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EFFECTS OF WHOLE-BODY γ -IRRADIATION ON THE
BIOSYNTHESIS OF CERTAIN SERUM PROTEINS

Progress Report

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ABSTRACT

Studies have been completed to demonstrate that whole-body irradiation stimulates only the Na^+ -dependent "A" system for the transport of amino acids into the liver. Distribution ratios for the *in vitro* AIB uptake in Na^+ -free media were the same for liver slices from normal and irradiated rats. This shows that irradiation did not influence the non-saturable or passive diffusion of AIB. Whole-body irradiation had no effect on the *in vivo* accumulation of the bicyclic model amino acid BCH which is transported exclusively by the Na^+ -independent "L" system. Studies have been performed using AIB as a competitive inhibitor for the *in vitro* transport of L-methionine and cycloleucine. These studies also showed that irradiation stimulates only the "A" system of amino acid transport. Studies of AIB transport in a variety of tissues indicate that stimulation is not relatable to radiation sensitivity but rather to their metabolic activities. The stimulated amino acid transport reflects an elevated serum amino acid level possibly following the breakdown of muscle tissue. Our data indicate that the elevated amino acid transport promotes an increased gluconeogenic activity culminating in glycogenesis.

Studies continue on improving the isolation of individual isoelectric species of α_{2u} . We now have the purified $\alpha_{2u}5.2$ species on hand in sufficient quantity to prepare a specific antiserum. Studies are being continued on the dietary requirements for α_{2u} production and the concept of biological priority in the hepatic biosynthesis of plasma protein. Other studies are in progress to establish the nature of the renal modification of α_{2u} .

The primary goals of the current program are to study the effects of whole-body γ -irradiation on:

- PROJECT I. Plasma protein synthesis via its action on the hepatic transport of amino acids.
- PROJECT II. Hepatic synthesis of specific plasma proteins, especially acute phase proteins and $\alpha_2\mu$ (sex-dependent urinary protein of the adult male rat).

SUMMARY OF PROGRESS TO DATE

DATES COVERED: November 29, 1967 through December 31, 1973

An effect of whole-body exposure to ionizing radiations on the hepatic transport of amino acids was first observed as an increased accumulation of amino acids in the liver of rats 60 minutes following an injection of ^{14}C -algal hydrolysate (1). The increased uptake of amino acids was not the consequence of a reduced food intake since pair-fed animals did not exhibit a comparable effect. This observation was confirmed using the non-metabolizable amino acid α -aminoisobutyric acid (AIB) (1, 2). Four days following exposure to 1300R, the accumulation of AIB in the rat liver was approximately seven times the normal while that of other tissues, kidneys, spleen, muscle, and testes, was suppressed (1). *In vitro* studies using liver slices showed the stimulated uptake of AIB to be the result of Na^+ -dependent active transport (3). Inhibition by the glycoside ouabain suggested the involvement of a Na^+ -pump mechanism (3). Both the K_m and V_{max} were increased by the irradiation process. Thus V_m for irradiated rats was 25 $\mu\text{moles AIB/ml intracellular fluid/min}$ compared with a normal of 10 $\mu\text{moles AIB/ml ICF/min}$; K_m was 9.2 and 5.1 (mM) for the irradiated and normal rats respectively (COO-1754-12). These data, in the light of the increased K_m , do not eliminate the possibility that irradiation induces a transport mechanism which either is not observed or is a very minor component in normal liver tissue.

Our initial results were consistent with the concept that only the Na^+ -dependent, alanine-preferring or "A" system was affected by whole-body irradiation. Preliminary *in vitro* studies using a bicyclic model acid, 2-aminobornane-2-carboxylic acid (BCH) showed that irradiation had no effect on the sodium-independent leucine-preferring or "L" system of transport (4).

