MASTER

HISTOPATHOLOGICAL CHANGES IN LABORATORY ANIMALS EXPOSED
TO PROTRACTED LOW-LEVEL TOTAL-BODY NEUTRON AND
GAMMA RAY FLUXES---A CONTINUING STUDY

ATOMIC ENERGY COMMISSION CONTRACT AT(11-1) 1487

for the period

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The University of Chicago Contract No. AT (11-1) - 1487

for the period from June 15, 1970 to June 14, 1971

- (1) Histopathological Changes in Laboratory Animals Exposed to Protracted Low-Level Total-Body Neutron and Gamma Ray Fluxes-- A Continuing Study
- (2) The University of Chicago
Departments of Pharmacology and Radiology
950 East 59th. Street
Chicago, Illinois 60637
- (3) The research program completed and proposed herein has been and will be associated with the Janus Reactor Program at the Argonne National Laboratory, Division of Biological and Medical Research, Argonne, Illinois. The program is identified there as the "Neutron and Gamma Ray Toxicity Studies." The portion of the program that the proposer will largely be associated with is the gross and histo-pathology and causes of morbidity and mortality. Some related preparatory studies will be or have been completed and are to be reported.

The completed study, but as yet not fully reported, is one with laboratory mice exposed to neutrons following the administration of bile salts. A short resume is included later in which the rationale and results will be presented (Appendix A). An incomplete study is that with the stressed multimammate mouse population, i.e., *Paraomys (mastomys) natalensis*. The protocol for the experimental design is given for information purposes (see Appendix B).

The second is an uncompleted report on the large mouse study. Several reports by Grahn, Sacher and others have been made on this work (see Appendix C) but their reports and assessments have been concerned with matters other than the pathology. We will start the analysis of the data which is now ready and hopefully complete the pathology report by the end of the 1971-72 contract period. Certain data will be examined, such as, the variety and multiplicity of tumors, the incidence of tumors, the incidence of the various tumor types as they relate to radiation dose, age, sex, and strain, and, the change in mortality rates as they are related to non-tumorous and tumor bearing animals. Further, we wish to inspect the data on lung tumors in order to establish whether or not that entity can be used to establish a useful morbidity rate.

In the "stressed" mastomys study we are now completing the histological examination of the animals killed at 18 months. In this examination we have had the excellent advice of Dr. Ward R. Richter, Associate Professor of Comparative Pathology at the University of Chicago. We plan to start the study of the nine months old animals as soon

as the older animals are completed. This should be by early summer of 1971. We have not completed the detailed assessment of the effect of the stress upon diseases or longevity. It can be said, however, that whenever six males are housed together they soon reduce their numbers to three by fighting. It has, therefore, been well established that three male mastomys is the greatest that number can be kept in one cage, without respect to cage size.

Based upon an inspection of gross pathological observations for all mastomys killed and in the approximately 250 mastomys, of the 18 months group, examined histologically and other supporting studies. Certain special broad and useful conclusions can be made. First, a general and summary conclusion can be made that the mastomys will be a very useful laboratory animal to submit to low level neutron and gamma ray fluxes in order to understand the latent response of animals to low-level total-body exposures.

The special observations and conclusions made are,

- (1) The cell turnover time in the gut of the mastomys is slower, i.e., mean life span longer, than in the mouse. This has relevance in the study of the induction of intestinal tumors and a comparison with the mouse,
- (2) The mastomys has a high incidence, possibly sex related, of the auto-immune disease of the thyroid, i.e., Struma Lymphomatosis (Hashimoto's Disease). The incidence in the 18 months group will probably range between 70-90 percent,
- (3) Disabling and deforming chronic arthritis is relatively a common disease in this species. The incidence has not yet been established,
- (4) Stomach cancer is not as common in our strain of mastomys as been reported by others, i.e., up to 40%. We have found about 1-3% of the animals with stomach cancer. In contrast to others, carcinoid of the stomach is not more common than adeno-carcinoma or squamous cell carcinoma,
- (5) Reticular cell tumors are not common. Thymic lymphoma were not seen. In this respect they resemble the guinea pig (see our earlier progress report on that species).
- (6) Kidney disease is unusually common. Most animals at the age of 18 months exhibit some evidence of the disease ranging from at least a moderate involvement of the glomeruli to severe cystic and interstitial changes. It is interesting that in even the most severe disease states, the parathyroid was not hypertrophic. This suggests that even though the upper nephron and glomerulus which are the prime sites of the disease, do not disturb the calcium-phosphate homeostatic equilibrium in the circulating plasma. It also seems quite possible that the intermediate (Henle's Loop) and distal tubule of the nephron may, in the mastomys, retain some of their primitive characteristics and function as an intact

