and reproductive hormones in the male rat. *Alcohol Clin. Exp. Res.*, v. 15(4), p. 685-692, 1991.

AHERNE, W. Methods of counting discrete tissue components in microscopical sections. *J. Roj. Micr. Soc.*, v. 87, p. 493-508, 1967.

AHERNE, W. Quantitative methods in histology. *J. Med. Lab. Techn.*, v. 27, p. 160-170, 1970.

ALLAMANI, A. et al. Alcohol carousel and children´s school drawings as part of a community educational strategy. *Subst. Use Misuse,* v. 35(1-2), p. 125-139, 2000.

ANDERSON JR., R.A. et al. Hormonal imbalance and alterations in testicular morphology induced by chronic ingestion of ethanol. *Biochem. Pharmacol.,* v. 29, p. 1409-1419, 1980.

ANDERSON JR., R. A., et al. Partial reversal of ethanol-induced male reproductive pathology following abstinence. *Alcohol & Alcoholism,* v. 20(3), p. 273-286, 1985.

ANDERSON JR, R.A.; PHILIPS, J.F.; ZANEVELD, L.J.D. Chronic ethanol ingestion during puberty alters the transient increase in testicular 5α-reductase in the Swiss-Webster mouse. *Journal of Andrology*, v. 10, (1), p. 28-36, 1989.

BANDERAS, J. A. et al. Effects of chronic ethanol consumption on the rat. *Arch. Bras. Biol.,* v. 37, p. 69-72, 1992.

BANNISTER, P.; LOWOSKY, M. S. Ethanol and hipogonadism. *Alcohol. Alcohol,* v. 22, p. 213-7, 1987.

BHALLA, V.K.; RAJAN, V.P.; MEREDITH, E.N. Alcohol-induced luteinizing hormone receptor deficiency at testicular level. *Alcohol Clin. Exp. Res.*, v. 7, p. 153-162, 1983.

BLANDY, J.P.; LYTTON, B. What is the prostate? In: *Comparactive Anatomy,* p.1-11, 1986

BOURNE, G.H.; DANIELLE, J.F. International Review of Citology. *New York and London Academic Press*, v. 20, p. 207-275, 1966.

BRANDES, D.; PORTELLA, A. The fine structure of the epithelial cells of the mouse prostate II Ventral lobe epithelium. *The Journal of Biophisical and Biochemical Cytology*, v. 7(3), p. 511-519, 1960.

BROWN, J. B.; CTREAN, G. P.; FINSBERG, J. Oestrogen metabolism and excretion in liver disease. *GUT.,* v. 5, p. 56-59, 1964.

CAGNON, V. H. A. et al. Ultrastructural study of the coagulating gland of wistar rats submitted to experimental chronic alcohol ingestion. *The Prostate* , v. 28, p. 341-346, 1996.

CAGNON, V. H. A. et al. Ultrastructural study of the lateral lobe of the prostate of Wistar rats submitted to experimental chronic alcohol ingestion. *J. Submicrosc. Cytol. Pathol.,* v. 30, (1), p. 77-84, 1998.

CAGNON, V. H. A. et al. Structure and ultrastructure of the ventral prostate of isogenic mice (C57B1/6J) submitted to chronic alcohol ingestion. *Tissue & Cell,* v. 33(4), p. 354-360, 2001.

CAMPANA, A.O. et al. Experimental protein deficiency in adult rats. *Rev. Bras. de Pesquisas Méd. Biol.*, v. 8 (3-4), p. 221-226, 1975.

CARVALHO, H.F.; LINE S.R.P. Basement membrane associated changes in the rat ventral prostate following castration. *Cell Biology International*, v. 20(12), p. 809-819, 1996.

CAVAZOS, F. Fine structure and functional correlates of male accessory sex glands of rodents. In: Washington Roy O. Greep, Edwin B. Astwood Handbook of Physiology. American Physiological Society, p. 353-381, 1975

CICERO, T. J.; BADGER, T. M. Effects of alcohol on the hypotalamic-pituitary-gonadal axis in the male rat. *The journal of pharmacology and experimental therapeutics*, v. 201 (2), p. 427-433, 1977.

