and ± 1.9 respectively. Thus, in the case of mephobarbital, the sleeping time was reduced 67% in three days, showing a definite development of tolerance. Two times the standard error of the mean (95% confidence limits) were used to indicate the significant difference of the means.

On the fourth day following the three daily injections of mephobarbital a butabarbital sodium injection caused the rabbits to sleep an average of 46.6 minutes. This is well below the normal sleeping time for the dose used(1). The fact that the sleeping time was greatly increased (85.7 minutes) with the same drug and the same dose on the fourteenth day, after a ten day interval without drugs, by which time the tolerance should be lost as was shown by Gruber and Keyser(1), would indicate that cross-tolerance also is produced.

Summary. (1) Repeated daily intravenous injections of mephobarbital produces tolerance in experimental animals (rabbits). (2) The development of tolerance to mephobarbital also produces cross tolerance to butabarbital sodium.

1. Gruber, C. M. and Keyser, G. J., *J. Pharm. and Exp. Ther.,* 1946, v86, 196.

2. Carmichael, E. B., *Anesthesiology,* 1948, v9, 532.

3. Hubbard, T. F. and Goldbaum, L. R., *J. Pharm. and Exp. Ther.,* 1949, v97, 488.

4. Holch, G. O,. Riedesel, C. C., Robidoux, F. A,. *J. Am. Pharm. Assn.* (sci. edit.), 1950, v39, 630.

5. Weese, H., *Deutschr. Med. Wch. schr.,* 1932, v58, 696.

6. Krantz, Jr., J. C., and Carr, C. J., *Pharmacologic Principles of Medical Practice,* Baltimore, Williams & Wilkins, 1951.

7. Millman, C. G., *Brit. Med. J.,* 1937, v2, 61.

8. Bonsmann, M. R., *Arch. f. exp. Path. u. Pharmakol.,* 1933, v171, 612.

9. Fitch, R. H. and Tatum, A. L., *J. Pharm. and Exp. Ther.,* 1932, v44, 325.

Received December 17, 1951. P.S.E.B.M., 1952, v79.

Post-Irradiation Studies on Mammalian Testes. Effect at Hourly Intervals for First 24 Hours.*† (19282)

LLOYD C. FOGG AND RUSSELL F. COWING. (Introduced by Shields Warren.)
From the Massachusetts Department of Public Health, Pondville Hospital, Walpole, Mass.

The differential response of the germinal elements in the testes of adult pure strain mice subjected to direct x-irradiation follows a characteristic pattern when examined by quantitative random sampling. This observation is based on a tabulation of the occurrence of a given type of germ cell in 100 observed cross-sections of testicular tubules. The tubules were chosen at random from multiple sections of both testes in order to give fair sampling.

In this investigation the incidence of the various germinal cells at hourly intervals in the 24-hour period post-irradiation was observed. The dose used is known to lower the incidence of germinal cells to a level of 3% (4) by 26 days post-irradiation. The present study is a continuation of the general project of accumulating quantitative data on the biological effects of direct x-irradiation as a contribution to the fundamental problems of normal cell growth and neoplasia. Sensitivity of germinal cells as a result of over-all irradiation, or by the use of radioactive isotopes, has been discussed(1). A review of the literature, with a summary of some of the findings as they apply to the general effect of irradiation on mammalian testicles, was given previously(5). It has long been clear that spermatogonia are the first to show effects following irradiation. The relative sensitivity of other germinal cells, however, needs further investigation. For example, Bloom(1) comments on the relative radioresistance, while

* The authors wish to express their appreciation to Miss Kathleen McCarthy, Pondville Hospital, for cooperation in collection of data and assisting in this project.

† This work was supported by Grant C-1047 from the U. S. Public Health Service and by the Department of Public Health, Pondville Hospital.

TABLE I. Incidence of Frequency of Germinal Cells in Irradiated Testes. Hourly interval study in 24 hr period post-irradiation.

