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

FINAL REPORT

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ABSTRACT

Whole-body exposure of rats to ionizing radiations yielded an increased incorporation of labeled amino acids into serum albumin in *in vivo* studies suggesting a stimulation of biosynthesis. Actually this may have been caused by an elevated hepatic transport of labeled amino acids (see below). A suppressed biosynthesis of albumin was observed when the experiments were performed *in vitro* using liver microsomes. Impaired biosynthesis appeared to be caused by a reduced mRNA production. Irradiation stimulated the biosynthesis of acute-phase plasma proteins (stress response) and inhibited the excretion of $\alpha_2\mu$ -globulin, the sex-dependent protein of the adult male rat.

Exposure of rats to γ -rays stimulated amino acid transport into the liver. This process which is Na^+ and energy-dependent was studied with α -aminoisobutyric acid, cycloleucine, and L-methionine among others. After irradiation the serum glucagon and insulin, as well as hepatic cAMP levels, were elevated. Amino acid transport may be an important factor in controlling the increased gluconeogenesis and glycogenesis observed in rats following whole-body irradiation.

PROGRAM GOALS

The primary goal of this program was to study the effects of whole-body exposure to ionizing radiations on the biosynthesis of plasma proteins in the liver. This program was divided into two phases which will be described individually as:

- PROJECT I. EFFECT OF WHOLE-BODY EXPOSURE TO IONIZING RADIATIONS ON THE HEPATIC BIOSYNTHESIS OF PLASMA PROTEINS; ACUTE-PHASE PROTEINS, SERUM ALBUMIN, AND $\alpha_2\mu$ -GLOBULIN, THE SEX-DEPENDENT URINARY PROTEIN OF THE ADULT MALE RAT
- PROJECT II. EFFECT OF WHOLE-BODY EXPOSURE TO IONIZING RADIATIONS ON THE HEPATIC TRANSPORT OF AMINO ACIDS

STATEMENT OF ORIGINAL OBJECTIVES: RATIONALE

In my original proposal of 1966, I called attention to the inconsistencies in the literature regarding the effect of whole-body exposure to ionizing radiations on the hepatic biosynthesis of plasma proteins. The original intent of this program was to describe the effects of whole-body irradiation on the biosynthesis of such proteins as serum albumin and to ascertain why there was no consensus as to the effects of irradiation on its hepatic biosynthesis. A review of the literature suggested that those investigations based on *in vivo* studies demonstrated a stimulation while those using *in vitro* systems showed an impaired synthesis of protein. Our initial objectives, therefore, were to verify this supposition and to resolve the reasons for this discrepancy.

PROJECT I: EFFECT OF WHOLE-BODY EXPOSURE TO IONIZING RADIATIONS ON THE HEPATIC BIOSYNTHESIS OF PLASMA PROTEINS

A. *Effect on Biosynthesis of Serum Albumin*

1. *In vivo* studies: In these the incorporation of ^{14}C -labeled amino acids into serum albumin was measured in rats exposed to varying

doses of X-rays (670 - 896 R). ^{14}C -labeled algal hydrolysate was injected 0, 4, 5, 6 and 10 days later and 1 hour prior to sacrifice. Proteins were precipitated from blood samples with 0.6 N HClO_4 ; the albumin was extracted with alcoholic trichloroacetic acid from the bulk of the proteins. Albumin was also obtained from the hepatic microsomal fraction, prepared by differential centrifugation of a liver homogenate, sonicated to liberate nascent proteins. Labeled albumin was extracted from the HClO_4 -precipitated proteins in the presence of ^{12}C -albumin as a carrier. In both serum and microsomal albumin there appeared to be an increased labeling as measured in terms of specific activity. Starvation produced no comparable effects.