CLAIR, H. R. Recognizing alcoholism and its effects: a mine-guide. Basel: S. Krager, 1991. 105p.

CLAVERT A.; CRANZ C.; BOLLACK C. Functions of the seminal vesicle. *Andrologia*, v. 22(1), p. 185-192, 1990.

COSTELLO, C. L.; FRANKLIN, B. R. Effects of Prolactin on the Prostate. *Prostate*, v. 24, p. 162-166, 1994.

CUKIERSKI, M.A. et al. Decreased fertility in male rats administered the 5a-reductase inhibitor, finasteride, is due to deficits in copulatory plug formation. *Reproductive Toxicology*, v. 5, p. 353-362, 1991.

DAHL, E.; KJAERHEIM,A.; TVETER, K .J. The ultrastructure of the accessory sex organs on the male rat. *Z. Zellforch Mikrosk. Anat.*, v. 137, p. 345-359,1973.

DAVIES, P.; EATON, L. C. Regulation of prostate growth (Review). *Journal of Endocrinology*, v. 131, p. 5-17, 1991.

EDWARDS, G.; PETERS, T.J. Alcohol and alcohol problems. *British Medical Bulletin*, v. 50(1), p. 186-199, 1994.

ELLINGBOE, J.; CAROLE, C.V. Ethanol inhibits testosterone biosynthesis by direct action on leidg cells. *Res.Commun. Chem. Pathol. Pharmacol.*, v. 24, p.87-102, 1979.

FARNSWORTH, W.E. Prostate Stroma. Phisiology. *The Prostate*, v. 38, p. 60-72, 1999.

FINK, R.; ROSALKI; S.B. Clinical biochemistry of alcoholism. *Clin. Endocrinol. Metab.*, v.7, p. 297-319, 1978.

FISH, V.B.; NELSON, V.E. The distribution of alcohol in the tissues of the rat. *Proc. Iowa Acad. Sci.*, v. 49, p.263-267, 1942.

GALVAO-TELES, A. et al. Gonadal consequences of alcohol abuse: Lessons from the liver. Special Article by the American Association for the Study of Liver Diseases. *Hepatology*, v.6(1), p.135-140, 1986.

GARCIA, P.J. et al. Ultrastructural observations of the dorasl lobe of the prostate of rats (*Rattus norvegicus*) submitted to experimental chronic alcohol ingestion. *Rev. Chil. Anat.*, v. 17 (2), p.153-159, 1999.

GARY, G.G.; ACTMAN, K.; SOUTHRGN, L. Effect of alcohol (ethanol) administration on Sex hormone metabolism in normal men. *N. Engl. J. Med.*,v. 295, p. 793-797, 1976.

GEOKAS, M.C. et al. Ethanol, the Liver and the Gastrintestinal Tract. *Annals of Internal Medicine*, v. 95(2), p. 198-211, 1981.

GIESBRECHT, N.; RANKIN, J. Reducing alcohol problems through community action research projects: contexts, strategies, implications and challanges. *Subst. Use Misuse,* v. 35(1-2), p. 31-53, 2000.

GOMES, I.C. et al. Stereology and ultrastructure of the seminal vesicle of C57/BL/6J mice following chronic alcohol ingestion. *Tissue & Cell*, v. 34(3), p. 177-186, 2002.

HARKIN, J.C. Prostatic ultrastructure. *Natl. Cancer Inst. Monograph*, v. 12, p. 85-97, 1963.

ICHINARA, I.; PELLINIEMI, L.J. Ultrastructure of the basal cell and the acinar capsule of rat ventral prostate. *Anat. Anz. Bd.*, v. 138, p. 355-364, 1975.

INSTITUTE FOR LABORATORY ANIMAL RESEARCH. Nutrient requerements of laboratory animals.1995, 4ªed. 11-15.

JARRED, R.A. et al. Evidence that estrogens directly alter androgen-regulated prostate development. *Endocrinology*, v. 141(9), p. 3471-3477, 2000.

