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Radiation Effect on Mortality and Some Body Compositions in Mice

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THIS STUDY was conducted to examine the hypothesis that deposition of fat and or protein in animals's body before irradiation or using radio-protector material such as soyasbean oil may reduce the hazard effects of radiation on life span and body compartments. Therefore, 286 mice (144 males and 142 females after chemical maturity were used in this study. The animals were divided to 4 major groups. The first group was fed on chow diet, the second group was fed on radioprotector diet (basel diet), the third group was fed on high energy diet and the forth group was fed on high protein diet for 7 weeks before the exposure to gamma rays. At the exposure day each nutritional group was divided to 3 exposure treatments (non-irradeated, 800 and 1200 rads).

The previous hypothesis was studied for 42 days after irradiation. The data showed that:

1. The male and the female of the chow diet group which were exposed to 800 and 1200 rads died within the first two weeks and the mortality percent in all the nutritional groups increased with increasing the post irradiation days and with increasing the dose level.

2. Feed additives (skin milk or starch) along with soyabean oil as the radioprotector prevented the injuries effect of radiation on the life span. This because at 7 weeks after irradiation, the mortality percent was 27-40 % in the dietary groups and on mortality was observed in the male basel diet group (Soyabean oil) which exposed to 800 rads.

3. There was a correlation between the decrease in body weight and the percent mortality, since increasing the percent weight loss was paralleled with increasing the mortality percent.

4. The reduction in body fat as percent body weight in the male and in the female mice which were exposed to 800 rads were pronounced in the chow diet group rather than in other groups.

5. The radioprotector substance (Soyabean oil) reduced the effect of radiation on the fat-free body.

6. The reduction in the fat-free body was mairly due to the reduction in the body cell mass rather than a reduction in the extracellular tissue. The reduction in the body cell mass was due to a reduction in the body cell mass was due to a reduction in the intracellular water and in total body protein.

7. The reduction in the body comportments was extended to the body ash. The reduction in this compartment was significant either as an absolute amount or as a fraction of body weight.

In recent years great attention has been given to the possible damaging effects upon mortality of farm animals (Curtes and Gebhard 1959, Patricia and Ratblat, 1959, Stearner and Christian, 1971, Marz, 1971 and James and Wilson, 1974.

The effect of radiation on life upon, and the way to modify this effect have been the subject of continuons studies for many years. Many literatures revealed that the effect of radiation on the life span might be affected by sex, age, species and dose rate. The data of Holland Mitchell (1976) in mice confirmed the said results and suggested a constant effect of sex.

The effect of radiation of the building units of some compartments may be taken as an indication for the changes induced in the components. The data of Turner and Fowler (1963) in rats, and Fiedberg *et al.* (1962) in mice showed an increase in protein leakage after the 3rd or 4*th* day post irradiation. The reduction in serum proteins of irradiated animals has been found in different species, in rats (Glinos, 1963), in hamster (Ditzel.. 1962) and in monkey (Leone *et al.*, 1959).

Rare of data are available on the effect of radiation on the absolute amounts of the major body compartments, (Total body water, total body fat, total body protein and ash). Esnouf *et al.* (1961) exposed male CBA mice to 750r x-irradiation at different ages rangeing from 3-9 months old. They found that there was no difference in fat or water content of the individual organs (test, heart and kidney) compared with control. Total fat content of the mice was greater in the irradiated mice than that of the control at the same weight (Esnouf *et al.*, 1961).

The objective of this study was to follow the effect of gamma irradiation in the different mice groups on the mortality life span and some body compaartments compared with the corresponding nonexposed group.

Material and Methods

The experiment was preformed on charles River mice bred in Experimental Animals Laboratory, Body composition unit, Radiobiology Departm-ent,Atomic Energy Establishment at Inshus. The mice were raised in aluminum cages 45 × 24 × 22 cm bedded with white wood shavings. The new hornmice were lift with their parents until 3 weeks of age, then sexed and the males were separated from the females. The animals were supplied with top water (Containing 0-1 terramycin) and regular chow diet. All animals were reared under similar conditions.

Two hundred and eighty six mice (144 males and 142 females) following the sexual maturity age (11 weeks) were used in this study. The animals were divided to four nutritional groups, where the average body weight between groups within sex was approximately the same as shown in Table 1. The nutritional groups were as follows :

1. Chow diet : The commercial prepared pelleted ration consisting of not less than 13% crude protein, 9.5% fat, 3%, crude fiber and 6.5 Ash.