2. *In vitro* studies: Microsomal fractions were prepared from liver homogenates of rats 4 days following exposure to 1300 R. Microsomes and post-microsomal supernatants (cell sap) were then incubated for 60 minutes in the presence of an energy generating system and ^{14}C -leucine. At the end of the incubation, the reaction was stopped with 0.6 N HClO_4 . Albumin was extracted from the HClO_4 -precipitate with alcoholic-trichloroacetic acid. The incorporation of leucine into albumin was reduced by 50%. However, it was of importance that the irradiated rats consumed only 30% as much food as the normals. A reduction in food provided to controls caused a comparable decrease in the incorporation of leucine into albumin. We were unable to demonstrate any changes in the endogenous pools of free amino acids of the liver pursuant to the treatment with X-rays. Further *in vitro* studies were performed in which the microsomes from irradiated rats were incubated with cell sap from normal controls and vice versa. In such crossover experiments it became apparent that the defect resided in the

liver microsomes. Starvation alone suppressed the synthesis of albumin. Possible defects in the ribosomes were studied by measuring the incorporation of ^{14}C -phenylalanine using poly U for mRNA. Whole-body irradiation, however, did not affect the ability of the ribosomes to incorporate phenylalanine in the presence of added mRNA (poly U). If the ribosomes were normal, how could the reduced albumin synthesizing capacity of the microsomal fraction be explained? Studies were initiated in which the incorporation of ^{14}C -orotic acid was measured into polysomal RNA. Whole-body irradiated rats (4 days post 1300 R) were given ^{14}C -orotic acid 45 minutes before sacrifice. A polysomal fraction was separated from the homogenate, treated with 5% deoxycholate, by sucrose-gradient ultracentrifugation. Total polysomal RNA was removed by extraction. Irradiation decreased the specific activity of the total RNA from the polysomes by 37% whereas starvation had no effect.

B. *Effect on the Biosynthesis of Acute-Phase Proteins*

1. Stimulation of the seromucoid fraction by whole-body irradiation: The stress responsive plasma proteins or acute-phase proteins were measured as the perchloric acid-soluble, phosphotungstic acid precipitable fraction or the so-called "seromucoid" fraction. Blood samples were collected at various times post-exposure to X-rays (745 R). ^{14}C -labeled a)gal hydrolysate was injected 1 hour prior to sacrifice. The seromucoid fraction showed a transient increase in concentration as well as in specific activity with time after exposure. At 4 days post-irradiation the rate of seromucoid production appeared to be 3 times the normal. There is an apparent contradiction here in that the synthesis of acute-phase proteins was stimulated by irradiation while that of albumin was suppressed.

2. Potential inhibition of the biosynthesis of acute-phase proteins by whole-body irradiation: As indicated previously, exposure of rats to large doses (1300 R) interfered with the biosynthesis of serum albumin, apparently at the level of the transcription of mRNA. If exposure to this dose of radiation has the general effect of inhibiting plasma protein synthesis, it should also have a comparable effect on the acute-phase proteins. However, the results could be complicated by the stress-response itself. To test if irradiation might inhibit seromucoid synthesis, we tested the system on pre-injured rats which were either already stimulated to produce stress proteins or were about to be. The biosynthesis of the seromucoid fraction was stimulated by surgical injury. Rats were exposed to γ -rays (1300 R) at various times before or after surgery. ^{14}C -glycine was injected 2 hours prior to sacrifice; samples of blood plasma were precipitated with 0.6 N HClO_4 . The soluble seromucoid fraction was then precipitated with phosphotungstic acid. As expected injury increased the labeled seromucoid fraction 5-fold in 24 hours post-injury. Irradiation at 8, 4, and 2 hours prior to injury and 2 and 4 hours post-injury had no effect on the production of the seromucoid fraction. Therefore, whole-body exposure to γ -rays had no apparent inhibitory effect on the stimulated biosynthesis of plasma proteins.

C. *Effect on the Biosynthesis of α_{2u} -Globulin*

To study further the potential effect of whole-body irradiation on hepatic protein biosynthesis, a more productive system should involve a single well-defined protein whose biosynthesis could be controlled by hormones in a selective manner. To this end, we extended our studies to the sex-dependent α_{2u} -globulin of the adult male rat. This protein is

synthesized in liver, secreted into the bloodstream, concentrated in the kidneys, and excreted in the urine. Its biosynthesis and excretion begin with puberty (ca 40 days of age).

