tions did not change with increasing age of the calf from 8 to 32 days.

1. Kesler, E. M., and Knodt, C. B., *J. Dairy Sci.*, 1951, v34, 145.

2. Association of Vitamin Chemists, *Methods of Vitamin Assay*, Interscience Publishers Inc., N. Y., N.Y., 1947, p69.

3. Loy, H. W., Jr., *J. Assn. Official Agr. Chemists*, 1947, v30, 392.

4. Steele, H. K., *Cereal Chem.*, 1945, v22, 448.

5. Allfrey, V., Teply, L. J., Geffen, C., and King, C. G., *J. Biol. Chem.*, 1949, v178, 465.

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Development of Tolerance and Cross Tolerance by Mephobarbital.* (19281)

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A review of the literature on the development of tolerance to the hypnotic action of many barbituric acid derivatives in experimental animals has been presented by Gruber and Keyser(1). That cross-tolerance between different barbiturates can also occur has been demonstrated by the same investigators. Since that publication further evidence on the development of tolerance and cross-tolerance between the barbituric acid derivatives has been demonstrated by Carmichael(2), Hubbard and Goldbaum(3) and Holck and his associates(4). There have been reports in the literature, however, which indicate that no tolerance can be developed with mephobarbital (Mebaral) 5-ethyl-1-methyl-5-phenylbarbituric acid(5-8) but we have been unable to find any report in which a study has been made to determine actually whether there can or can not be developed tolerance to this chemical in experimental animals. This investigation was therefore undertaken to answer this question and also if tolerance can be developed with mephobarbital in experimental animals does it also produce cross-tolerance.

Methods. Seventeen rabbits, 8 males and 9 females, weighing between 2.1 and 2.6 kg were used. The sodium salt of mephobarbital was employed for injection. This salt was formed by neutralizing the acid with sodium hydroxide and back-titrating with hydrochloric acid to the lowest pH possible without precipitation. Solutions were made up in distilled water and the concentrations adjusted so that 2 cc/kg were given as the final dose. Mephobarbital (40 mg/kg once daily for three days) was injected rapidly, intravenously and the length of induced sleeping time noted following each injection. On the fourth day butabarbital sodium (36 mg/kg) was similarly administered. The same dose of butabarbital was injected on the fourteenth day as a control for this drug. The criterion for development and abolition of sleep was the loss and gain of the righting reflex respectively(9).

Results. Evidence recorded in the literature indicates that there is no essential sex difference in rabbits in response to the action of certain barbiturates(1,9,10). Our results are in accord with these findings. Following the injections of mephobarbital on the first, second and third days, the males slept an average of 19.5, 7.7 and 7.3 minutes respectively while the females slept an average of 16.2, 10.1 and 4.8 minutes respectively. The results with the use of butabarbital also showed no significant difference in the sleeping times between the males and females. Therefore the results obtained from the male and female animals were combined. The mean sleeping times for the first, second and third daily injections of mephobarbital were 17.8, 8.9 and 5.9 minutes respectively and two times the standard errors were $\pm$ 5.4, $\pm$ 1.9

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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.

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

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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

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