A NOTE ON THE SYNTHESIS OF FATTY ACIDS IN BONE MARROW HOMOGENATES AS AFFECTED BY X-RADIATION*

by

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In the course of studies on the effect of X-radiation on hemoglobin synthesis in bone marrow homogenates[1], experiments were carried out with the aim to explore the effect of radiation on synthesis of bone marrow fats. Histological observations indicate that starting at the third or fourth day after exposure of the animal to radiation fat begins to fill the marrow cavity to a degree proportionate with the prevailing myeloid hypoplasia. An investigation of the ability of animals previously exposed to X-radiation to synthesize marrow fats is, therefore, of interest inasmuch as such information may shed light upon the question of whether the increased amounts of marrow fat are due to *in situ* synthesis or are derived from fats mobilized elsewhere in the body.

Since it has been shown by GOLDINGER, LIPTON, AND BARRON[2] that bone marrow utilizes acetate, and since fat synthesis in bone marrow slices as well as in homogenates has been demonstrated[3,4], bone marrow homogenates were used in this study on the effect of radiation on fat synthesis. The homogenates were obtained from the marrow of the long bones of rabbits at varying times after exposure to X-rays. In these experiments $^{14}CH_3COONa$*** was used as a fatty acid precursor. The results of this investigation are reported in this paper.

METHODS

Six rabbits weighing 6 to 8 pounds each were used in this investigation. Two rabbits served as controls, whereas the other rabbits received 800 r (250 kv.) total body X-radiation and were sacrificed either immediately, 48 hours, 72 hours, or 1 week after exposure to radiation. Homogenates from the marrow of the long bones were prepared by a procedure described previously[3,5] and were incubated in modified 300 ml Warburg vessels at 38° C for 3 hours with constant shaking. The experimental design permitted manometric measurement of oxygen consumption. The composition of the vessel content is shown in Table I.

After the incubation period the vessel content was high-speed centrifuged at 4° C and the top layer containing the bone marrow fats was removed. The liquid intermediate layer was set aside for

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the isolation of protoporphyrin IX dimethyl ester, and the residue at the bottom of the centrifuge tube was discarded.

TABLE I

COMPOSITION OF VESSEL CONTENT

Additions	Amount added per g wet weight of bone marrow	Final concentrations
Main Compartment		
0.1 *M* Phosphate buffer pH 7.3	2.2 ml	0.0250 *M*
0.5 *M* Glycine	0.2 ml	0.0013 *M*
0.1 *M* Sodium Acetate	0.5 ml	0.0063 *M*
α-^{14}C-Acetate	0.16 μc	0.02 μc/ml
Side Arms		
2 *N* Sulphuric Acid	2.0 ml	
5 *N* Potassium Hydroxide	2.0 ml	

The total fatty acids were isolated essentially as described by KORRODI[6]. A separation of the saturated and unsaturated fatty acids was effected by the method of TWITCHELL as described by HILDITCH[7]. Further purification of the saturated and unsaturated fatty acid fractions was achieved by low temperature fractional crystallization of the fatty acid methyl esters in accordance with the procedures of BROWN AND SHINOWARA[8]. On the basis of saponification equivalents an average molecular weight of 274 and 270 was calculated for the saturated and unsaturated fatty acid fractions, respectively, in the untreated marrows. A determination of iodine numbers revealed that the saturated fatty acid fractions contained no unsaturated components and that the iodine numbers obtained for the unsaturated fractions were in the range usually observed for unsaturated fatty acids.

Carbon dioxide was collected in KOH in one of the side arms of the Warburg vessel and was isolated as $BaCO_3$. In order to compare the response of the animals to radiation with that of another, larger series[1], protoporphyrin IX dimethyl ester was isolated according to GRINSTEIN[9], except that no chromatography was carried out. Carbon-14 activity was determined by means of an ionization chamber as described briefly elsewhere[10].

RESULTS AND DISCUSSION

In order to facilitate the comparison of the biosynthetic capacity of organs with respect to one specific process, and also in order to make possible a comparison of the synthetic capacity with respect to two or more different processes within the same organ, the term W^* has been introduced[1]. It is felt that the term W might be found generally useful in comparing the biosynthetic capacity of different organ systems under various physiological and pathological conditions. On this basis it is then possible to compare saturated and unsaturated fatty acid synthesis in bone marrow homogenates before and after exposure to radiation. The pertinent experimental findings are presented in Table II.