1. Hormonal control: Studies were conducted on the hormonal control of the biosynthesis and excretion of $\alpha_2\text{U}$ -globulin. Castration of adult male rats resulted in a 50% reduction in the excretion of $\alpha_2\text{U}$; supplementation with testosterone and other androgenic steroids restored its excretion to normal. Since some androgens may be of adrenal origin, rats were also adrenalectomized. Removal of adrenals alone led to a total cessation in urinary excretion of $\alpha_2\text{U}$ indicating that other adrenal hormones are important besides the androgenic ones. Replacement therapy with hydrocortisone restored the excretion; aldosterone was without effect. Castration and adrenalectomy suppressed the excretion of $\alpha_2\text{U}$; neither androgen nor glucocorticoid alone could restore the excretion of this protein. Supplementation with both hormones restored the normal level of excretion. It was concluded that glucocorticoid acts synergistically with androgen in controlling the hepatic synthesis of $\alpha_2\text{U}$.

2. Preliminary studies on the effects of whole-body irradiation on the excretion of $\alpha_2\text{U}$: At one day post-exposure to γ -rays (1300 R) the total excretion of $\alpha_2\text{U}$ was reduced by 80%. Normally the $\alpha_2\text{U}$ represents 32% of the total excreted urinary protein while in the irradiated rat the $\alpha_2\text{U}$ level was only 13% of the total. At 4 days post-irradiation the urinary $\alpha_2\text{U}$ was only 3%. However, these results are of dubious value because of problems of renal shutdown. Irradiated rats were also bilaterally nephrectomized and the blood level or accumulation of $\alpha_2\text{U}$ was measured immunologically. After 4 hours the blood serum level increased from a

normal of 3 mg% to 18 mg% compared with nephrectomized non-irradiated control rats in which it increased from 3 mg% to 30 mg%. However, there must have been extensive loss of plasma proteins from the damaged intestinal mucosa which could not be evaluated. Therefore, we could not establish if the effect of irradiation was on the hepatic biosynthesis of α_{2u} .

3. Importance of diet in controlling the biosynthesis of α_{2u} -globulin: A major concern in studying the effect of whole-body irradiation is the effect of starvation or reduced food intake on hepatic protein synthesis. In fasted rats the excretion of α_{2u} -globulin was reduced within 36 hours from a normal of 10-15 mg/24 hours to 1 mg. To test the importance of dietary protein, either on the hepatic synthesis of α_{2u} or its renal excretion, experiments were performed using rats kept on a normal 20% casein diet, a protein-free or 0% casein diet and a high protein (50% casein) diet. Rats on a 20% casein diet excreted 10-15 mg α_{2u} /24 hours while those on a 0% casein diet excreted only 0.5-1.5 mg/24 hours. The percentage of α_{2u} in total urinary protein declined from a normal of 35% to a minimum of 7%. When the depleted rats were bilaterally nephrectomized, the serum levels for α_{2u} increased from 2.7 mg% to 9.5 mg% while in rats on a 20% casein diet the levels increased from 5.6 mg% to 34 mg%. Therefore the 0% casein diet resulted in a sharp decline in the biosynthesis of α_{2u} . Similar results were obtained on 20% zein diets. Supplementation with L-lysine and L-tryptophan nearly restored the biosynthesis of α_{2u} to normal levels. Rats on a 50% casein diet increased the α_{2u} excretion over 100% namely from 10-15 mg/day to 30-35 mg/day; however, the total urinary protein increased proportionately and therefore the ratio of α_{2u} in total urinary protein remained normal. Our data show that any reduced synthesis of α_{2u} pursuant to whole-body exposure to γ -rays can be ascribed to a

reduced food intake.

RESUMÉ

Our studies of the effect of whole-body exposure to ionizing radiations on the biosynthesis of serum albumin corroborated the problems of interpretation evident from the earlier literature. Stimulation was observed in *in vivo* studies; impairment of synthesis was observed in *in vitro* studies. It appears, however, that the actual suppressive effect of irradiation was on the production of mRNA; the defect occurred at the level of transcription. The results obtained *in vivo* might be explainable on the basis of an increased transport of amino acids into the liver cell thereby increasing the specific activity of the intracellular amino acid pool. Increased specific activity would result in an increased labeling of albumin as well as all hepatic protein. To demonstrate that amino acid transport was a factor, a preliminary study was initiated in which ^{14}C -labeled algal hydrolysate was injected into an irradiated rat at various times post-exposure (1300 R); the rats were sacrificed 1 hour later. The liver was homogenized and counted. The accumulation of ^{14}C -amino acids was increased by 80% 4 days after irradiation.