Although optimal Na^+ concentrations are required for AIB uptake by livers of both normal and irradiated rats, a stoichiometric relationship has not been demonstrated to date (5). Whole-body irradiation results in an apparent delipidation of plasma membrane from the livers of the experimental animals. Thus the total lipid content and cholesterol is reduced by approximately 30-40%. However, the ouabain-sensitive Na^+ , $\text{K}^+(\text{Mg}^{++})\text{ATPase}$ and the K^+ -p-nitrophenylphosphatase activities were not affected by this treatment. Furthermore, there was no evidence of an elevated ATPase to compensate for the increase in amino acid transport. To date our results are not in accord with the concept that the increased AIB transport is dependent on an elevated Na^+ gradient maintained by a stimulated ATPase system.

It is anticipated that studies of the effect of whole-body irradiation on

hepatic protein synthesis will be more productive if a system is used involving a single protein which can be controlled by hormones in a selective manner. To achieve this goal, we have studied the hepatic biosynthesis of $\alpha_2\text{u}$, a sex-dependent protein of the adult male rat. This protein is synthesized in the liver, secreted into the bloodstream, concentrated by the kidneys, and excreted in the urine (6-9). Its synthesis is under the synergistic control of androgens and glucocorticoids (10). $\alpha_2\text{u}$ is produced in the liver as a single species having an isoelectric point of 5.2 (11, 12). Upon excretion by the kidneys it is converted to a number of species having isoelectric points varying from 4.8 to 6.0 (13). The chemical difference between species and the nature of the renal modification are presently unknown. The hepatic synthesis of $\alpha_2\text{u}$ is dependent on an adequate protein diet. An amino acid deficiency results in a suppression of $\alpha_2\text{u}$ synthesis. A 50% casein diet yields an increased excretion of $\alpha_2\text{u}$; however, this is caused not by an increased synthesis but by an increased output of urine and a consequent elevated loss of total proteins (14).

PROGRESS REPORT FOR THE PERIOD COVERING JANUARY 1, 1974-DECEMBER 31, 1974
(extended to April 30, 1975)

PROJECT 1: RADIATION-STIMULATED UPTAKE OF AMINO ACIDS BY THE LIVER

The purpose of these studies is to establish the nature of the effect of whole-body irradiation on the transport of amino acids in various organs, particularly the liver. It is desirable to establish whether the irradiation-stimulated hepatic uptake of amino acids represents an increase in the normal transport processes or the imposition of an abnormal component system. It is also desirable to determine any chemical changes in the plasma membrane which might predispose the altered amino acid transport. Furthermore, I intend to determine the role played by the increased amino acid transport in changes in general metabolism elicited by whole-body irradiation.

A. Is the Radiation-Stimulated Uptake of Amino Acids Exclusively a Sodium-Dependent Mechanism?

To establish whether or not the irradiation-stimulated hepatic transport of amino acids is exclusively Na^+ -dependent or not, the following amino acids were used:

AIB (α -aminoisobutyric acid) transported principally by the "A" system
BCH (2-aminonorbornane-2-carboxylic acid) transported by the "L" system
Cycloleucine - transported by both the "A" and "L" systems
L-Methionine - transported by both "A" and "L" systems

The determination of which transport systems are involved depends primarily on studies using Ehrlich ascites cells (15, 16). AIB is transported primarily by the Na^+ -dependent "A" system in the tumor cells and the liver (17). There is, however, some alanine transport by a related Na^+ -dependent mechanism called the "ASC" system (18).

The distribution ratio (DR) for AIB in liver slices from normal rats was 2.25-3.97 in a medium which contained 143 mM Na^+ , 1.00 at 25 mM, and 0.70 in a Na^+ -free medium. Irradiation increased the distribution ratio to 6.5-7.8 in 143 mM Na^+ , 4.0 in 25 mM, and 0.63 in Na^+ -free medium. These data show that irradiation

increases the Na^+ -dependent transport. The transport was still active at 25 mM in liver slices from both normal and irradiated rats. There is a distinct possibility that irradiation stimulates two Na^+ -dependent systems one of which may function optimally at low (25 mM) Na^+ concentration. The fact that the DR's are reduced to 0.63-0.70 for liver slices from both normal and irradiated rats shows that irradiation had no effect on any Na^+ -independent transport processes. This also suggests that irradiation did not influence the non-saturable AIB uptake (passive diffusion); only the saturable transport mechanism was affected.