kidney,

- (7) Adrenal tumors of the cortex and medulla were seen on occasion. It is probable that the medullary tumors were largely pheochromocytomas. Most striking was a degenerative necrosis and proliferation of a unique characterless cell type which seemed to be replacing the degenerated area. We have made no attempt to associate this with stress at this time. We tentatively have considered these to be proliferating modified or primitive cells of the zona reticularis. It will take further observations and possibly added studies to properly identify these cells. They are unique and have not been reported, to our knowledge, in the medical and biological literature before. It is undoubtedly a regenerative or reparative process of some sort,
- (8) Lung tumors of any variety were never seen in contrast to the mouse, rat, guinea pig, hamster, gerbil etc. This observation was made from about 1200 animals and is of considerable interest in comparative pathology of rodentia. It is also of much interest in radiobiology for it may be possible to add information which will help settle the question of the induction of lung tumors by total body irradiation,
- (9) Hepatomas were quite common. It is not possible at this time to determine whether or not they were more or less common than in other rodentia,
- (10) Several cases of pituitary tumors were seen. No attempt will be made now to classify them. They are to be studied in detail and classified later. They often were quite large and deformed the brain and skull.
- (11) No amyloidosis was observed in any animal, and
- (12) Other tumors, and disease processes (on some occasions an absence of these) will be studied in greater detail before any attempt will be made to report them. One obvious fact of great importance is that there is a complete lack of information on microbiologically induced disease in this animal species. Much of what will be said in the future will therefore be descriptive and most certainly not definitive.

Work is in progress with the Janus program. Mice have now been exposed that are destined to come to the necropsy table. Some should be available within the next month, i.e., mid-March. There will be large numbers of animals exposed starting within a few weeks which should produce many deaths from thymic lymphomas next fall and winter. The success of the histopathologic portion of this program, no matter how well planned the rest may be, will flounder if histology

technicians are not available to prepare the tissues. At the moment this appears to be a soft spot and is likely to delay that part of the program.

The proposer is presently receiving support from the National Institutes of Health on the following grant,

USPHS 5-PO6-FR-00409-03
and this contract
AT (11-1) 1487.

- (4) The scientific and professional activities and background of the proposer is best given in the attached curriculum vitae and bibliography.

APPENDIX A

Pathological Findings after Single Dose Neutron Irradiation.

R. J. Michael Fry, J. H. Rust, J. Newlin and E. Staffeldt

Purpose and Methods.

This investigation had two purposes (1) to determine whether increased proliferative activity in the biliary tract and hepatocytes altered the incidence of tumors arising from these cells after irradiation. (2) to obtain information for a sacrifice series on the incidence of tumors and lens abnormalities after exposure to a single dose of neutron irradiation in the BCF mouse, the strain chosen for the Janus program.

Male and female, BCF₁ mice 155-180 days of age were used. Mice were exposed at CP-5* to approximately half the LD 50/30 dose-170 rads. Apart from those sacrificed at about 250 days of age for seriological examination for viral antibodies and of course those dying, the mice were sacrificed at 699-723 days of age about 550 days after irradiation. The choice of time of sacrifice is a compromise, it is early enough that a reasonable sized sample was obtained and sufficiently long after the irradiation to include the latent period of most tumors.

Increased proliferative activity of the biliary tract, hepatocytes and littoral cell was stimulated by giving a diet containing 2% deoxycholic acid⁽¹⁾ for 48 hours prior to irradiation.

Progress Report

The pathological findings for the pooled data of the irradiated groups are compared to those of pooled data for the control group of this experiment and data for BCF₁ mice of a similar age group and are shown in Table I.

The points of interest are the following: 1. There was no change in the incidence of tumors of the reticular tissue but six cases of myelogenous leukemia were found. We have not found this condition in any of the large number of unirradiated BCF₁ examined, nor in those exposed to chronic gamma irradiation but have diagnosed it after single exposures to X-rays (100R and 395R). An increased incidence of myelogenous leukemia with single dose neutron irradiation compared to protracted exposures has been reported. 2. There was an increase in the incidence of ovarian tumors and cysts and miscellaneous tumors, which included two in the area of the gastro-duodenal junction. The latter type of tumor has not been found in this age group of unirradiated or chronically irradiated mice. 3. Abnormalities of the lenses were found in all of the eyes examined in the irradiated group including those sacrificed about 100 days after irradiation and very slight abnormalities were found in 35% of the control animals of about 700 days of age. 4. There were 10% hepatomas in the irradiated groups compared to 4.5% in the control mice. The incidence of hepatomas was 18% in the mice given the diet 48 hours prior to irradiation compared to 6% in the mice receiving only irradiation. 5. One tumor of the gall bladder was found

*We are grateful to Dr. H. Vogel and R. Barhost for exposing the mice.

and perhaps of interest it was one of the groups given the diet after irradiation. Accelerated induction of hepatomas after neutron irradiation and carbon tetrachloride, which results in proliferation secondary to liver degeneration, has been reported.³ Significant titres for antibodies to PVM, G.D. VII, Sendai, and Mouse hepatitis were found in both irradiated and control mice.