JAYASINGHE, S. Alcohol consumption and risk of coronary heart disease. *Jama,* v. 283(6), p. 745-746, 2000.

JERNIGAN, D.H. et al., Towards a global alcohol policy: alcohol, public health and the role of who. *Bulletin of the World Health Organization*, v.78, p. 491-499, 2000.

JESIK, C.J.; HOLLAND, J.M.; LEE, C. An anatomic and histologic sudy of the rat prostate. *Prostate*,.v. 3, p.81-97, 1982.

JUARRANZ, M.G. et al. G-Protein regulation of adenylate cyclase activity in rat prostatic membranes after chronic ethanol ingestion. *Prostate*, v. 36, p. 226-234, 1998.

KALANT, H. Absortion, diffusion, distribution and elimination of ethanol: Effects on biological membranes. *Biol. Alcoholism Biochem.*, v. 1, p. 1-62, 1983.

KLASSEN, R.W.; PERSAUD, T.V.N. Influence of alcohol on the reproductive system of the male rat. *International Journal of Fertility*, v. 23 (3), p. 176-184, 1978.

KURKIEWICZ, A.; LANGELOH, A.; GUEDES, A.D. Time-response curves for barium and noradrenaline in vas deferens of castrated rat. *Eur. J. Pharmacol.*, v. 45(2), p. 145-151, 1977.

LANGWORTHY, O.R. Innervation of the pelvic organs of the rat. *Invest. Urol.*, v. 2: p. 491-511, 1965.

LEHNINGER, A.L. , 1991. Principios de Bioquimica. Editora Savier. P. 543-544.

LEO, M.A.; SATO, M.; LIEBER, C.S. Effect of hepatic vitamin A depletion on the liver in men and rats. *Gastroenterology*, v. 84, p. 562-572, 1983.

LI, T.K. The absorption, distribution and metabolism of ethanol and its effects on nutrition and hepatic function. In: Tabakoff B., Sutker p.b., Randall C.L., eds. Medical and social aspects of alcohol abuse, 1983. New York: Plenum Press, 47-78.

LIEBER, C.S.; SHAW S. General nutritional status in the alcoholic including disorders of minerals and vitamins. In: Lieber C.S., ad. Medical disorders of alcoholism, pathogenesis and treatment. Major problems in internal medicine, Series XXII. Philadelphia: W.B. Saunders, p. 555-568, 1983.

LIEBER, C.S. Alcohol and the liver. *Hepatology,* v.4, p. 1243-1260, 1984a.

LIEBER, C.S. Alcohol-nutrition interaction. *Alcohol*, v. 1, p. 151-157, 1984b.

LLOYD, C.W.; WILLIAMS, R.H. Endocrine changes associated with Laennec's cirrhosis of the liver. American J. Med., v. 4, p. 315-330. apud: Van Thiel, D. H., Lester R.,1979. Effect of chronic alcohol abuse on sexual function. Clin. Endocrinol. Metab., v. 8, p. 499-510, 1948.

LUNG, B.; CUNHA ,R.,G. Development of Seminal Vesicle and Coagulating Glands in neonatal mice. I. The morphometris effects of various hormonal conditions. *Anat. Records,* v. 199, p. 73-88, 1981.

MADDEN, J.S. The definition of alcoholism. *Alcohol & Alcoholism*, v. 28(2), p. 617-620, 1992.

MAINWARING, W.I.; MANGAN, F.R. A study of the androgen receptors in a variety of androgenous-sesitive tissues. *J. Endocrinol.*, v. 59(1), p. 121-139, 1973.

MANN, T.; LUTWAK-MANN, C. Male reproductive function and semem. *Berlim: springer-verlog,* p.495, 1981.

MARKS, V.; WRIGHT, J. Metabolic effects of alcohol. *Clin. Endocrinol. Metab.,* v. 7, p. 245-466, 1978.

MARKUS, R.P.; GONÇALO, M.C.; LAPA, A.J. Castration atrophy and pharmacological reactivity of the rat coagulating glans. *Brazilian J. Med. Biol. Res.*, v. 14, p. 181-185, 1981.