2. Basel diet : Consisted of the regular chow diet with an additive of 3% soyabean oil as radioprotector.

3. High energy diet : Consisted of the regular chow diet with an additive of 40% rice starch.

4. High protein diet : Consisted of the regular chow diet with and additive of 30% skim milk.

For the said reasons each animal group was fed *ad libitum* for 7 weeks on its special diet before the exposure to gammarays. At the end of the nutritional treatments, the averages of body weight for the different groups increased (Table I). On the day of exposure, each nutritional group within sex was divided to three exposure groups with approximately the same body weight. Whole body irradiation was applied for each animal under study. The source of irradiation was 60° gamma cell 220 located in the Nuclear Physics Deptarment A.R.F. Atomic energy Establishment. The dasimetry of the source at the exposure day was 1002 + 10^5 Rad/hour.

TABLE I Effect of feeding addiitives on body weight in the three dietary regime groups compared with chow diet group.

Item	Chow diet 88		Basel diet		High energy diet		High protein diet	
	Male	Female	Male	Female	Male	Female	Male	Female
Before feeding treatment. . . .	27.81± 3.21 (30)	23.23± 2.31 (27)	27.42± 2.49 (35)	23.24± 2.81 (40)	27.74± 2.77 (39)	23.16± 3.20 (36)	27.88± 3.56 (40)	23.23± 5.0 (39)
After (7 weeks) . . .	28.67± 2.52 (30)	28.24± 2.4 (27)	29.24± 3.01 (35)	25.58± 2.61 (40)	30.84± 1.12 (39)	26.78± 3.14 (36)	31.34± 3.20 (40)	26.93 4.0 (39)

a=Mean (g) I.S.D.
The number between brackets is the No. of animals.

Following the exposure, the same regime diet for each group was used. Individual body weight of the surviving animals was recorded daily for the first 2 weeks, ever couple of days for the third week, every three days for the fourth week and at the fifth and the sixth weeks after the exposure which was the end of the experiment.

Statistical analysis were punched on IBM cards for each animal under study as the input data, where the 201 N.C.K. computer a tthe, American University Computer Center in cairo was used. All of the statistical analysis were made according to Snedecor and Cachran (1968).

Results and Discussion

Effect on cumulative mortality

Table 3 shows the cummulative mortality percentages. It is observed that mortality increased with advancing in days post-irradiation. It is interesting to note that all the animals in the chow diet groups which exposed to 800 rads and/or 1200 rads died within the first two weeks. On the other hand, feed additives (Skim milk or starch) along with the radioprotector improved the injurys of radiation since only 27-40% mortality was observed after 6 weeks of radiation (Table 2). However, no mortality was observed in the male of the basel diet exposed to 800 rads. Moreover, it is worth mentioning that there was difinit relation between mortality and decreasing body weight. These findings are in agreement with Ellinger (1952) Chapman (1955) and Douglas and Tyree (1954), who reported a direct relationship between the depression in weight gain and increasing the mortality rats in mice.

The data represented in Table 2 cleared that the basel diet group was more resistant to the radiation injury than the other groups especially at 800 rads. This finding is in agreement with Stearner and Azuma (1968), who found that survival percent could be increased by using an antiprotease (Soyabean trypsin inhibitor).

Effect on body composition

It is a well known phenomenon that the biological systems are generally affected by ionizing radiations. Body weight has been introduced as a one compartment system in biological science. Table 3 shows that exposing male and or female mice to gamma-rays was responsible for significant reduction (Table 4) in body weight. However, neither the nutritional treatments high starch or high protein) not the radioprotector (Soyabean) could help in reducing the effect of gammarays on body weight, since the interaction group $\times$ treatment was insignificant (Table 4). Since the animal body is composed of different components, it was expected that there must be a reduction in these components. In this respect, Chaffee *et al.*, (1966) and Brisbin (1969) indicated that the exposure to ionizing radiation induced reductiion in body fat. The data in Table 3 confirmed the above mentioned finding. Body fat either as an absolute amount or as a fraction of body weight (Table 3) decreased significantly (Table 4,5) in both sexes. However, nutritional treatment before and after the exposure were responsible to decrease the injury of gamma-rays. In this respect the averages of body fat in gams in the four nutritional treatments irrespective of the dose level as calculated from Table 4 were 0·896, 0·988, 0·880 and 0·616 for the chow diet, basel diet, high energy diet and high protein diet, respectively.