The absence of any effect on the Na^+ -independent "L" system was demonstrated using the bicyclic model amino acid BCH.

Experiment	Normal Rat	Irradiated Rat
1. <i>In vivo</i> (accumulation in total liver in 60 min)	2.1% of injected dose	2.4% of injected dose
2. <i>In vitro</i> (DR)	0.9 - 1.2	0.9 - 1.0

These results were obtained 3 days following exposure to 1300R. The absence of any effect on transport confirms the fact that irradiation did not influence the specific Na^+ -independent "L" transport system.

Of equal importance to the use of model amino acids transported almost exclusively by a specific transport mechanism, is the use of amino acids transported to some degree by both "A" and "L" systems. Thus, L-methionine and also the model amino acid, cycloleucine, are known to be transported by both the "A" and "L" systems in Ehrlich ascites cells(15,18a). Their transport in liver has been studied but the mechanism is not clear (17, 19). Liver slices from normal and irradiated (3-4 days post 1300R) rats were incubated for 1 hour with ^{14}C -cycloleucine. The media used were Krebs-Ringer buffers of 143 and 25 mM Na^+ concentration. In some studies 20 mM AIB was added to the incubation system to serve as a competitive inhibitor. Excess AIB competed for that component of the total transport of cycloleucine which was carried by the "A" or Na^+ -dependent system but not for the "L" or a Na^+ -independent transport mechanism. Normal rat liver slices showed little accumulation of cycloleucine (DR = 1); low Na^+ or added AIB had no effect. Irradiated rats showed a stimulated uptake (DR = 2.75); in the presence of 25 mM Na^+ the distribution ratio was reduced to 1.75 while AIB reduced the uptake to 1.2. There is a possibility of two Na^+ -dependent systems, both abolished by AIB, but only one affected by the reduced Na^+ concentration. When similar experiments were performed using L-methionine (^{14}C), the liver slices from normal rats showed a DR of 1.5 when incubated in 143 mM but only 0.8-1.0 when incubated with either 25 mM Na^+ or 20 mM AIB as competitive inhibitor of the "A" system component. These results can be interpreted to mean that L-methionine is transported in liver cells by a Na^+ -dependent system that is non-functional at a low Na^+ concentration (25 mM). Irradiation leads to an increase in DR to 2.5, while in the presence of 25 mM Na^+ or 20 mM AIB the ratio is 1.25-1.35. These results suggest that irradiation stimulates only a Na^+ -dependent system. It seems clear that whole-body γ -irradiation in some way stimulates only the Na^+ -dependent amino acid transport mechanisms. Our data exclude a contribution of the "L" system but do not exclude the possible stimulation of a second Na^+ -dependent system such as described by Riggs and McKirahan for muscle (20). We need to repeat

these studies in media completely free of Na^+ to establish the lowest DR's before any real conclusions may be made.

In addition to the effects on the active transport of amino acids, it is likely that whole-body irradiation may change the passive diffusion of amino acids. To determine the possible existence of an increase in AIB accumulation by an alteration in diffusion, ^{14}C -AIB accumulation was determined in the complete absence of Na^+ . In the Na^+ -free medium (25 mM KHCO_3 , 236 mM sucrose), the DR's using slices from both irradiated and normal rats were unchanged in AIB concentrations ranging from 0.001 mM to 0.1 mM. The DR's at 0.007 mM AIB using a Na^+ -free medium were 0.4-0.5 for liver slices from normal and irradiated rats. These values are to be compared with 2.6 and 6.4 obtained when normal and irradiated livers respectively are incubated in 143 mM Na^+ . Irradiation had no apparent effect on the non-saturable diffusion of AIB.