At this point a critical reappraisal of our direction was required. One course of action would require an in depth study of the effect of whole-body irradiation on the transcription of RNA while another would mean a complete changeover to a study of amino acid transport and its response to irradiation. There were a number of reasons why the latter course of action was chosen.

1. Practically no work had been reported on the effect of whole-body irradiation on amino acid transport in tissues. In fact, little was

known concerning amino acid transport and its control in liver cells.

2. Amino acid transport would serve as an important regulatory process for controlling the biosynthesis of plasma proteins. In fact amino acid transport should influence hepatic metabolism in general following whole-body irradiation.

3. It would be preferable to study a stimulation rather than a metabolic defect. Stimulation of transport would allow a study of the responsible factors, *e.g.*, hormones.

4. The area of the biosynthesis of RNA had progressed to such a point that we no longer had the necessary capacity to contribute effectively in this field.

PROJECT II: EFFECT OF WHOLE-BODY IRRADIATION ON THE HEPATIC TRANSPORT OF AMINO ACIDS

A. *Characteristics of Hepatic Amino Acid Transport*

1. *In vivo* studies: The uptake of metabolizable amino acids in an *in vivo* study is difficult to assess. In many instances the intracellular metabolism is simply too great. For this reason we used ^{14}C - α -aminoisobutyric acid (AIB) a non-metabolizable analogue of alanine. AIB is thought to be carried by the so-called A or alanine-preferring system. Therefore, AIB may monitor a transport system shared by serine, threonine, glycine, proline, asparagine, glutamine, and alanine. The elevated accumulation of ^{14}C -amino acids observed using algal hydrolysate was confirmed using ^{14}C -AIB. Four days following exposure to 1300 R, the accumulation of AIB in the rat liver was approximately 7 times the normal; uptake in kidneys, spleen, muscle, and testes was not affected or suppressed. Similar studies were also performed in rats subjected to surgical stress

and adrenalectomy. Sham adrenalectomy caused a stimulation in uptake reaching a maximum 8 hours following injury. Adrenalectomy delayed the stress response to 48 hours post-operation.

To determine if AIB transport reflects a general tissue sensitivity to irradiation, we studied the accumulation of ^{14}C -AIB by various tissues at different doses (500 - 2500 R) and times post-irradiation. Of the tissues tested (liver, kidneys, musculature, testes and spleen) only the liver and kidneys exhibited an elevated accumulation of AIB. In the liver the uptake of AIB increased in a linear fashion throughout 72 hours post-exposure to 500, 1000, and 1500 R. After exposure to 2000 and 2500 R, the response was transient reaching maximal uptake 48 hours post-exposure and being reduced to near normal values after 72 hours. It is significant that the only tissues responding were the most radio-resistant and were those capable of gluconeogenesis. The significance of this observation will be evident in section II B. The transient stimulation of AIB uptake was not the consequence of a reduced food intake; pair-fed animals did not exhibit a comparable effect.

2. In vitro studies: The increased uptake of amino acid could have been the consequence of physiologic changes other than transport. For this reason it was essential to develop *in vitro* experiments using livers from irradiated rats versus unirradiated, starved controls. In these studies livers were sliced (0.4 mm thickness) and incubated in the presence of ^{14}C -AIB. After 1 hour of incubation the ^{14}C -AIB Distribution Ratios (cpm intracellular fluid/cpm in extracellular fluid) were 2 - 2.5 times greater in livers from irradiated rats compared with the normal. Furthermore the stimulated uptake was Na^+ - and energy-dependent. An active Na^+ -pump was implicated because the addition of the glycoside ouabain inhibited

the stimulated uptake.