MARTINEZ, F.E. et al. Ultrastructural study of the ventral lobe of the prostate of rats submitted to experimental chronic alcoholism. *Prostate.*, v. 22, p. 1-8, 1993.

MARTINEZ, F.E. et al. Morfhometric ultrastructural study of the seminal vesicle of rats submitted to experimental chronic alcoholism. *J. Submicrosc. Cytol. Pathol.*, v. *29* (4), p. 537-42, 1997.

MARTINEZ, F.E. et al. Morphology of testis and epididymis in an ethanol-drinking rat strain (UchA and UchB). *J. Submicrosc. Cytol. Pathol.*, v. 32(2), p. 175-184, 2000.

MARTINEZ, M. et al. Morphometric analysis of the endometrial epithelium of rats submmited to chronic experimental alcoholism. *J. Submicrosc. Cytol. Pathol.*, v. 31(4), p. 469-475, 1999.

MARTINEZ, M. et al. Morphology of the seminal vesicle of *Calomys callosus* submitted to experimental chronic alcoholism. *J. Submicrosc. Cytol. Pathol.*, v. 33(4), p. 453-461, 2001.

MARTINS, T.H.; VALLE, J.R. Contractilité et pharmacologie comparée *in vitro* dês canaux déférents de *Macacus rhesus* normaux castrés au injectés d'hormones sexuelles. *Comptes Rendus des Séances de la Société de Biologie et de Ses Filiales.* Paris. V. 129, p.1122-1125, 1938

MARTINS, T.H.; VALLE, J.R. Endocrine control of the motility of the male accessory genital organs. *Endocrinology*, v. 25, p. 80-90, 1939.

MELLO JUNIOR, W. et al. Morphologic changes in the vesical transition epithelium of alcoholic rats. *J. Submicros. Cytol. Pathol.*, v. 29 (3), p. 393-399, 1997.

MENENDEZ-ABRAHAM, E.; VELASCO, A.P.; MARIN B. Effect of alcohol treatment on sexual behaviour of male rats. *Indian J. Med. Res.*, v. 94, p. 320-326, 1991.

MONTGOMERY, D.C. Design and analysis of experiments. 3th Ed. Jonh Wiley, New York. 649 p, 1991.

MURAKOSHI, M. et al. Effects of testosterone, and testosterone plus estrogen, in the castrated rat ventral prostate – Histopatological and immunocytochemical studies. *Tokai J. Exp. Med.*, v. 17 (3-4), p. 133-137, 1992.

MURAKOSHI, M. et al. Expression of glutathione-peroxidase (GSH-PO) in the rat ventral prostate – effect of castration and administration of testosterone. *Tokai j. Exp. Clin. Med.*, v. 22(3), p. 111-118, 1997.

OKAMOTO, S. et al. Proliferative pattern of seminal vesicle cells and production testosterone and androgens from birth to adult-hood in mice. *Endocrinology*, v. 111, p. 1230-1234, 1982.

ORLOWSKI, J., BIRD, C.E., CLARK, A.F. Androgen metabolism and regulation of rat ventral prostate growth and acid phosphatase during sexual maturation. *J. Endocrinol.*, v. 116, p. 81-90, 1988.

ORLOWSKI, J.; CLARK, F. Androgen metabolism and actions in rat ventral prostate epithelial and stromal cell cultures. *Biochem. Cell. Biol.*, v. 64(6), p. 583-593, 1986.

ORLOWSKI, J., CLARK, A.F. Differential androgen modulation of acid phosphatase isozimes in primary cultures of rat ventral prostate epithelial and stromal cells. *Endocrinology*, v. 127(4), p. 2009-2016, 1990.

PALMER, T.N. The biochemistry of alcohol and alcohol abuse. *Scci. Prog.*, v. 73, p. 1-15, 1989.

PICARELLI, Z.P.; VALLE J.R. Hormonal regulation of rat seminal vesicle sensitivity to adrenaline, noradrenaline, acetylcholine and acetyl-beta –methylcholine. *Braz. J. Pharmacol.*, v. 35(3), p. 468-475, 1969.