Conclusion: Studies with ^{14}C -AIB, ^{14}C -cycloleucine, ^{14}C -BCH, and ^{14}C -L-methionine indicate that whole-body irradiation of rats leads to a stimulated transport of amino acid across the liver cell membrane exclusively via Na^+ -dependent systems. Passive diffusion is not influenced by irradiation. At this time there is a possibility that more than one Na^+ -dependent transport mechanism is affected. These results will be presented at the Federation Meetings, April 1975 (Flory, W. and Jennewein, S.M. "The Effect of γ -Irradiation on the Hepatic Transport of Amino Acids").

B. Effect of Whole-Body Irradiation on Amino Acid Transport in Extrahepatic Tissues

In an earlier Progress Report (COO-1754-8), I described the radiation-stimulated uptake of amino acids as "hitherto unrecognized radiobiological effect". We now know that a stimulated-uptake of ^{14}C -glycine by the rat liver was observed by Maass *et al.*, in 1967 (21). Maass exposed rats (60 gm) to doses of 800R. Bekhor and co-workers exposed rats (150-160 gm) to 150-600R and observed some increase in AIB uptake by heart and skeletal muscle (33) and a considerable elevation in AIB accumulation in the kidneys but did not work with liver. Thymus, on the other hand, exhibited a distinct decrease in AIB uptake. Our studies using 1300R (140-160 gm rats) showed that the uptake by skeletal muscle and kidneys was reduced while only the uptake in the liver was increased (23). This raised a question concerning an apparent tissue sensitivity to radiation as measured in terms of amino acid transport. Thus, thymus appeared to be very sensitive while kidney exhibited a stimulus as long as the dosage level was below 1300R; only the liver survived the exposure to 1300 R. The sensitivity of amino acid transport mechanisms may reflect the general tissue sensitivity to irradiation (24). Of the tissues studied, the liver appeared to be the most resistant (25).

To establish whether or not the AIB uptake reflects a general tissue sensitivity to irradiation, we studied the accumulation of ^{14}C -AIB by various tissues at differing doses and times post-irradiation. All injections of ^{14}C -AIB were made 1 hour prior to sacrifice. The tissues in question were removed, homogenized and counted. Of the tissues tested [liver, kidneys, musculature (gastrocnemius and diaphragm), testes and spleen (intestine was not used)] only the liver and kidneys exhibited an elevated accumulation of AIB.

The kidneys exhibited a 67 to 100% increase in AIB accumulation (representing 6-12% of the injected dose) 48 hours after exposure to 500-2500R. Seventy-two hours after the exposures to dosages of 1000R or more, the accumulation of AIB was reduced to normal level or well below (less than 1% of the dose). Why should this stimulation observed at 48 hrs be restricted to the liver and kidneys? Apparently this phenomenon is not related to the radiation-sensitivity of tissues. The fact that these are the gluconeogenic tissues suggests an important role of the transport of L-alanine to supply glucose to the organism. The increase in alanine transport may in fact be pursuant to an elevation of blood amino acids resulting from the breakdown of carcass protein (26). Elevated amino acid levels in the blood and tissues might reflect the sum of the starvation effect and an irradiation-induced catabolism of peripheral carcass proteins. It is likely that the elevated amino acid levels trigger the secretion of glucagon which, in turn stimulates the "A" transport mechanism (27). A subsequent increase in hepatic pyruvate level would increase gluconeogenesis. Conceivably, an excessive breakdown of protein caused by irradiation would lead to an overabundance of glucose-6-phosphate which, in turn, would stimulate glycogenesis.

Conclusion: Studies of AIB transport using a variety of tissues indicate that a stimulation of amino acid transport is not relatable to tissue radiation sensitivity, rather it appears to be involved in gluconeogenesis and glycogenesis.