Kinetic studies were performed using both Na^+ -containing and Na^+ -free incubating media. These studies established the V_{max} for AIB transport in livers of irradiated rats to be 42.3 ± 5.2 $\mu\text{moles/ml/hr}$ compared with 19.5 ± 2.9 for liver slices from normal (unirradiated) rats. The K_m values were 11.6 ± 2.5 mM and 10.0 ± 1.9 for normal and experimental livers. Therefore, there was no difference in K_m for the two systems. Irradiation had no effect on AIB transport in the absence of Na^+ . Furthermore, the transport of the model amino acid BCH (2-aminobicyclo(2,2,1)heptane-2-carboxylic acid) which monitors the L or leucine-preferring, Na^+ -independent transport, was not affected. Cycloleucine and L-methionine, transported by both the A and L-systems, were increased following whole-body irradiation. Again the stimulation was observed only in the presence of Na^+ . It was the Na^+ -dependent component of the overall transport which was affected. On the other hand, phenylalanine, leucine, and histidine, which also showed considerable Na^+ -dependent as well as Na^+ -independent transport were not stimulated following whole-body irradiation. These results suggested the existence of both inducible and non-inducible Na^+ -dependent systems.

Methionine was added in excess to a system for the transport of ^{14}C -AIB to study the potential action of the natural amino acid as a competitive inhibitor for the model amino acid. Methionine competitively inhibited all of the Na^+ -dependent AIB transport in liver slices from normal and irradiated rats. This suggests that the Na^+ -dependent transporter carries both amino acids. If this were true, AIB also should have inhibited ^{14}C -L-Met transport. However, an excess of AIB only inhibited the stimulated transport of Met. Therefore, the stimulated, Na^+ -dependent transport system is the same for both amino acids. We propose the existence of

radiation-inducible and non-inducible Na^+ -dependent transport systems.

In another study of the hepatic transport system, we compared the uptake of ^{14}C -AIB, ^{14}C -cycloleucine (CL), ^{14}C -L-methionine and ^{14}C -phenyl-alanine in the presence of competitive inhibitors using liver slices from normal and irradiated rats. For example, the transport of AIB was measured in the presence of CL, Met or Phe, etc. AIB, CL, and Met inhibited each other's stimulated transport. Although the uptake of ^{14}C -L-Phe was not affected by irradiation (non-inducible, Na^+ -dependent), it served as an inhibitor for the stimulated transport of the others. This indicates that Phe can bind to the stimulated transporter but is itself not transported. It appears that the radiation-stimulated system carries AIB, CL, and Met by a common, Na^+ -dependent process.

The role of Na^+ in the radiation-induced transport is still unknown. One explanation involves a co-transport of Na^+ and amino acids. In this hypothesis the actual energy requirement for transport is the maintenance of a Na^+ gradient via the function of a sodium-potassium pump. The stimulated amino acid transport pursuant to whole-body irradiation could involve, among other factors, a disturbance in the gradient characterized by an increased efflux of K^+ accompanied by an increased influx of Na^+ . This would continue only so long as a downhill Na^+ -gradient persists. To maintain such a gradient would require a sodium pump, namely, a membrane-bound Na^+ , K^+ -ATPase system. Of the two constituent ATPase reactions, namely,

reaction 2 is discharged by the glycoside ouabain. As already indicated the radiation-stimulated transport is inhibited by ouabain indicating the

need for the Na^+ , K^+ ATPase. Preliminary measurements of ATPase in preparations of plasma membranes isolated from livers of normal and irradiated rats showed no compensatory increase in enzyme activity in the latter. Furthermore, no increased influx of Na^+ could be demonstrated using $^{22}\text{Na}^+$ nor could a stoichiometric relationship be established relating AIB transport to Na^+ concentration using liver slices from irradiated rats. At this point we conclude that Na^+ is required in some way for the radiation-stimulated uptake of AIB but the sodium gradient probably does not supply the driving energy. The importance of the ouabain-sensitive ATPase to the stimulated AIB uptake remains unknown.