In the untreated animals saturated fatty acid synthesis proceeds somewhat faster than unsaturated fatty acid synthesis. This is in agreement with the findings of PIHL AND BLOCH[11] for liver slices although the difference between the incorporation of ^{14}C into saturated and unsaturated fatty acids is considerably smaller in the case of the bone marrow homogenate. The relationship between saturated and unsaturated fatty acid synthesis changes considerably during the post-radiation period. Immediately after radiation there is a marked increase in fatty acid synthesis similar to the increase in

* Definition and calculations are given in the Appendix.

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TABLE II

Series	Saturated Fatty Acids		Unsaturated Fatty Acids		Hemin		O_2 Uptake	$^{14}CO_2$ Activity
	$W^* \cdot 10^{-1}$	Per cent pre-radiation value	$W^{**} \cdot 10^{-1}$	Per cent pre-radiation value	10^4 disint./min per mm per g wet weight bone marrow	Per cent pre-radiation value	μl O_2 per g wet wt. per 3 hrs	10^3 disint. min/mm
No radiation	6.31		5.50		1.22		300	5.66
0 hour	14.60	231	18.90	344	1.90	156	810	8.40
48 hours	5.96	108	—	—	0.31	25	280	4.70
72 hours	20.00	363	1.00	18	0.21	17	80	1.14
158 hours	3.21	58	15.60	283	0.05	4	280	0.18

$^* W^{\text{acetate}}_{\text{sat'd. fatty acid}}$ $^{**} W^{\text{acetate}}_{\text{unsat'd. fatty acid}}$

hemin synthesis observed at this time. The changes in rate of hemin synthesis observed throughout the experimental period are in agreement with those encountered in another more extensive investigation[1], thus indicating that the animals are exhibiting a behavior characteristic for the post-radiation period studied. Unfortunately, it is not possible to calculate $W^{\text{acetate}}_{\text{hemin}}$ since information is as yet lacking concerning the number of carbon atoms contributed by acetate to protoporphyrin synthesis. It is of interest to note that immediately and one week after radiation the incorporation of the isotope into the unsaturated fatty acid fraction is greater than the incorporation into the saturated fatty acid fraction. The unsaturated fatty acid fraction into which ^{14}C has been incorporated consists largely of the monounsaturated fatty acids (primarily oleic acid) since poly-unsaturated fatty acids should not have incorporated any isotope[12]. Although there is good evidence that C_{16} and C_{18} monoethenoid fatty acids can be formed by desaturation of the corresponding or closely related saturated fatty acids[13], it is as yet uncertain whether desaturation is an obligatory pathway in the biosynthesis of monoethenoid fatty acids. The high isotope concentration found in the unsaturated fatty acids, particularly one week after radiation at a time when saturated fatty acid synthesis proceeds at a substantially slower rate, suggests that saturated fatty acids need not constitute the main source of unsaturated fatty acids.

The high W values observed immediately, 72 hours, and 1 week after radiation tend to support the postulate that bone marrow fats which fill a hypoplastic marrow cavity can be synthesized *in situ*. The data presented do not eliminate the possibility that depot fats at other sites of the body might have been mobilized and transported to the marrow. However, since marked lipemias have only been observed 1 to 2 days after radiation[14,15], and since these lipemias are transitory and occur at times when there is neither histological nor biochemical evidence for increased marrow fat, these lipemias are probably not related to the process of intramedullary deposition of fat mobilized from extramedullary depots.

It is evident from the increased O_2 consumption and CO_2 evolution that not only the anabolic but also the catabolic phases of bone marrow metabolism are stimulated immediately after radiation. That this stimulating effect might be a general one, is seen from the increased hemin synthesis shown in Table II. The nature of this stimulation of over-all metabolic activity by radiation cannot be explained adequately at this time.