TABLE 2 Cummulative mortality percentage in the four nutritional treatments of both sexes under different exposures

Dayes after exposure	Chow diet				Basel diet				High energy diet				High protein diet			
	Male		Female		Male		Female		Male		Famale		Male		Female	
	800	1200	800	1200	800	1200	800	1200	800	1200	800	1200	800	1200	800	1200
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
2	—	—	—	—	—	13	—	—	—	—	7	—	—	—	—	
3	—	—	—	—	—	13	—	—	—	—	7	—	—	—	—	
4	—	—	—	—	—	13	—	—	—	—	7	7	—	—	—	
5	—	—	—	—	—	13	—	7	—	—	7	7	—	—	—	21
6	—	20	—	—	—	20	7	7		20	7	20	—	7	—	24
7	—	20	—	—	—	27	7	7		33		40	—	13	—	36
8	20	80	10	40	—	47	7	7		53		47	7	40	—	71
9	20	80	10	40	—	47	7	7	20	93	13	53	—	53	—	71
10	30	80	20	40	—	67	7	27	20	93	15	67	—	87	—	86
11	30	80	20	40	—	80	13	40	27	100	20	100	—	100	—	100
12	40	80	20	40	—	92	13	73	27		82				7	
13	40	80	20	40	—	100	20	93	33		27		7		13	
14	50	80	50	60			20	93	33		33		20		13	
15	100	100	100	100			20	93	33		33		20		20	
17							20	93	33		33		27		29	
18							20	93	33		33		27		20	
21							20	100	33		40		33		20	
22							27		33		40		33		27	
25							27		33		40		33		27	
28							33		33		40		33		27	
30							33		33		40		33		27	
35							33		33		40		33			
42							33		33		40					

TABLE 3 Effect of gamma-irradiation on body weight: body fat, fat free body, lean dry body, total body protein, total body ash and total body water.

a. In males

Group	Dose leve	Body weight	Fat	Fat free Body	Total body			Lean dry body
					Protein	Ash	Water	
CD	0	27.125	1.336	25.789	5.821	0.978	18.990	6.799
	800	20.924	0.463	20.461	4.610	1.000	14.851	5.610
	1200	21.488	0.967	20.521	4.308	1.054	15.154	5.367
BD	0	27.794	2.645	25.149	5.351	0.853	18.946	6.204
	800	26.293	2.196	24.097	5.041	1.040	18.015	6.081
	1200	20.032	0.079	19.953	4.747	0.924	14.281	5.672
HED	0	30.320	1.186	29.134	6.981	1.139	21.014	8.121
	800	23.634	0.934	22.700	6.081	1.072	16.547	6.153
	1200	20.973	0.293	80.680	4.570	0.952	15.158	5.522
HPD	0	24.342	1.054	28.288	6.876	1.201	20.211	8.076
	800	23.554	1.009	22.545	5.207	1.009	16.329	6.216
	1200	20.215	0.068	20.147	4.627	0.986	14.534	5.613

b. In Females

CD	0	23.538	1.491	22.047	4.838	0.865	16.344	5.703
	800	20.088	0.593	19.495	4.299	0.896	14.300	5.195
	1200	18.626	0.367	18.259	3.805	1.023	13.431	4.827
BD	0	25.794	1.680	24.114	5.503	1.038	17.573	6.541
	800	14.774	1.314	18.460	4.002	0.996	13.463	4.998
	1200	16.412	0.189	16.223	3.475	0.888	11.860	4.363
HED	0	26.021	1.903	24.118	5.303	1.044	17.772	6.347
	800	19.809	1.205	18.604	3.803	1.077	13.724	4.880
	1200	17.545	0.617	16.428	3.648	0.902	12.378	4.550
HPD	0	27.419	0.922	26.497	6.187	1.172	19.137	7.354
	800	14.871	1.130	18.741	3.927	0.946	13.868	4.873
	1200	17.446	0.072	17.374	3.741	0.914	12.719	4.655

CD Chow diet. Bm Basel diet

HED High energy diet.

HPD High protein diet.

TABLE 4. Mean squares of the absolute amounts of some body composition.

Source of vartance	df	Body weight	Body fat	Fat free body	Lean dry body	Total body protein	Total body asn	Total body water
Group	3	13.857	1.618*	22.856	2.989	2.178*	0.074**	10.514
Treatment	2	1409.553**	30.503**	1065.705**	78.418**	17.689**	0.175**	11.446**
Sex	1	603.853**	0.140	631.133**	57.707**	52.213**	0.185**	0.278
Group X Treat	6	28.523	3.419**	22.646	3.454**	2.279**	0.162**	4.937*
Group X Sex	3	8.631	0.437	13.278	1.649	1.705	0.039	2.524
Treat. X Sex	2	28.259	0.029	20.176	13.345	1.096	0.011	0.039
Group X Treat. X Sex	6	8.563	1.474	4.907	0.711	0.542	0.035	5.595
Error	262	15.219	0.446	12.890	0.781	0.645	0.024	2.206
Total	285							

* = Significant at (P 0.05).
** = Significant at (P 0.01).