B. *The Hormonal Regulation of Amino Acid Uptake and its Impact on Hepatic Metabolism*

An enhanced gluconeogenic and glycogenic state in rats following whole-body irradiation is a well documented phenomenon. It is thought that radiation induces a general degradation of peripheral protein leading to elevated blood amino acid levels. An elevated incorporation of ^{14}C -labeled L-alanine into blood glucose and hepatic glycogen shows that whole-body irradiation increases gluconeogenesis and glycogenesis.

Since the control of gluconeogenesis/glycogenesis may be dependent on substrate concentration, it seemed plausible that an important point of regulation should be the transport of amino acids. As indicated above the radiation-induced amino acid transport affected the Na^+ -dependent A or alanine preferring system which carries such glucogenic amino acids as alanine, serine, threonine, proline, glycine, glutamine and asparagine.

The accumulation of hepatic glycogen and the incorporation of carbon chains from ^{14}C -L-alanine exhibited the same time-dose profiles as did the uptake of AIB. The blood serum and liver amino acid levels were also

determined in rats following exposure to 1500 and 2500 R. Both serum and liver amino acid levels were elevated 60-65% at 12 hours post-irradiation; both gradually diminished from 12-48 hours during the time of maximum AIB uptake and glycogenesis. At 72 hours post 2500 R the AIB transport was impaired, consequently the liver amino acids declined and the serum levels increased. In the absence of liver amino acids to maintain glycogenesis, the glycogen level also declined. There appears, therefore, to be a time-dose relationship between AIB transport, serum and liver amino acid levels and glycogenesis.

Since it is well known that AIB transport can be triggered by glucagon, it was desirable to measure the effect of whole-body irradiation on the blood levels of this and other hormones. Plasma levels of growth hormone, corticosteroid, insulin, and glucagon (all of which are able to stimulate hepatic amino acid transport) were determined for the first 72 hours after exposure to 1500 and 2500 R. Plasma glucose and hepatic cAMP content were also measured. Corticosteroids and growth hormone were not changed by exposure to γ -rays. Glucagon was increased 5-fold in 47 hours after exposure to 2500 R, while no significant elevation was seen after 1500 R. Between 48 and 72 hours after 2500 R the levels declined whereas the content increased after 1500 R. Insulin was increased following both doses for the first 48 hours post-irradiation. At 72 hours, however, the insulin levels continued to increase after 1500 R while those after 2500 R decreased to control values. Hepatic cAMP levels followed a pattern similar to glucagon. The exact role of glucagon and/or insulin in mediating the radiation-induced amino acid transport is under investigation.

In the course of our studies regarding the role of hormones in mediating

amino acid transport, we considered a parallel but related question, namely, whether or not AIB transport might also be affected in experimental diabetes. Rats were made diabetic by treatment with alloxan. AIB uptake was stimulated in the diabetic rat following a pattern parallel to the hyperglycemia. Subsequent treatment with insulin restored both the hyperglycemia and AIB uptake to normal levels.

RESUMÉ

Whole-body exposure of rats to ionizing radiations appears to cause a degradation of peripheral proteins and a concomitant rise in serum amino acid levels. Consequently glucagon and/or insulin are released which in turn stimulate the hepatic amino acid transport. Stimulated transport is Na^+ and energy-dependent and involves the so-called A or alanine-preferring system which may carry primarily glucogenic amino acids. An increased hepatic amino acid pool plus the action of glucagon leads to a stimulation in gluconeogenesis and glycogenesis.

GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS

A. Graduate students trained:

1. Zakariya K. Shihabi: Ph.D., thesis: "Effects of ionizing radiation on albumin biosynthesis and cell permeability", June 1970.
2. Robert J. Pattison: M.A., thesis: "Isolation and characterization of liver parenchymal cells from injured and adrenalectomized rats", June 1970.
3. Kao-Pao Yang: M.A., thesis: "Effect of whole-body γ -irradiation on amino acid transport in rat liver slices", January 1971.
4. Michael S. Kilberg: M.A., thesis: "The effect of amino acid transport on the accumulation of liver glycogen in rats after whole-body irradiation", May 1975. Ph.D., 1973 to present.

B. Postdoctoral fellows:

1. J. Foster Irwin, Ph.D., November 1969 through August 1971.
2. Wayne Flory, Ph.D., May 1973 to present.

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