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The rapid rate of isotope incorporation into the unsaturated fatty acid fraction is contrasted with the considerably slower rate of incorporation into saturated fatty acids one week after radiation. This observation points toward a possible change in marrow composition one week after radiation in the direction of an increase in unsaturated fatty acids. Although this point remains to be tested experimentally as regards radiation anemia, it would be analogous to the findings of KRAUSE[16] who has observed higher than normal iodine numbers in the bone marrow fats of anemic cats.

The present data suggest that the synthesis of fatty acids as well as their oxidation in bone marrow homogenates is affected by radiation. The experiments described illustrate how X-radiation may serve as a useful tool in the elucidation of fundamental processes.

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SUMMARY

1. The synthesis of saturated and unsaturated fatty acids in bone marrow homogenates from untreated and X-radiated rabbits has been studied with α-^{14}C-acetate as a precursor.

2. Two and three-fold increases, respectively, over preradiation levels in saturated and unsaturated fatty acid synthesis were observed immediately after radiation.

3 Whereas saturated fatty acid synthesis, which approached control values at 48 hours after radiation, again increased $3\frac{1}{2}$ times 72 hours after radiation, unsaturated fatty acid synthesis had fallen to 18% of the preradiation value. At 158 hours after radiation, the relationship between saturated and unsaturated fatty acid synthesis was reversed since unsaturated fatty acid synthesis had risen to 283% and saturated fatty acid synthesis had dropped to 58% of the preradiation value.

4. Oxygen uptake and $^{14}CO_2$ production increased immediately after radiation, reaching preradiation values 48 hours after exposure. $^{14}CO_2$ evolution decreased steadily throughout the experimental period while oxygen uptake was unchanged 158 hours after exposure.

5. Hemin synthesis after radiation has been found to behave in a manner similar to that observed in other studies.

6. The term *W* has been introduced to denote the capacity of the bone marrow to synthesize fatty acids from α-^{14}C-acetate.

RÉSUMÉ

1. Nous avons étudié la synthèse des acides gras saturés et non-saturés à partir de l'acétate α-^{14}C dans la moëlle osseuse homogénisée de lapins non traités et de lapins irradiés aux rayons X.

2. Nous avons observé que la synthèse des acides gras saturés avait doublé et celles des acides gras non-saturés triplé par rapport à la valeur initiale (avant irradiation).

3. Tandis que la synthèse des acides gras saturés qui, après 48 heures, approchait les valeurs de l'essai de comparaison, augmentait de nouveau $3\frac{1}{2}$ fois au bout de 72 heures après l'irradiation, la synthèse des acides gras non-saturés étaient tombée à 18% de la valeur initiale. 158 heures après l'irradiation la relation entre la synthèse des acides saturés et non-saturés était inversée: en effet, la synthèse des acides gras non-saturés était montée à 283% et celle des acides gras saturés était tombée à 58% de la valeur initiale.

4. La consommation d'oxygène et la production de $^{14}CO_2$ augmentaient immédiatement après l'irradiation, atteignant les valeurs initiales 48 heures après l'exposition. Le dégagement de $^{14}CO_2$ diminuait régulièrement pendant toute la durée de l'expérience, tandis que la consommation d'oxygène était inchangée 158 heures après l'exposition.

5. Nous avons trouvé que la synthèse d'hémine après irradiation se comportait d'une manière semblable à celle observée dans d'autres travaux.

6. Nous avons introduit le terme *W* pour représenter la capacité de la moëlle osseuse de synthétiser des acides gras à partir de l'acétate α-^{14}C.

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ZUSAMMENFASSUNG

1. Die Synthese von gesättigten und ungesättigten Fettsäuren in Knochenmarkhomogenaten von nicht behandelten und Röntgen-bestrahlten Kaninchen, mit α-^{14}C-Acetat als Ausgangsmaterial wurde untersucht.

2. In der Synthese von gesättigten und ungesättigten Fettsäuren wurde unmittelbar nach der Bestrahlung eine respektive 2- und 3-fache Zunahme wahrgenommen.