C. Is the Stimulation of Amino Acid Transport Relatable to Glycogenesis?

The accumulation of hepatic glycogen after exposure to 2500R follows a profile comparable to the stimulation in AIB uptake. An increased AIB uptake is already evident at 12 hrs post-irradiation. At this time the specific activity of glycogen following the administration of ^{14}C -L-alanine has reached a maximum whereas the actual amount of glycogen (mg/gm of liver) is only 11 mg. A maximum of 18 mg/gm is reached by 23-48 hrs. These results were obtained after exposure to 2500R which is obviously a super-lethal dosage of radiation. At 1500R, on the other hand, the AIB uptake was increased, not suppressed, at 72 hrs post-irradiation. Exposure to yet lower levels such as 500-1000R yielded a gradual and linear increase in AIB uptake which reached 300% above normal 72 hrs post-irradiation (1000R). Yet Sobocinski and Altman (26) observed a transient increase in glycogen formation, maximal 24-48, but suppressed at 72 hrs. The lack of a temporal relationship between our AIB transport data and the glycogenesis data of Sobocinski and Altman suggests that these phenomena may be interdependent but not "coupled".

Sobocinski and Altman (26) showed that glycogenesis occurred at the expense of carbon chains from protein-bound amino acids, especially alanine. They proposed that a major response to irradiation was a degradation of peripheral carcass protein. Amino acids were released into the bloodstream and transported to the liver. In support of this proposal Nadkarni and Deshpande (28) showed amino acid levels in the blood of 79 ± 12 and 125 ± 5 mg% for normal and irradiated (600R:24 hrs post-irradiation) rats. We have determined the blood serum and liver amino acid levels for rats at various times after exposure to 2500R. These data were obtained as ninhydrin-positive material following deproteinization of blood serum and liver homogenates. Data are presented in the accompanying figure. It is evident that both serum and liver amino acid levels were elevated 60-65% at 12 hrs post-irradiation. Both gradually diminished from 12-48 hours during the period of maximum AIB uptake and glycogenesis. At 72 hrs transport was impaired as shown by AIB accumulation. Consequently, liver amino acids declined and serum amino acids increased. In the absence of liver amino acids to maintain glycogenesis, the glycogen level also

declined.

Conclusion: Whole-body irradiation degrades peripheral protein leading to an increased serum amino acid level. This may trigger a stimulated transport as shown by the elevated AIB accumulation. Amino acids increase in the liver followed by an elevated gluconeogenesis and glycogenesis. These results will be presented at the Federation Meetings, April 1975 (Kilberg, M. and Neuhaus, O.W., "Effect of Amino Acid Transport on the Accumulation of Liver Glycogen in Rats After Whole-Body Irradiation").

D. Effect of Whole-Body Irradiation on the Composition of the Liver Cell Plasma Membrane

Plasma membrane from normal rats has been separated successfully and assays of 5'-nucleotidase were performed. Our recent efforts have not been extended as yet to the livers of irradiated rats.

PROJECT II: HEPATIC SYNTHESIS OF SPECIFIC PLASMA PROTEINS: α_{2u} --THE SEX-DEPENDENT URINARY PROTEIN OF THE ADULT MALE RAT

A. Studies on the Effect of Whole-Body Irradiation on the Hepatic Synthesis of Acute-Stress Proteins

No further studies have been conducted in this area. In a previous Progress Report (COO-1754-8) I indicated that whole-body irradiation did not inhibit the injury-induced production of the seromucoid fraction of the plasma protein.

B. α_{2u} --The Sex-Dependent Urinary Protein of the Adult Male Rat

1. *Multiple Forms of the Sex-Dependent Protein.*

The α_{2u} prepared from urine migrates in an immunoelectrophoretic system as a single component while on acrylamide gel from 5 to 6 species were observed (6, 13). Isoelectric focusing was used to separate these species into individual forms having isoelectric points of 5.0 to 5.8 (11). Efforts are underway to develop an improved procedure whereby these individual species may be isolated in higher recovery.

