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MASTER

"EFFECT OF WHOLE BODY X AND γ -IRRADIATION
ON THE BIOSYNTHESIS OF CERTAIN SERUM PROTEINS"

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In our original proposal we suggested that whole body X (or γ) irradiation might serve as a unique system for studying the nature of control mechanisms involved in the hepatic biosynthesis of plasma proteins in response to stimulation. The compelling interest in using ionizing radiation was to explain an apparent dichotomy of effects of radiations in which albumin synthesis was suppressed while at the same time the production of acute-phase proteins was stimulated. It is hoped to use pharmacological doses of glucocorticoids to reverse the suppressed synthesis of albumin. New knowledge concerning the effect of glucocorticoids on protein synthesis would be gained if the radiation-suppressed albumin synthesis could be reversed in this manner.

Studies using liver perfusion of X-irradiated rats, however, suggested that the inhibition of albumin synthesis was primarily related to inanition (1). On the other hand ionizing radiations do affect the biosynthesis of RNA and could be expected to have a profound inhibitory effect on protein synthesis because of a deficiency of messenger RNA (2).

Some of the following studies are a continuation of those reported previously (COO-1754-1) and are intended to clarify the role of irradiation versus inanition. Most of these studies were conducted by Mr. Zakariya Shihabi in partial fulfillment of his requirements for the Ph.D. degree. Initial X-irradiation studies were conducted with the collaboration of Dr. J.P. Steele, Chief Radiologist, Sacred Heart Hospital, Yankton, South Dakota. More recent studies were performed in collaboration with Dr. Wm. Thoman, Radiologist, St. Vincent's Hospital, Sioux City, Iowa, using a Cobalt - 60 therapy unit. We now possess a Gammator Model 38-1, gamma irradiator (Radiation Machinery Corp.), containing 800 curies of Cesium - 137. This apparatus is extremely useful for the irradiation of small animals.

I. IN VITRO STUDIES: Liver microsomal fractions from normal and X (and γ) irradiated rats were used to extend the original work of Zamyatkina with liver slices (3). ^{14}C -leucine incorporation was measured using microsomal and cell sap fractions prepared by differential ultra-centrifugation as described by us (4). These mixtures were incubated at 37° C for 1 hour. The reactions were stopped with 0.6 N HClO_4 and the albumin was extracted from precipitates with ethanol-trichloroacetic acid. The data reported previously (COO-1754-1) were obtained using Sprague-Dawley rats from a single supplier. In the course of this study the quality of these rats deteriorated making it necessary to change to a new source of animals. Consequently all previously reported work was repeated. In general the data were

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the same as reported but the new rats were more resistant to irradiation, necessitating somewhat higher dosages.

A. Subcellular fractions were prepared from rats irradiated at dosages of 700, 900, 1200, 1300 and 1500r. All X-irradiations were performed at 220 KV, 15 ma. using a 4mm. Al. filter. At the lowest level (700r) the amino acid incorporating capacity was elevated 10% at 2 days and suppressed 6%, 4 days post irradiation. At the higher dosages the amino acid incorporating capacity was clearly suppressed, e.g. 56% at 1300r 4 days post irradiation. These results are similar to those reported previously (COO-1754-1) and are in accord with those of Zamyatkina (3). However, control animals pair-fed to the 1300r experiment likewise showed an inhibition of 60% suggesting that the effect was primarily one of reduced food intake and would be in accord with the report of John and Miller(1). It appears unlikely at this time that such studies using amino acid incorporation into subcellular systems can distinguish the effect of starvation from a presumed special effect of the ionizing radiation.

B. Crossover studies using liver microsomal fractions from irradiated (745r, 6 days; 896r, 4 and 6 days; 1300r 4 days), normal and pair-fed rats confirmed our earlier conclusions that irradiation affected primarily the microsomal fraction (av. 50% reduction). However, microsomal fractions from pair-fed rats likewise showed this degree of suppression. Microsomes from normal rats incubated with cell sap from irradiated or pair-fed animals yielded an average of 87% of the homologous control values.

Comment Although X-irradiation creates an apparent functional defect in the microsomal fraction, this may actually occur as a result of a depleted food intake. In either event the effect may be due to one or more of the following factors:

1. Functional defect in the ribosomes
2. Decrease in m-RNA production
3. Decrease in the aggregation of polysomes responsible for albumin synthesis.