3. Während die Synthese der gesättigten Fettsäuren, welche 48 Stunden nach der Bestrahlung ähnliche Werte zeigte wie in den Parallelversuchen, 72 Stunden nach der Bestrahlung wieder auf das 3½-fache anstieg, war die Synthese der ungesättigten Fettsäuren auf 18% des Wertes vor der Bestrahlung gefallen. 158 Stunden nach der Bestrahlung war das Verhältnis zwischen der Synthese der gesättigten und der ungesättigten Fettsäuren umgekehrt, da die Synthese der ungesättigten Fettsäuren auf 283% gestiegen und die der gesättigten auf 58% des Wertes vor der Bestrahlung gefallen war.

4. Der Sauerstoffverbrauch und die $^{14}CO_2$-Entwicklung nahmen gleich nach der Bestrahlung zu, während sie nach 48 Stunden dieselben Werte erreichten wie vor der Behandlung. Die $^{14}CO_2$-Entwicklung nahm während der ganzen Versuchsdauer regelmässig ab, während der Sauerstoffverbrauch 158 Stunden nach der Bestrahlung unverändert war.

5. Die Häminsynthese verhielt sich nach der Behandlung in ähnlicher Weise wie in anderen Untersuchungen beobachtet worden war.

6. Die Fähigkeit des Knochenmarkes Fettsäuren aus α-^{14}C-Acetat zu synthetisieren wird mit *W* bezeichnet.

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APPENDIX

1. *Calculation of the number of* ^{14}C *atoms incorporated in fatty acids.* The ^{14}C concentration was determined with the aid of an ionization chamber. The charge, Q, collected on the plates is given by

$$Q = C\Delta V \qquad (1)$$

where C is the capacity in farads and ΔV the change in potential per minute. Q is also given by n, the number of atoms disintegrating per minute, multiplied by the average energy in ev per disintegration, divided by 32.5 ev, the ionization potential of air, and reduced to coulombs by multiplication by $1.6 \cdot 10^{-19}$.

The capacity, C, is determined by means of a standard sample of ^{14}C to be $7.5 \cdot 10^{-12}$ farads. From this value and from equation (1) it is found that

$$n = 3.07 \cdot 10^{4} \Delta V \qquad (1a)$$

Since the number of atoms decaying, ΔN, per unit time, Δt, is proportional to the number of radioactive atoms present, N, ΔN can be shown to be

$$\Delta N = -\lambda N \Delta t \tag{2}$$

and, therefore,

$$N = N_0 e^{-\lambda t} \tag{2a}$$

where λ is the decay constant ($2.59 \cdot 10^{-10}$ for ^{14}C).

From (2) and with the use of the value for n previously obtained, N can now be calculated and is found to be $1.19 \cdot 10^{14} \Delta V$.

2. *Calculation of the number of fatty acid molecules synthesized.* Assuming that the biological half-life of the total rat lipids (9 days [17]) also holds for rabbit bone marrow fats and that the newly formed fat is synthesized from acetate, it is possible to calculate the rate of synthesis of fatty acids. Thus, in 6 g of bone marrow containing 1.8 g of fat, 12.5 mg of fat are replaced every 3 hours. If one assumes that the acetate necessary for the resynthesis of fats is supplied by the bone marrow in addition to the acetate added during the incubation period, it can be calculated that 57.5 mg of acetate are present throughout the experimental period. Since

$$1\ \mu c = 9.24 \cdot 10^{-4}\ \text{mg}\ ^{14}CH_3COOH$$

the ratio of non-radioactive acetate molecules to radioactive acetate molecules is, therefore, $6.1 \cdot 10^4$. From this value and from the value obtained for N, the total number of acetate residues incorporated into the fats can be calculated to be $7.26 \cdot 10^{18} \Delta V$ acetate residues. Since the average fatty acid of the bone marrow contains 9 acetate residues, the amount of fatty acid synthesized is $0.134 \Delta V$ micromoles of fatty acid.

3. *Calculation of the capacity of bone marrow homogenates to synthesize fatty acids.* The capacity of the system to synthesize saturated and unsaturated fatty acids in unit time from acetate as a precursor is expressed in terms $W^{\text{acetate}}_{\text{saturated fatty acids}}$ and $W^{\text{acetate}}_{\text{unsaturated fatty acids}}$, respectively. The synthetic capacity of the system has been calculated on the basis of μM fatty acids synthesized per g wet weight of tissue in 3 hours.