	Spermatogonia			Spermatocytes	
Time, hr	Resting	Mitotic	Reticulated or prophase-like	Nucleated	Meiotic
1	44	0	42	88	8
2	34	1	38	88	8
3	38	0	45	87	16
4	31	0	48	86	13
5	31	0	43	85	12
6	36	0	47	86	10
7	21	0	44	85	9
8	38	0	47	87	5
9	40	0	48	83	10
10	28	0	41	93	9
11	42	0	39	90	9
12	43	0	44	91	12
13	37	0	38	91	13
14	35	0	41	94	7
15	32	0	44	85	14
16	41	0	41	92	11
17	38	0	43	87	13
18	41	0	47	80	12
19	23	0	50	88	14
20	29	0	45	94	13
21	15	0	33	96	12
22	12	0	26	97	18
23	12	0	35	99	16
24	16	0	38	92	10

Eschenbrenner and Miller(3) suggest that all stages of spermatogenesis may be equally sensitive. Tullis and Barrow(9) note that the basement membrane cells survive longer than the "prespermatogonia" (Bloom) after total-body exposure to 1100 r of x-rays.

Procedure. The technic employed for direct irradiation of the testes has been described in detail(4). It involves direct irradiation of the testes with the rest of the animal shielded. Adult C57 black mice, 50 to 80 days of age were used. Radiation was obtained from a G.E. Maximar, 250 KvP x-ray unit. The following factors were used: 100 KvP, 15 Ma Hvl 2.6 mm Al, 187 r per minute at TSD of 20 cm, for 18 min. 40 sec., total dose 1440 r. All irradiation procedures were carried out from 9 a.m. to 12:00 noon. Two animals were killed at hourly intervals through the 24-hour period post-irradiation (Table I). Untreated animals, 2 for each interval, were killed at hourly intervals for a period of 24 hours. The 2 animals used at each interval were considered adequate for random sampling unless data revealed marked biological variation.‡ Sections representative of the whole of both testes were prepared for microscopic study. The material was fixed in Zenker's solution and stained with Heidenhain's iron haemotoxylin.

Observations and discussion. The tabulation of the frequency of occurrence in untreated animals of resting, mitotic and prophase-like spermatogonia and of the nucleated and meiotic spermatocytes was calculated. The results may be summarized as follows: at any interval approximately 34 tubules out of any 100 observed will reveal the presence of

‡ In determining whether the sample used in this experiment was large enough, trends were computed from the data and tested for significance. Two methods were used in computing trends: (1) Trends were constructed for both normal and irradiated cells, using averages of the 2 figures in a sample; (2) Two individual trends were constructed for each sample of the irradiated group, and the 2 individual probabilities were multiplied together. No significant trends were found in the control group. It would appear that with differences as great as those found in the irradiated group, the number of mice used is significant. The only overall significant downward trend is in the irradiated resting spermatogonia. (Report by Dr. Herbert L. Lombard, Massachusetts Division of Cancer and Other Chronic Diseases, Boston, Mass.)

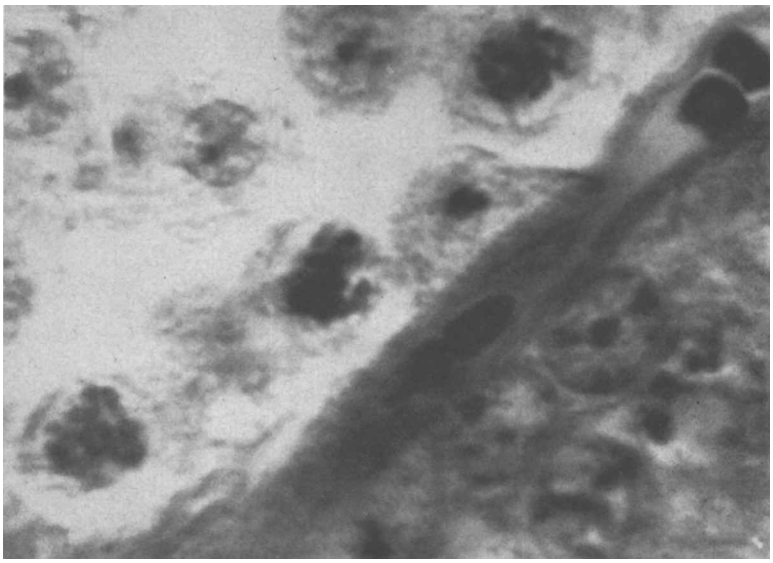

FIG. 1. Normal untreated testis from C-57 pure-lined black mouse. Zenker fixation. Section—6/u. Heidenhain's hematoxylin. The tubule on the left shows 2 typical resting spermatogonia. These can be seen at the periphery. The tubule on the right shows the prophase-like cells lining the periphery.

resting spermatogonia, mitotic spermatogonia will appear 2-3 times out of 100, prophase-like spermatogonia 41 times out of 100, nucleated spermatocytes 86 times and meiotic spermatocytes 10 times.

