References and Notes

1. B. F. Skinner, *The Behavior of Organisms* (Appleton-Century, New York, 1938), p. 306; M. Sidman, *Science* **122**, 925 (1955).
2. A fourth rat died on the second day following the first nicotine injection.
3. Since the graphed data represent a third cycle in the experiment, the rats may have acquired a tolerance for the drug and therefore show little if any immediate nicotine effects.
4. T. Sollmann, *A Manual of Pharmacology* (Saunders, Philadelphia, 1957), p. 454.

5. The authors wish to express gratitude to Larry Stein for his critical reading of the paper.

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Autoradiographic Investigation of Incorporation of H^3-Thymidine into Cells of the Rat and Mouse

Abstract. The application of H^3-thymidine results in labeling of those nuclei of cells in which deoxyribonucleic acid (DNA) is synthesized during the interval between application and the sacrifice of the animal (*1–3*). This paper reports autoradiographic investigation with H^3-thymidine of rats and mice. This method permits a more exact statement of the number of dividing cells than does the microscopic estimate of mitosis. The latter method is practically impossible in tissues with small fusiform cells. Moreover, it is possible to obtain information about the relative time of DNA synthesis in different cells.

Adult rats and mice received 460 and 50 μc, respectively, of H^3-thymidine by intraperitoneal injection, and were sacrificed after 90 and 60 minutes, respectively. Autoradiograms were prepared from 5-μ, paraffine-imbedded slices, by use of the stripping-film method (Kodak autoradiographic plates AR 10), or with liquid emulsion (Ilford G 5). Exposure times were up to 20 days.

The autoradiographic blackening was exclusively limited to a certain percentage of nuclei and matched the distribution of the chromatin in the nuclei. Nucleoli with a diameter above 2 μ appeared as white spots within the silver grain covered chromatin. A remarkable result was that the nuclei either were labeled rather uniformly or were absolutely free from silver grains. For that reason the percentage of labeled nuclei in a section was independent of the exposure time.

The relative numbers of labeled nuclei (H^3 index) and of mitosis (mitosis index) were determined for different tissues and the percentages these formed of the total number of examined nuclei were calculated. Table 1 gives the results.

The examined tissues can be classified in three groups with a distinct difference in the percentage of H^3-labeled nuclei. These three groups correspond to the scheme given by Cowdry (*4*).

Fixed postmitotic cells (central nervous system, musculature). No labeled nuclei are found in ganglionic cells, in cells of striated muscle, or in cells of the heart muscle. However, in all three tissues there are some mesenchyme cells with labeled nuclei. Likewise, some labeled nuclei were found in the choroid plexus and in cells of the subependymal regions of the brain. Contrary to the case of striated muscle, there are few labeled nuclei in the cells of smooth muscle.

Reversible postmitotic cells (parenchymatous organs). In the liver the distribution of labeled nuclei does not favor certain lobes nor parts of lobules. Liver cells with two nuclei were rather often seen in which both nuclei were equally covered with silver grains. The percentage of labeled Kupffer cells is markedly higher than that of the labeled epithelial cells of the liver. In the kidney the labeling of the nuclei occurs considerably less often in the epithelial cells of the canaliculi than in the endothelial cells of the glomeruli and of Bowman's capsule. No preference of labeling is given to any parts of the canaliculi. There are no accumulations of labeled nuclei in the different layers of the adrenal cortex. In the pancreatic epithelia and in the islets of Langerhans the number of the labeled nuclei corresponds with the number in the organs mentioned above.

Accumulations of labeled nuclei are found in the peribronchial tissue of the lungs. Within the bronchial tubes the labeled epithelial cells belong partly to the basal and partly to the upper layer of cells of the ciliated epithelium. Labeled nuclei are only sporadically distributed in the single-layered pleural endothelium. There are increased numbers of labeled pleural endothelia over subpleural inflammatory infiltrations. There is a remarkably high number of labeled nuclei of alveolar cells.

It is remarkable that in all parenchymatous organs the number of labeled cells is much smaller in the parenchyma than in the interstitial connective tissue and reticuloendothelial system.

Vegetative intermitotic cells (epithelium of skin and mucous membranes, lymphatic tissues, testicle, connective tissue). In the abdominal skin and in the esophagus, labeled nuclei are found in the basal cellular layer only. In the stomach, labeled cells appear more often in the lower third of the gastric foveolae than in the glands. Only very few labeled nuclei are seen in the Brunner's glands of the duodenum. Almost every other cell of the Lieberkühn's crypts is labeled. The epithelia of the villi are free of labeled nuclei. Similar statements are true for the jejunum and the ileum. In the colon the major density of labeled cells is found just above the bases of the crypts. Some nuclei of large basophil stem cells were labeled in the germinative centers of the spleen and of the lymphatic glands. Few labeled nuclei of large basophil reticular cells were detected in the peripheral zones of the lymphatic follicles and of the Malpighian corpuscles. Accumulations of labeled nuclei are specially found in the sinus of the spleen and originate in the relatively small nuclei of the prolymphocytes (*5*). Here, according to the more solid arrangement of the chromatin, the silver grains are closer

Table 1. Percentage of nuclei labeled by H^3-thymidine and percentage of mitosis, for cells of various organs.