PINCUS, I.J.; RAKOFF, A.E.; COHN, E.M. Hormonal studies in patients with chronic liver disease. *Gastroenterology,* v. 19, p. 735-54, 1951.

PRICE, D.; WILLIAMS-ASHMAN, H.G. The accessory reproductive Sex glands in mammals. In: Young W.C. (ed.). Sex and internal secretions, 3. Ed. Baltimore: Williams & Wilkins, p. 488, 1961.

PRINS, G. S. Neonatal estrogen exposure induces lobe specific alterations in adult rat androgen receptor expression. *Endocrinology*, v. 130 (4), p. 2401-2412, 1992.

PURINTON, P.T.; FLETCHER, T.F.; BRADLEY, W. E. Gross and light microscopic features of the pelvic plexus in the rat. *Anat. Rec.* v. 175, p. 697-706, 1972.

RAJER, J.; COFFEY, D. S. Sex steroid imprinting of the immature prostate. Long term effects. *Invest. Urol.,* v. 16, p. 186-190,1978.

RATCLIFFE, F. The effect of chronic ethanol administration on the growth of rats. *Arch. Int. Pharmacodyn.*, v. 197, p.19-30 , 1972.

REITER, E. et al. Effects of pituitary hormones on the prostate. Review article. *The Prostate*, v. 38, p. 159-165, 1999.

REYNOLDS, E .S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biology*, v. 17, p. 208, 1963.

RICHTER, C. P. A study of moderate doses of alcohol on the rat. *J. Exp. Zool.*, v. 44, p. 397-418, 1926.

RITTMASTER, R. S. et al. Evidence for atophy and apoptosis in the ventral prostate of rats given the 5α reductase inhibitor finasteride. *Endocrinology*, v. 136 (2), p. 741-748, 1995.

RIVIER, C.; VALE, W. Influence of ethanol on reproductive junctions of the adult male as juction of body weight. *Alcohol. Clin. Exp. Res.*, v. 7, p. 210-1, 1983.

SALONEN, I., HUHTANIEMI, I. 1990. Effects of chronic ethanol diet on pituitary-testicular function of the rat. *Biology of Reproduction*, 42, 55-62.

SAXENA, S. . Ethanol has direct inhibitory effects an steroidogenesis in human granulosa cells: specifc inhibition of LH action. *Alcohol. Clin. Exp. Res.*, v. 14, p. 522-7, 1990.

SEMCZUK, M.; RZESZOWSKA G. Chronic alcohol intoxication and the morphological state of seminal vesicles and prostate gland in a white rat. *Materia Medica Polona*, v. 2, p. 130-135, 1981.

SETCHELL, B.P.; BROOKS, P.E. Anatomy, vasculature, inervation and fluids of the male reproductive tract. In: Knobil e., Neil J. *The Phisiology of reproduction*. New York Raven Press, p. 753-836, 1988.

SJOSTRAND, N.O.; SWEDIN, G. Influence of age, growth, castration and testosterone treatment on the noradrenaline levels of the ductus deferens and the auxiliary male reproductive glans of the rat. *Acta Physiologica Scandinavica*, v. 98, p. 323-338, 1976.

SONNENREICH, J.C. Contribuição para o estudo da etiologia do alcoolismo. São Paulo. Ed. Revista de Tribunais, 168, 1971.

SPRANDO, R.L. Perfusion of rat testis through the heart using heparin. In: Russel I.D., Ettlin A.P.S., Cleeg E. D., eds. Histological and histopatological evaluation of the testis. 1990. Clearwater: Cache River Press. p. 277-280.

SYMONS, A.M., MARKS V. The effects of alcohol on weight gain and the hipothalamic-pituitary-gonadotrophin axis in the maturing male rat. *Biochemical Pharmacology*, v. 24, p. 955-958, 1975.

TADIC, S.D. et al. Hypogonadism precedes liver feminization in chronic alcohol-fed male rats. *Hepatology*, v. 31(5), p. 1135-1140, 2000.

TENNISWOOD, M.; BIRD, C.E.; CLARCK, A.F. Acid phosphatases: androgen dependent markers of rat prostate. *Can. J. Biochem.,* v. 54, p. 350-357, 1975.