Conclusions

The induction of myelogenous leukemia with the single exposures to neutron irradiation and X-rays in a strain which shows no such disease either in the unirradiated or in mice exposed to protracted gamma irradiation raises some interesting questions about the etiology of the disease that can be investigated in the Janus program. The results for the incidence of tumors of the liver and of the single tumor of the gall bladder after irradiation and with induced proliferation. Suggest that this method of altering proliferation in these cell systems will be useful for investigating the inter-relationship of proliferative activity and radiation induction of tumors.

References

1. Fry, R. J. M. and E. Staffeldt. Effect of diet containing sodium deoxycholate on the intestinal mucosa of the mouse. *Nature*. 203: 1396-1398 1964.
2. Upton A.C., M. L. Randolph and J. W. Conklin. Late effects of fast neutrons and gamma rays in mice as influenced by the dose rate of irradiation. Induction of neoplasia. *Rad. Research* 41: 467-491 1970
3. Cole, L. J. and P. C. Nowell. Carcinogenesis by fast neutrons relative to X-rays in mice in Biological effects of proton irradiation. IAEA Vienna, 1964 pages 129-141.

TABLE I

Pathological Findings: Percent Incidence

BCF ₁ Mice 650-750 days old	Tumors of the Reticular Tissue				Tumors				
	Thymic lymphoma	Lympho- sarcoma ¹	Myelogenous leukemia	Unclassi- fied	Pulmonary	Hepatic	Ovarian ²	Miscel- laneous	Lens abnormalities ³
0.0 Rads									
47	0.0	8.5	0.0	0.0	17.0	8.5		0.0	15
42	2.4	16.7	0.0	0.0	11.9	0.0	2.4	4.8	56.5
Total 89	1.1	12.4	0.0	0.0	14.6	4.5			34.8
	Pooled: 13.5								
170 Rads neutrons									
63	0.0	4.8	7.9	0.0	27.0	14.3		11.1	100.0
66	0.0	7.6	1.5	3.0	9.1	6.1	31.8	18.1	100.0
Total 129	0.0	6.2	4.7	1.5	17.8	10.1		14.7	150
	Pooled 12.4								

¹Includes all tumors, for example reticulum cell sarcoma, arising in lymphoid tissue other than the thymus.

²The data for ovarian tumors and cysts has been pooled.

³The degree of abnormality is markedly less in the control mice and the incidence is based on the presence of any deviation from normal microscopic appearance.

APPENDIX B

The Original Experimental Design and its Requirements for Mastomys Population Pressure Study

NOMINAL DESIGN: 3 level block design with 1 missing block; 3 levels of area per animal x 4 animal(s)/cage densities x 3 sample times

		Animals/cage			
		1	2	3	6
Cage area (IN ²)	20				
	35				
	60				

x 3 levels of sampling time { 4 months
9 months
18 months

ACTUAL DESIGN (actual areas, sampling times, cage types)

		1	2	3	6
Cage area (IN ²)	20	19.6 Y	20.0 B*	20.0 B*	19.8 M
	35	35.0 B*	37.1 B	35.0 M	31.5 R
	60	60.0 B*	59.5 M	63.0 R	

sampling times { 4 months
9 months
18 months (9-18 months due to deaths)

cage types: Y = gamma cages
B = breeder cages
M = mouse plastic cages
R = rat cages

*indicated cage modified to size

REPLICATIONS (by number of cages and types using animals as units rather than cages)

Example: at one level only

		1	2	3	6	
Cage area (IN ²)	20	6Y	3B	2B	1M	
	35	6B	3B	2M	1R	
	60	6B	3M	2R		

← animals/cage
← No. cages sampled
No. animals/sample = animals/cage x cages/sample

All ♂♂ (no sex test)
35 cages
66 ♂♂

		1	2*	3	6**
Cage area (IN ²)	20		3B (x2)		1M
	35		3B (x2)		
	60		3M (x2)		

where (*) indicates animals/cage will be ♀♀ for one replication and ♀/♂ for a second replication, and (**) indicates ♀♀ for one replication

19 cages
33 ♀♀/♂♂

The above data indicates that one sex-tested replication will contain:

Cages:	54	(= 35 + 19)	$\left\{ \begin{array}{l} 6Y \\ 32B \\ 13M \\ 3R \end{array} \right\}$
Animals:	75♂♂	(= 66 + 9)	
	33♀♀	(= 27 + 6)	

Replications/sampling period: 4 months (2 reps.)
 9 months (4 reps.)
 18 months (4 reps.)