Spermatogonia. A typical resting spermatogonium, observed in C57 black mice, is seen in Fig. 1. The nucleus is characterized by a definite nuclear membrane, several nucleoli, and dust-like chromatin scattered on a delicate fibrillar network. This is suggestive of the "dust-like" nucleus described by Bloom(2) and also noted by Tullis and Barrow(9). Location of this cell is limited to the periphery of the tubule. The other predominant spermatogonial cell is also represented in Fig. 1. It is characterized by apparent prophasic chromatin threads and may or may not have a nucleolus. Preliminary observations cast some doubt on the identity of this cell. It is possible that it may either proceed into active mitosis and produce two new spermatogonia, or increase in size, develop a prominent spermatocytic nucleolus and evolve into a spermatocyte. Further cytological studies of this cell are in progress. In the examination of 100 cross-sections of tubules of normal testes, spermatogonial mitosis is found to be infrequent. When it does occur there may be several mitotic figures in one median cross-section of a tubule. It is indicated, for example, that of 100 cross-sections examined, only one tubule revealed mitotic spermatogonial cells. This emphasizes the need for investigation of the regenerative rate of the spermatogonial cells. No attempt has been made to differentiate between the various types of resting spermatogonia that have been described in the literature, such as, for example, the large and small cells, or the spermatogonia and the spermatogonia of Bloom(1), nor to differentiate between the variations in intensity of stain.

Spermatocytes. In assembling data on spermatocytes, only two types were considered: (1) the spermatocyte in any phase where the nuclear wall was still present and (2) the active meiosis, whether late prophase, metaphase or telophase. No attempt was made to distinguish between primary and secondary spermatocytes. The nucleated spermatocytes showed little variation in incidence at any interval during the 24-hour period. The meiotic spermatocytes showed some variation but were more or less in a given range for frequency. It is interesting

to note that the incidence for meiotic spermatocytes was higher than for mitotic spermatogonia.

Post-Irradiation. The effects at hourly intervals for 24-hours after acute irradiation are summarized in Table I. It is apparent that (Table I): (a) Spermatogonial mitoses disappear. (b) The resting spermatogonia maintain normal incidence for 20 hours. By 24 hours there is a marked decrease. (c) Prophase-like cells, resting spermatocytes and meiotic spermatocytes appear as frequently as in the controls. These data are in agreement with the general belief that, of the male germ cells, the spermatogonia are the first to react to irradiation. They give further statistical confirmation to the observation that dividing spermatocytes "are on the whole quite radioresistant at least for the first 24 hours" Bloom (1). The reticulated or prophase-like cells in this study do not appear as sensitive as either the mitotic spermatogonia or the resting spermatogonia in the later intervals of the 24-hour period. This is in seeming contradiction to the general belief that cells in prophase are the most sensitive to irradiation (Henshaw) (6). The reticulated or prophase-like spermatogonium may, therefore, represent a differentiated stage, not a typical prophase. Disappearance of cells post-irradiation can be due to progression through differentiation to another type of cell or to degeneration. Therefore, some preliminary observations have been made on cells which are apparently in process of degeneration and on cells which show abnormalities.

Such observations should contribute to earlier evidences of biological effects of radiation than was possible by the presence-or-absence tabulations. Abnormal cells are considered to be those which have fragmented chromosomes, multipolar spindles and variation in size, shape and staining capacity. It is possible that these abnormal cells will recover. Degenerating germinal cells are those which are patently undergoing destruction. The criteria for degeneration are pyknosis, eosinophilic cytoplasm, giant cell formation or cellular disorganization.

The occurrence of degenerating cells is recorded in Table II. In some fields both normal and degenerating forms of any one type of germinal cell are present, but the data are kept separate to avoid confusion.

TABLE II. Incidence of Frequency of Degenerating Germinal Cells in Irradiated Testes. Hourly interval study in 24 hr period post-irradiation.