It has been accepted since 35 years ago that the animal body is composed of two compartments (Fat and fat-free body) as reported by pace and Ruthbun (1945). Moreover, the fat-free body is composed on two compartments (body cell mass and extracellular tissue) as pointed out by Moore *et al.*, (1963). Furthermore, the body cell mass is mainly the body protein and the intra cellular water while the extracellular tissue is mainly the extracellular water and the body ash as reviewed by Shebaita (1971). On the other hand, the fat-free body consisted of about 73% water (Pace and Rathun, 1945). Therefore the observed reduction in body weight in this study due to the ionizing radiation must be due to the above mentioned comportments in the body. Table 4 shows the values of the fat-free body in the four nutritional treatments and under the different dose levels. It is clear from Table 4 that the mice which had soyabean oil in their diet (Basel diet) was less sensitive to the injury of the ionizing radiation compared with the chow diet, high protein diet and high energy diet.

TABLE 5. Mean squares of body composition as a fraction of body weight

Source of variance	df	Body fat	Fat fre body	Lean dry body	Body protein	Body ash	Body water
Group	3	30.230**	30.230**	20.987**	17.532**	0.565	6.173
Treatment	2	368.596**	368.596**	31.725**	22.490**	30.715**	192.324**
Sex	1	23.289**	23.289**	30.522**	86.462**	13.784**	1.481
Group XTreat	6	449.480**	49.480**	23.801**	20.790**	1.369	19.665**
Group X Sex	3	7.951	7.951	7.861	10.113*	3.363*	17.828*
Treatment X Sex	2	2.414	2.414	2.196	2.861	1.066	3.146
Group X Treat. X Sex	6	15.993*	15.993*	14.059	3.213	0.556	3.957
Error	262	5.766	5.766	5.096	3.259	0.887	5.978
Total	285						

* = Significant at (P 0.05).
** = Significant at (P 0.01).

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تأثير الاشعاع على معدل النفوق وبعض مكونات الجسم في الفئران

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أجريت هذه الدراسة بهدف معرفة تأثير أشعة جاما المؤينة على مكونات جسم الحيوانات الزراعية وخصوصا الثدييات ولاختبار صحة الافتراض القائل بأن زيادة نسبة الدهن أو البروتين فى جسم الحيوان أو استخدام بعض المواد المضادة للاشعاع مثل زيت فول الصويا قبل التعريض للاشعاع يؤدى الى تقليل الآثار الضارة للاشعاع على كل من وزن الجسم ، نسبة النفوق ومكونات الجسم المختلفة ٠

ولاجراء هذه الدراسة استخدم ٢٨٦ جرزا من نوع تشارلزويفر (١٤٤ ذكر ، ١٤٢ أنثى) وصلت جميعها الى عمر النضج الكيماوى ٠ حيث قسم كل من الذكور والاناث الى أربعة مجاميع رئيسية متقاربة فى متوسط وزن الجسم حيث تم تغذية المجموعة الأولى على العليقة العادية الخاصة بالجرزان وغذيت المجموعة الثانية على عليقة عادية تحتوى على ٣٪ زيت فول الصويا (كمادة مضادة للاشعاع) وغذيت المجموعة الثالثة على عليقة عادية تحتوى على ٤٠٪ نشا أرز أما المجموعة الرابعة فقد غذيت على عليقة عادية تحتوى على ٣٠٪ لبن مجفف ٠ وقد استمرت كل مجموعة فى التغذية على العليقة الخاصة بها لمدة ٧ أسابيع قبل التعرض لأشعة جاما المؤينة وهذا يهدف زيادة نسبة الدهن والبروتين فى جسم الجرزان كوسيلة لتلافى الآثار الضارة للاشعاع على الجسم٠

وفى اليوم المحدد للتعرض للاشعاع قسمت كل مجموعة من المجموعات الغذائية الأربعة داخل كل جنس الى ثلاثة مجاميع متساوية تقريبا فى متوسط وزن الجسم حيث عرضت المجموعة الأولى الى ١٢٠٠ راد والثانية الى ٨٠٠ راد من أشعة جاما المؤينة واستخدمت المجموعة الثالثة كمجموعة للمقارنة دون تعريض للاشعاع وذلك لتقدير مكونات الجسم بها ٠ هذا وقد استمر البرنامج الغذائى السابق الخاص لكل مجموعة عقب التعريض للاشعاع ولمدة ٦ أسابيع تم خلالها اختبار الافتراض السابق وذلك بدراسة أثر الاشعاع على كل من :