SAMPLING DATES

Summer 1968 sampling:

9 mo. group (4 replications) = animals:	300♂/132♀	Total: 450♂/198♀
cages:	$\left\{ \begin{array}{l} 24Y \\ 128B \\ 52M \\ 12R \end{array} \right\}$	$\left\{ \begin{array}{l} 36Y \\ 192B \\ 78M \\ 18R \end{array} \right\}$
4 mo. group (2 replications) = animals:	150♂/66♀	
cages:	$\left\{ \begin{array}{l} 12Y \\ 64B \\ 26M \\ 6R \end{array} \right\}$	

Summer 1969 sampling: animals should be 18 months of age (4 replications as above) in July-August 1969

CAGING SCHEDULE AND REQUIREMENTS

9 month (4 replication sample)

CAGE TYPE	NO. OF CAGES	NO./CAGE	SEX COMPOSITION	
Y ----	24	1	♂	→ indicates ♀♀ groups in cage composition
B (B-35)	24	1	♂	
(B-60)	24	1	♂	
(B-20)	12	2	♂	
----	12	2	♂	
(B-60)	8	3	♂	
→ (B-20)	12	2	♀♀	
→ ----	12	2	♀♀	
→ (B-20)	12	2	♀♂	
→ ----	12	2	♀♂	
M ----	12	2	♂	
→ ----	12	2	♀♀	
→ ----	12	2	♂♀	
→ ----	8	3	♂	
→ ----	4	6	♂	
→ ----	4	6	♀♀	

<u>CAGE TYPE</u>	<u>NO. OF CAGES</u>	<u>NO./CAGE</u>	<u>SEX COMPOSITION</u>
R: ----	8	3	♂
----	8	6	♂

→ indicates
♀♀ groups
in cage
composition

4 month (2 replication sample)

<u>CAGE TYPE</u>	<u>NO. OF CAGES</u>	<u>NO./CAGE</u>	<u>SEX COMPOSITION</u>
----	12	1	♂
B (B-35)	12	1	♂
(B-60)	12	1	♂
(B-20)	6	2	♂
----	6	2	♂
(B-60)	4	3	♂
→ (B-20)	6	2	♀♀
→	6	2	♀♀
→ (B-20)	6	2	♀♂
→	6	2	♀♂
M ----	6	2	♂
→	6	2	♀♀
→	6	2	♂♀
----	4	3	♂
----	2	6	♂
→	2	6	♀♀
R ----	4	3	♂
----	2	6	♂

216 animals (150♂/66♀) will have births from 1 March, 1968 through 30 April, 1968, with exp. entry at weaning (~30 days); thus entry will be made during April-May, 1968.

APPENDIX C

"Analysis of Neoplastic Disease in Mice Following Daily Gamma Irradiation".

D. Grahn, R. J. M. Fry, J. H. Rust and G. A. Sacher,
presented at IV International Congress on Radiation
Biology, Evian, France, 1970

"Applications, Interpretations and Problems in the
Actuarial Analysis of Survival Data of Mice Under Single
Dose and Daily Gamma Irradiation".

D. Grahn, R. J. M. Fry, and R. A. Lea,
to be presented at Radiation Research Meeting-Boston (1971).

"Some Problems of Analysis of Survival Data" (Working Title)
to be presented at Symposium on Effects of Whole Body
Irradiation, Oak Ridge, Tennessee (1971)

"Epidemiological and Cellular Effects of Chronic Radiation:
A Search for Relationship"

G. A. Sacher, D. Grahn, R. J. M. Fry and J. H. Rust,
for Proceedings of First European Symposium on Late Effects
of Radiation, Rome, 1970.

Time (Effort) of Investigator

The principle investigator will have spent about 500 hours on this project during its effective period. These hours can be divided approximately into the following,

Gross Pathology (autopsy) -	50 hours
Histo-pathology -	350 hours
Preparation of Reports and Records	50 hours
Attendance at meetings and/or conferences and in the library	50 hours

The planned utilization will be approximately the same except that there probably will be a somewhat greater portion of effort spent on gross pathology.