a. Improvements in isolation and characterization. α_{2u} was originally isolated by processes involving concentration of the urine against Carbowax, $(NH_4)_2SO_4$ fractionation, precipitation by exhaustive dialysis against water, and gel filtration (Sephadex G-100). A major peak (Peak B) was obtained, free of carbohydrates and albumin, which contained the sex-dependent proteins; the recovery of sex-dependent protein was only 4% by immunoassay (11). Modification of this procedure increased the recovery (ca. 9%) but resulted in contamination with albumin. Since these preparations contained predominantly the 5.2 or parent species, it was thought that the procedure selectively concentrated this species at the expense of the others. To get at the 96% of α_{2u} lost and to obtain large amounts of the other isoelectric species (especially the 5.8), the isolation procedure was completely revised. The following is now being used:

Concentrated, dialyzed, and lyophilized urinary protein was fractionated twice with $(NH_4)_2SO_4$ (50-75%; 65-75%) followed by gel filtration on

Sephadex G-100. The fraction obtained was free of albumin, had little carbohydrate, and yielded an overall recovery of approximately 40%. Passage through CM-Sephadex (pH 5.0, discontinuous gradient, 0.1 to 1 M ammonium acetate) removed non-sex-dependent proteins of isoelectric point lower than 5 as well as residual carbohydrates. Two sex-dependent protein fractions were eluted, 1) enriched in 5.2 species (α_{2u} /protein = 1.3) in 20% recovery and, 2) enriched in 5.8 species (α_{2u} /protein = 1.4) in 16% recovery. These were subsequently fractionated on DEAE Sephadex (pH 5.8 continuous sodium chloride gradient, 0-400 mM in 5 mM potassium phosphate buffer). The DEAE fractions were then separated by preparative acrylamide gel electrophoresis at pH 8.3-8.4 (29). Both the 5.2 and 5.8 species are being accumulated for further studies. The α_{2u} 5.2 species is currently being used to produce a specific antiserum.

b. Renal modification of α_{2u} . As a working hypothesis, based on the apparent distribution of isoelectric species in kidney and urine (13), I propose that the 5.2 and 5.0 species are reabsorbed by the renal tubules while the 5.2 to 6.0 species are excreted in the urine. Perhaps this discrimination between retained and excreted forms is fundamental to the physiological function of α_{2u} . To test this hypothesis, the changes in renal and urinary α_{2u} were studied under circumstances in which synthesis was totally curtailed by adrenalectomy (10). As the α_{2u} synthesis was suppressed, the urinary α_{2u} profile appeared to change in favor of the 5.4 and 5.8 species with little of the parent 5.2 species being excreted. However, determinations of total α_{2u} showed that as the hepatic production decreased, the concentrations in serum, kidney, and urine also declined. There appeared to be no selective retention in the kidney at the expense of the urinary excretion. The changes in profile observed are inconclusive because of difficulties in aligning acrylamide gels. No progress has been made on this project. I plan to use affinity chromatography (30) to extend these studies as soon as we have the Sepharose-anti- α_{2u} in hand.