TENNISWOOD, M. et al. Effects of castration and androgen replacement on acid phosphatase activity in the adult rat prostate gland. *J. Endocrinol.*, v. 77, p. 302-308, 1978.

THOMPSON, S.A.; HEIDGER JR., P.M. Synergistic effects of prolactin and testosterone in the restoration of rat prostatic epithelium following castration. *Anat. Rec.*, v. 191, p. 31-46, 1978.

TSUJI, M. et al. 5α reductase activity in developing urogenital tracts of fetal and neonatal male mouse. *Endocrinology*, v. 134 (5), p. 2198-2205, 1994.

VAALASTI, A.; HERVONEN, A. Inervation of the ventral prostate of the rat. *Am. J. Anat.*, v. 154, p. 231-244, 1979.

VAN THIEL, D.H. Ethanol; its adverse effects upon the hypothalamic-pituitary-gonadal axis. *J. Lab. Clin. Med.*, v. 101, p. 21, 1983.

VAN THIEL ,D.H. et al. Alcohol-induced testicular atrophy. An experimental model for hypogonadism occuring in chronic alcoholic men: *Gastroenterology,* v. 69, p. 326-332, 1975.

VAN THIEL, D.H. et al. Alcohol-induced testicular atrophy in the adult male rat. *Endocrinology*, v. 105(4), p. 888-895, 1979.

VAN THIEL, D.H.; LESTER, R. The effect of chronic alcohol abuse on sexual function *Clinics in Endocrinolgy and Metabolism,* v. 8 (3), p. 499-510, 1979.

VAN THIEL, D.H.; LESTER, R.; SHERINS, R.J. Hypogonadism in alcoholic liver disease: evidence for a double defect. Gastroenterology, v. 67, p. 1188-99, 1974.

WATSON, M.L. Staining of tissues sections for eletron-microscopy with heavy metals. *J. Biophys. Biochem. Cytol.,* v.4, p. 475, 1958.

WAKADE, A.R.; GARCIA, A.G.; KIPERKAR S.W. Effect of castration on the smooth muscle cells of the internal sex organs of the rat: influence of the smooth muscle on the sympathetic neurons innervating the vas deferens, seminal vesicle and coagulating glans. *Journal of Pharmacology and Experimental Therapeutics*, v. 193, p. 424-434, 1975.

WALQVIST, R.; DAHL, E.; TVETER, K.J. Scanning electron microscopy of the acessory sex glands of the adult male rat. *Scanning Microscopy*, v. 10(4), p. 1143-1154, 1996.

WANG, Y.J. et al. Gonadal disfunction and changes in sex hormones in postnecrotic cirrotic men: a matched study with alcoholic cirrotic men. *Hepato-Gastroenterol.* v. 38, p. 531-534, 1991.

WEIBEL, E.R. Stereological principles for morphometry in electron microscopy cytology. *Int. Rev. Cytol.*, v. 26, p. 235-302, 1979.

WEXBERG, L.E. A critique of physiophatological theories of the etiology of alcoholism. *Q.J. Study Alcohol.*, v.11, p. 113-118, 1950.

WICHERN, D.W.; JOHANSON R.A. 1992. Applied multivariate statistical analysis. 3rd ed. In: N.J. Prentice Hall. P. 642.

WILLIS, B.R. et al. Ethanol-induced male reproductive tract pathology as a function of ethanol dose and duration of exposure. *Journal of Pharmacology and Experimental Therapeutics*, v. 225 (9), p. 470-478, 1983.

WRIGHT, A.S. et al. Relative potency of testosterone and dihidrotestosterone in preventing atrophy and apoptosis in the prostate of the castrated rat. *J. Clin. Invest.*, v. 98(11), p. 2558-2563, 1996.

WRIGHT, J. Endocrine effects of alcohol. *Clin. Endocrinol. Metab.*, v. 7, p. 351-367, 1978.

7. APÊNDICE

Tabela 1. Média diária dos consumos sólido e líquido (etanol 20% ou água).