C. Study of a potential functional defect. Microsomal fractions and cell saps from normal and irradiated (1300 r., 4 days) rats were incubated with ^{14}C -phenylalanine in the presence and absence of a synthetic messenger RNA, poly U. The poly U stimulation in the homologous irradiated system was 63% of that with normal controls. Crossover studies using irradiated microsomes and control cell sap showed 132% while normal microsomes plus irradiated cell sap was 70% of the homologous control. This experiment allows 2 conclusions: 1) microsomes from liver of irradiated rats are not defective and 2) irradiation also

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affects the cell sap.

D. Study on "messenger" RNA. In the preceding experiment poly U also caused a 32% increase in phenylalanine incorporation when using irradiated microsomes and control cell sap. This suggests that m-RNA had been stripped from the ribosomes. The cause may be a multiple one beginning with a retardation in RNA transcription (2). The uptake of ^{14}C -orotic acid (45 minute pulse) was measured in total liver polysomal RNA from irradiated (1300r, 4 days) and pair-fed rats. Polysomes were separated from the post-mitochondrial supernatant by centrifugation through a discontinuous 0.5 - 2.0M sucrose gradient. The polysomal pellet was suspended in 0.6 N HClO_4 and the RNA hydrolyzed by 0.2N KOH. Protein was removed by further treatment of the hydrolysate with HClO_4 . The supernatant was then counted and used for determination of RNA. The specific activity of polysomal RNA was reduced 38% and 21%, 2 and 4 days post irradiation, respectively. At the same times, pair-fed rats showed no change in specific activity. Simultaneously nuclei were separated, purified and the nuclear RNA separated by an operation similar to that for the polysomes. The RNA specific activity was reduced 30% and 24%, 2 and 4 days post irradiation. Pair-fed rats showed no significant effect on RNA synthesis. This experiment provides an explanation for the suppressed amino acid incorporating capacity of liver microsomal fractions from irradiated rats. It also distinguishes the effect of the X or γ irradiation from inanition.

E. Effect on the polysomal profile: Conflicting data have been reported in the literature concerning the effect of ionizing radiations on polysomal profiles. It would appear logical that a suppression of RNA synthesis would prevent any increase in polysomal aggregation (5) yet increases in aggregation have been reported. Polysomal fractions, previously separated from the post mitochondrial fraction using a 0.5 - 2.0 M discontinuous sucrose gradient, were then centrifuged at 90,000xg through a 0.5 - 2.0M continuous gradient. The profile was produced by passing the gradient through a continuously recording spectrophotometer (260m μ). A preliminary, qualitative evaluation of these profiles showed an increase in heavy aggregates with a concomitant suppression of the monomer-dimer peaks. Pair-fed rats however, showed the opposite effect, an increase in the monomer-dimer peaks. We have observed, however, that the polysomal pellets from irradiated rats are invariably contaminated with glycogen (6) yielding exaggerated profiles of heavy aggregates. For this reason we have doubts as to the validity of current studies on the

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polysomal profiles of irradiated rats.

II. IN VIVO STUDIES: The synthesis of albumin was also studied in vivo both in terms of albumin bound to liver microsome and as circulating albumin. Rats were irradiated at 700 and 1300 r, 2 and 4 days before sacrifice.

A. Microsomal - bound. Microsomes were separated as before by differential centrifugation and treated with trichloroacetic acid and ethanol. In our previous report we reported a 45% increase 4 days after irradiation with 670r. This study was extended using 700 and 1300r. Two days following 700r the synthesis of albumin appeared to be elevated 42% and 76% for 1300r. In pair-fed rats the change was not significant.

B. Circulating Albumin: The specific activities of albumin 2 days after irradiation at 700 r and 1300 r were increased 40% and 105% respectively. At this time the concentration was reduced only by 8 and 10%. Four days after injury the increases were 11% and 54% while the concentration was reduced 12% and 35% for 700 and 1300r respectively. Pair-fed rats showed no changes either in specific activity or concentration.

Comment. The elevation of specific activities in both the circulating and hepatic microsomal-bound albumin appear to contradict directly the observation of the in vitro studies. It appeared to us that the increase in specific activity might be more apparent than real and not actually reflect an increased synthesis of protein at all. This would be so if irradiation caused a major change in cell permeability leading to a significant increase in specific activity of the hepatic amino acid pool.