2. *Effect of Diet on the Synthesis and Excretion of α_{2u}*

In the previous Progress Report (C00-1754-12) results were described regarding the effect of dietary protein on the biosynthesis and excretion of α_{2u} . In fasted rats the excretion of α_{2u} was reduced from a normal of 10-15 mg/24 hrs to 1 mg within 36 hrs. To test the importance of dietary protein, either on the hepatic synthesis of α_{2u} or its renal excretion, experiments were performed comparing the excretion on a normal diet (20% casein) and that on a protein-free diet (0% casein) and a high protein (50% casein) diet. Rats on a 20% casein diet excreted normal levels (10-15 mg α_{2u} /24 hrs) while those on 0% casein excreted only 0.5-1.5 mg/24 hrs. When the regimen was reversed, the repleted rats rapidly increased their excretion to normal while the newly depleted ones no longer excreted a significant amount. Simultaneously the percentage of α_{2u} in TUP decreased from 30% to less than 10% again showing that dietary depletion had an important effect on the biosynthesis of α_{2u} . To confirm the effect of the 0% casein diet on the hepatic α_{2u} production, the plasma accumulation of α_{2u} was measured in nephrectomized rats. On a 0% casein diet the blood level was 2.7 mg% compared with 5.7 mg% (20% casein). Four hours after nephrectomy the level was 9.5 mg% (0% casein) compared with 34 mg% (20% casein). In another experiment, rats on a 20% casein diet were transferred to the 0% diet and then were nephrectomized after 0, 1, 3, 5, and 10 days. The accumulation of α_{2u} in the nephrectomized rats declined with remarkable rapidity as the depletion time increased. No such change was observed in serum albumin levels (31). Apparently in the event of an amino acid deficiency, the α_{2u} production is expendable and curtailed at the hepatic level. Miller (32) has suggested a biological priority among plasma proteins synthesized in the liver. It appears that the α_{2u} protein enjoys a very low biological priority. The total urinary proteins declined from a normal of 45-50 mg to 5-10 mg on the 0% casein diet.

In a parallel experiment rats were placed on 50% casein; the level of $\alpha_2\text{u}$ increased from a normal of 10-15 mg/24 hr to 30-35 mg/24 hr. Restoration of the 20% casein diet reduced the excretion to normal. The accumulation of $\alpha_2\text{u}$ in the blood plasma of nephrectomized rats kept on a 50% casein diet was the same as in the normals on a 20% casein diet. In accord with earlier studies (33, 34) we observed an increased excretion of total urinary proteins, from 50-60 mg/24 hrs to 140-170 mg/24 hrs. However, the percentage of $\alpha_2\text{u}$ in TUP remained in the normal range (25-30%). The normal percentage of $\alpha_2\text{u}$ in total urinary protein as well as the normal accumulation of blood $\alpha_2\text{u}$ following nephrectomy showed that the elevated excretion of $\alpha_2\text{u}$ on the high protein diet reflected an increased loss of plasma proteins and not an increased synthesis of sex-dependent protein. This conclusion is supported by the fact that the total output of urine was approximately doubled and the ratio of $\alpha_2\text{u}$ /creatinine was almost doubled. To establish if the biosynthesis of $\alpha_2\text{u}$ is responsive to a deficiency of specific amino acids, studies were undertaken using zein as a source of protein instead of casein. Zein supplemented with lysine and tryptophan was also used. Adult male rats on the zein diet showed a marked reduction of $\alpha_2\text{u}$ excretion from 10-15 mg/24 hrs to 0.5-1.0 mg/24 hrs. Supplementation with lysine (1.7% total diet) and tryptophan (0.24% total diet) increased the excretion to 5-10 mg/24 hrs. Determination of the accumulation of $\alpha_2\text{u}$ in the plasma of nephrectomized rats kept on zein and supplemented diets showed that the zero time level on zein alone was 17 $\mu\text{g/ml}$ compared with a normal of 30 $\mu\text{g/ml}$. After 4 hrs the rats fed zein showed 50-80 $\mu\text{g/ml}$ compared with 200-250 $\mu\text{g/ml}$ in 20% casein-fed rats. The rats fed diets of zein supplemented with lysine and tryptophan exhibited intermediate levels, namely, 21-27 $\mu\text{g/ml}$ at 0 time and 105-190 $\mu\text{g/ml}$ 4 hrs post-nephrectomy. These data show that diets deficient in lysine and tryptophan are not adequate for the biosynthesis of $\alpha_2\text{u}$. Zein contains a large concentration of leucine, therefore, our results may also reflect an inhibitory effect of an imbalance in amino acids (35).

Conclusion: The hepatic synthesis and urinary excretion of $\alpha_2\text{u}$ is dependent on an adequate protein diet. An amino acid deficiency results in a suppression of $\alpha_2\text{u}$ synthesis. A 50% casein diet yields an increased excretion; however, this is caused not by an increased synthesis but by an increased output of urine and a consequent increase in the loss of total proteins.

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