CS : consumo sólido (g)

CL: consumo líquido (ml)

Grupos: C – controle; A – alcoolista 20%; AH – Alcoolista tratado com hormônio.

Grupo	Animal	CS	CL
C	C1	26.7	39.2
C	C2	28.5	42.8
C	C3	28.9	43.9
C	C4	27.2	44.2
C	C5	32.5	37.8
A	A11	13.1	30.0
A	A12	13.8	19.9
A	A13	14.7	19.4
A	A14	12.9	19.0
A	A15	12.9	16.5
AH	AH21	14.2	31.1
AH	AH22	12.4	27.6
Ah	AH23	12.5	24.4
AH	AH24	13.8	27.5
AH	AH25	13.5	30.3

Tabela 2. Média de kilocalorias (kcal) ingeridas diariamente através dos consumos sólido e léquido.

kcal CS : kilocalorias provenientes do consumo sólido.

kcal CL: kilocalorias provenientes do consumo líquido.

Grupos: C – controle; A – alcoolista 20%; AH – Alcoolista tratado com hormônio.

Grupo	Animal	kcal CS	Kcal CL
C	C1	72.09	0.0
C	C2	76.95	0.0
C	C3	78.03	0.0
C	C4	73.44	0.0
C	C5	87.75	0.0
A	A11	35.37	33.44
A	A12	37.26	22.18
A	A13	39.69	21.62
A	A14	34.83	21.17
A	A15	34.83	18.39
AH	AH21	38.34	34.66
AH	AH22	33.48	30.76
AH	AH23	33.75	27.19
AH	AH24	37.26	30.65
AH	AH25	36.45	33.77

Tabela 3. Acrésimo de peso corpóreo durante os 150 dias de tratamento ns três grupos experimentais (C – controle, A – alcoolista 20% e AH – alcoolsta tratado com hormônio)

ΔP – diferença entre peso final e peso inicial (g).

Grupo	Animal	ΔP
C	C1	287.2
C	C2	196.5
C	C3	215.1
C	C4	267.2
C	C5	273.0
C	C6	248.3
C	C7	211.8
C	C8	223.8
A	A9	142.8
A	A10	164.4
A	A11	183.3
A	A12	151.3
A	A13	194.6
A	A14	167.2
A	A15	157.6
A	A16	163.0
AH	AH17	142.2
AH	AH18	155.7
AH	AH19	120.9
AH	AH20	117.2
AH	AH21	197.0
AH	AH22	138.7
AH	AH23	147.3
AH	AH24	110.7

Tabela 4. Média dos volumes nuclear, citoplasmático e celular total; frações celulares ocupadas pelo núcleo e pelo citoplasma ($\mu m^2/\mu m^3$).

Vol. Ncl. – volume do núcleo.

Vol Cit. – volume do citoplasma.

Vol. Tt. – volume celular total.

%Ncl. – fração celular ocupada pelo núcleo.

%Cit. – fração celular ocupada pelo citoplasma.

Grupos: C – controle; A – alcoolista 20%; AH – Alcoolista tratado com hormônio.

Grupo	Animal	Vol Ncl.	Vol Cit.	Vol. Tt.	% Ncl.	% Cit.
C	C6	49.5	235.8	285.4	17.3	82.6
C	C7	54.4	278.6	333.0	16.3	83.7
C	C8	46.2	234.6	280.8	16.4	83.5
C	C9	55.1	272.0	355.0	16.9	83.1
C	C10	51.6	263.0	314.6	16.4	83.6
A	A16	30.2	64.5	99.7	35.3	64.7
A	A17	27.3	81.0	119.0	31.5	68.5
A	A18	31.2	88.0	124.4	29.3	70.7
A	A19	37.3	135.2	181.0	25.3	74.7
A	A20	35.5	118.1	154.8	23.7	76.3
AH	AH26	49.1	215.8	264.9	18.5	81.5
AH	AH27	45.6	213.4	259.0	17.6	82.4
AH	AH28	49.5	208.9	258.4	19.2	80.9
AH	AH29	45.8	183.2	229.0	20.0	80.0
AH	AH30	51.9	202.5	254.4	20.4	79.6