C. Amino acid pool: ^{14}C -Algal hydrolysate (1 μC /100 gm body weight) injected intravenously 60 minutes prior to sacrifice. The livers were perfused and then homogenized in 0.44M of sucrose. A post 11,250 rpm supernatant was collected and used for counting. Results were expressed as percentage of radioactivity recovered in the liver (cpm recovered) / (cpm injected) x100. Two days following 700r the recovery was increased 50% while after 1300r it was increased 125%. Pair-fed rats did not show this change. These results confirm our opinion that irradiation stimulates an increase in cell permeability.

D. Studies on cell permeability. Since an effect of whole body irradiation on the permeability of the liver to amino acid has, to our knowledge, never been described, a series of experiments were undertaken to determine the nature

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of this phenomenon. Amino acids do not in themselves serve as a good index for studies of cell permeability because they are rapidly incorporated into protein and/or catabolized. For this reason all of the following studies were undertaken using a non-metabolizable model amino acid, aminoisobutyric acid (AIB). AIB is incorporated into the liver cell by an active transport mechanism (7).

1. Use of AIB: AIB was injected and counted as was the algal hydrolysate (cf. C. above). The incorporation of AIB was also studied at various times following a sham operation. In this case a 225% increase was observed 4 and 8 hours post operation. The rate of cellular incorporation then decreased reaching normal levels 48 hours post operation. The validity of these observations was established in two experiments. First, 4 hour injured and control livers were forced through a 100 mesh nylon screen to produce a suspension of hepatocytes in a buffer- sucrose medium. These cells were centrifuged and washed by resuspension in fresh medium. After sedimentation, the washed cells were suspended and ruptured in 0.1N NaOH. The liver cells from injured rats contained 122% more radioactivity than the normal. In a second experiment, ¹⁴C-labelled Inulin was injected intravenously and the liver was minced, homogenized and centrifuged at 11,250RPM. Prior perfusion was omitted in this instance. Inulin is used to measure extracellular space and is known not to enter the cell. 4 hour-injury had no effect upon the inulin-radioactivity recovered in pulses of 15, 30, or 60 minutes. AIB (60 min. pulse) recovery was increased 190%, 2 days and 670%, 4 days after irradiation with 1300 r. An increase of 70% was obtained at both 2 and 4 days in pair-fed animals.

COMMENT. These data show a major stimulation of hepatocyte permeability by irradiation which is not affected similarly by inanition. Since the permeability to AIB and amino acids generally (algal hydrolysates) is so greatly elevated following irradiation, it is apparent that the specific activity of the amino acid pool, following a pulse label, would be greater than normal. This would be reflected in an increased specific activity in proteins synthesized, thereby accounting for the apparent increased "synthesis" of albumin observed in vivo.

2. Factors affecting permeability. Adrenalectomy greatly suppressed the 4-8 hour stimulation of AIB incorporation. This, however, was a delayed effect. A 160% increase in recovery was observed 48 hours post injury. Reinjury (4 hour) of chronic, 4-day adrenalectomized rats resulted in a 235% increase in AIB recovery indicating that the adrenal gland actually was not required for this response. Neither actinomycin D nor puromycin had any effect on AIB uptake indicating

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that RNA or protein synthesis were not involved. Replacement therapy of adrenalectomized rats with either epinephrine or hydrocortisone caused a significantly stimulated uptake of AIB.

III. Studies are in progress regarding the separation of parenchymal cells from reticuloendothelial cells. Cell suspensions of normal rats have been prepared by maceration of liver through 100 mesh nylon cloth. These suspensions are first placed on a continuous sucrose gradient to separate hepatocytes from RBC's and debris. The hepatocytes are then placed on a second continuous sucrose gradient to separate the cells. Studies in progress involve determinations of numbers of cells, cellular DNA, RNA and total proteins. Preliminary evidence suggest a major increase in the numbers of cells sedimenting in the light layers.

CONCLUSIONS

1. X-irradiation suppresses the amino acid incorporating capacity of microsomal subcellular systems. This effect is also observed in pair-fed rats. Suppression of albumin synthesis was observed to occur primarily in the microsomes and to a lesser degree in the cell sap. The effect is not to restrict the functioning of the microsomes since they act normally with added poly U.

2. Irradiation does inhibit the biosynthesis of liver polysomal and nuclear RNA. This effect is not reproducible with inanition alone.

3. Preliminary results show that irradiation leads to an increase in polysomal aggregation while inanition results in depolymerization.

4. X and γ -irradiation result in a marked increase in the uptake by the liver of α -aminoisobutyric acid showing a marked change in cellular permeability.

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