وزن الجسم ، نسبة النفوق ومكونات الجسم المختلفة وهى (كمية الدهن الكلية ، وزن الجسم الخالى من الدهن ، كمية الماء فى الجسم ، وزن الجسم الجاف الخالى من الدهن ، كمية البروتين فى الجسم ، كتلة الأنسجة الموجودة خارج الخلايا ، الماء خارج الخلايا ، رماد الجسم وكمية البوتاسيوم فى الجسم) ٠ ويمكن تلخيص نتائج هذه الدراسة كما يلى :

١ – ذكور واناث المجموعة التى غذيت على عليقة عادية وعرضت الى ٨٠٠راد، ١٢٠٠ راد نفقت جميعها خلال الأسبوع الأول والثانى من التعريض أما بالنسبة لبقية المجاميع ففى خلال الأسبوع الأول والثانى فقد زادت نسبة النفوق بزيادة جرعة الاشعاع وزيادة الأيام بعد التعريض ٠

٢ – أظهرت المجاميع المعاملة غذائيا أو المضاف اليها المادة المضادة للاشعاع مقاومة للأثر الضار للاشعاع على طول فترة الحياة ففى نهاية الأسبوع السادس عقب التعريض للاشعاع بلغت هذه النسبة ٣٣٪ ، ٣٣٪ ، صفر٪ فى ذكور المجاميع التى غذيت على عليقة غنية فى البروتين ، عليقة غنية فى النشا وعليقة

بها زيت فول الصويا • وعرضت لـ ٨٠٠ راد أما بالنسبة للاناث فقد بلغت هذه النسبة عقب الأسبوع السادس للتعرض ٤٠٪ ، ٣٣٪ ، ٢٧٪ فى اناث المجموعة التى غذيت على عليقة غنية فى النشا ، عليقة بها زيت فول الصويا وعلى عليقة غنية فى البروتين على الترتيب •

٣ ـ أظهرت الدراسة أن هناك علاقة بين الانخفاض فى وزن الجسم ونسبة النفوق حيث زادت نسبة النفوق بزيادة معدل النقص فى وزن الجسم •

٤ ـ أظهر التحليل الكيماوى أن المجموعة التى غذيت على عليقة عادية أن نسبة الدهن فى الذكور والاناث التى عرضت لـ ٨٠٠ راد من أشعة جاما المؤينة قد انخفضت انخفاضا ملحوظا عن بقية المجاميع الأخرى المعاملة غذائيا أو بمادة مضادة للاشعاع فقد بلغت نسبة الدهن ٢ر٢٪ ، ٨٪ ، ٧ر٣٪ ، ٩ر٣٪ فى ذكور المجموعة التى غذيت على عليقة عادية ، والتى غذيت على عليقة بها زيت فول الصويا والتى غذيت على عليقة غنية فى النشا والتى غذيت على عليقة غنية فى البروتين على الترتيب • أما بالنسبة للاناث فكانت نسبة الدهن فى المجاميع الغذائية السابقة هى ٩ر٢٪ ، ٦ر٦٪ ، ٦٪ ، ٦ر٥٪ على الترتيب •

٥ ـ المجموعة التى غذيت على عليقة غنية فى زيت فول الصويا كانت أقل حساسية لأثر الاشعاع على نسبة وزن الجسم الخالى من الدهن عن بقية المجاميع الأخرى •

٦ ـ النقص الحادث فى وزن الجسم الخالى من الدهن نتيجة للتعرض للاشعاع يرجع معظمه الى نقص فى كتلة خلايا الجسم أكثر من النقص الحادث فى الأنسجة الموجودة خارج الخلايا وأن النقص الحادث فى كتلة خلايا الجسم انما يرجع الى النقص الحادث فى نسبة بروتين الجسم ونسبة الماء داخل الخلايا •

٧ ـ بالاضافة الى النقص المعنوى (على مستوى معنوية ٠١ر) الحادث فى مكونات الجسم المختلفة (الدهن الكلى ، الجسم الخالى من الدهن ، كتلة خلايا الجسم ، الأنسجة الموجودة خارج الخلايا ، الماء خارج الخلايا ، البروتين والبوتاسيوم) نتيجة التعرض لأشعة جاما المؤينة فقد امتد هذا النقص المعنوى الى كل من رماد الجسم والماء خارج الخلايا •