	Spermatogonia			Spermatocytes	
Time, hr	Resting	Mitotic	Reticulated or prophase-like	Nucleated	Meiotic
1	4	0	3	7	0
2	10	0	3	5	0
3	2	0	15	2	0
4	1	0	7	3	1
5	4	0	4	3	1
6	5	0	10	5	3
7	7	1	22	7	2
8	17	1	28	7	2
9	9	0	31	10	0
10	13	0	20	7	3
11	18	0	14	4	2
12	29	0	35	30	9
13	24	0	29	23	6
14	24	0	33	10	6
15	22	0	17	6	9
16	23	0	11	4	7
17	20	0	11	3	10
18	19	0	7	5	11
19	13	0	10	7	10
20	14	0	14	13	11
21	3	0	5	7	9
22	3	0	6	20	15
23	4	0	7	14	13
24	2	0	8	7	4

The frequency of degenerating spermatogonia increased notably in the period from 8 to 20 hours. Emphasis is attached to the observation that a decline in incidence of resting spermatogonia follows the 20-hour interval. This suggests that some of the spermatogonia are actually undergoing degeneration and not proceeding into spermatocytes. The reticulated or prophase-like spermatogonia also show increased degeneration 6 to 20 hours after irradiation but without the consequent effect noted above for resting spermatogonia. This suggests that these spermatogonia-like cells are reacting to irradiation more like spermatocytes than spermatogonia.

In the 24-hour interval there was no lowering of incidence of spermatocytes although evidences of degeneration were present.

It is recognized that degeneration does occur in the testes of untreated animals. The frequency appears to be of the order of 1 to 6 in 100 tubules. These degenerating cells are few in number in any given tubule.

Summary. Quantitative data are presented to show incidence of spermatogonia and spermatocytes in normal mouse testicles at stated intervals in a 24-hour period, and compared with data for testicles of mice killed at hourly intervals the 24 hours post-irradiation. Testicles were exposed to a single dose of 1440 r for 18 min. 40 sec.

The data indicate that within the 24 hours a pattern of response can be demonstrated which is limited to the spermatogonia. Active mitosis is inhibited for at least the first 24 hours and resting spermatogonia decrease in incidence after 20 hours. Reticulated spermatogonial cells, resting and meiotic spermatocytes do not decrease during the interval studied. Supplementary effects of irradiation are noted by a tabulation of the frequency of degenerating cells, confirming the belief that mitotic spermatogonia and resting spermatogonia are the most sensitive germinal cells. Degeneration of the reticulated spermatogonial cells does not result in a lowered incidence of these cells at any time in the 24-hour period.

1. Bloom, W., *Radiology,* 1947, v49, 346.

2. Bloom, W., *Histopathology of Irradiation from External and Internal Sources,* (1948) pp.55, 568-69, 1st Ed. New York, Toronto and London, McGraw-Hill Book Co.

3. Eschenbrenner, A. and Miller, E., *Arch. Path.,* 1950, v50, 736.

4. Fogg, L. C. and Cowing, R., *Cancer Research,* 1951, v11, 23.

5. ———, *Cancer Research,* 1951, v11, 81.

6. Henshaw, P. S., *Am. J. Roent. and Radium Ther.,* 1940, v6, 913.

7. Marshak, A., PROC. SOC. EXP. BIOL. AND MED., 1938, v39, 194.

8. Maximov, A. and Bloom, W., *A Textbook of Histology,* (1948) p510, 5th Ed., Philadelphia and London, W. B. Saunders Co.

9. Tullis, J. L., and Barrow, J., *The Sequence of Cellular Response to Injury in Mice Exposed to* 1100r *Total Body X-Radiation.* Naval Medical Research Inst., 23:NM 007 039, 1949.

10. Warren, S., 1943, 4-7, Chicago, Cancer Commission of Harvard University, Reprint No. 560.

Received November 26, 1951. P.S.E.B.M., 1952, v79.

Effect of Methylcellulose on Growth Response of Rats to Low Vitamin Intakes. (19283)

R. C. ELLINGSON AND O. N. MASSENGALE. (Introduced by Warren M. Cox, Jr.)
From the Research Laboratories, Mead Johnson and Co. Evansville, Ind.

With increasing use of methylcellulose as a laxative in the treatment of chronic constipation(1-4), the question naturally arises—will continued ingestion significantly decrease the intestinal absorption of certain essential nutrients? Perhaps the question has already been answered in the negative by the fact that no clinical symptoms of vitamin deficiencies have been reported in patients who have ingested methylcellulose for long periods of time. Musick(1) cites the unpublished work of Shapiro, who showed that daily ingestion of methylcellulose did not interfere with the absorption of vit. K. However, for more