Organ	Kind of cells	H^3 index (% of labeled nuclei)	Mitosis index (% of mitosis)	H^3 index ÷ mitosis index
	Group 1			
Central nervous system	Ganglionic cells	0		
Heart muscle; skeletal muscle	Cells of striated muscle	0		
Gastrointestinal tract	Cells of smooth muscle	0.28		
	Group 2			
Liver	Liver epithelia	0.4	0.04	10
	Kupffer cells	1.2		
Kidney	Epithelia of canaliculi	0.6	0.05	12
	Endothelia of glomeruli	4.0		
Suprarenal gland	Cells of the cortex	0.4	0.04	10
Pancreas	Pancreatic epithelia	0.83	0.073	11
	Cells of Langerhans' islets	0.8		
Lung	Surface cells of alveoli	1.8		
	Bronchial epithelia	1.0		
	Group 3			
Abdominal skin	Basal layer	4.0	0.52	8
Esophagus	Basal layer	20.0		
Duodenum	Epithelia of mucous membrane	17.0	1.1	15
Jejunum	Epithelia of mucous membrane	15.0		
Colon	Lieberkühn's crypts	8.2	0.72	11

packed than over the large nuclei of cells of the germinative centers. The testicles contain canaliculi, in which practically all spermatogonia are labeled with almost an equal number of silver grains. On the other side there are canaliculi without a labeled spermatogonium. Nuclei of spermatocytes and sperms are never labeled within the chosen experimental time intervals. In the connective and adipose tissues, labeled nuclei are found in a surprisingly great number. They apparently form a reservoir of undifferentiated mesenchyme cells with a marked tendency to proliferation (*2, 6*).

As far as the same organs were examined, these results agree very well with those of Leblond *et al.* (*6*) and of Pelc (*3*) obtained from rats after a single application of H^3-thymidine.

The figures given in Table 1, columns 3 and 4, are in close relationship with the time interval during which deoxyribonucleic acid (DNA) is synthesized, with the duration of the microscopically detectable stages of the metaphase and anaphase of the mitosis, and with the life span of the cell. One may express the H^3 index and the mitosis index as

$$H^3 = \frac{\text{duration of DNA synthesis}}{\text{life span of cell}} \quad (1)$$

$$\text{Mitosis} = \frac{\text{duration of mitosis}}{\text{life span of cell}} \quad (2)$$

If the cell formation in a tissue is due to mitosis only, the divisors in Eq. 1 and Eq. 2 are equal. Then the division of Eq. 1 by Eq. 2 results in

$$\frac{H^3}{\text{Mitosis}} = \frac{\text{duration of DNA synthesis}}{\text{duration of mitosis}} \quad (3)$$

Equations 1 and 2 have a different meaning in the eventual case of amitosis and the formation of nuclei with polyploid chromosome numbers. However, this case cannot be discussed here.

It is remarkable that the quotient H^3 index/mitosis index, which is given in column 5 of Table 1, is almost equal in all investigated tissues and is approximately equal to 10. Even the liver does not differ from this general rule. This means that the duration of the DNA synthesis should be 10 times longer than the duration of the mitosis. This agrees with the present opinion that the DNA synthesis occurs during the interphase and is not connected with the much faster mitosis.

Knowlton and Widner (*7*) reported that the duration of mitosis is equal in different tissues of mice and lasts 20 to 36 minutes. Our own autoradiographic work with H^3-thymidine and H^3-cytidine on the percentage of labeled mitosis figures resulted in a value of 20 to 30 minutes. Assuming that these figures represent the real conditions, the time that DNA synthesis requires would be about the same for all cells and would last about 5 hours. Moreover, the life span of cells of the third group in Table 1 should amount to 1 to 5 days, and that of the cells of the second group 30 to 50 days. These theoretical estimates based on data of the mitosis index are in accord with the results of work already accomplished by others (*7, 8*). But while mitosis is often not detectable at all, or at least not with certainty, by microscopic examination, it is traceable by radioactive labeling.

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Genetic Control of Two γ-Globulin Isoantigenic Sites in Domestic Rabbits

Abstract. Results of immunochemical analysis of sera from 335 offspring of 81 litters of rabbits are consistent with the hypothesis that the isoantigenic sites, RGG-I and RGG-II, of the γ-globulins are controlled by a single allelic pair of autosomal genes with both specificities exhibited by the heterozygote. The three genotypes may be designated γ^I/γ^I, γ^{II}/γ^{II}, and γ^I/γ^{II}.

Recent investigations have shown that components from individual rabbits are antigenic in other rabbits (*1–3*). Subsequently, 500 domestic rabbits (*Oryctolagus cuniculus*) of several breeds could be separated into three groups on the basis of two isoantigenically different γ-globulin specificities (*4*). These γ-globulin specificities, designated RGG-I and RGG-II, were demonstrated with specific isoprecipitins by agar gel immunochemical methods. Individual rabbits were found to contain either RGG-I, RGG-II, or both RGG-I and RGG-II in their sera but never lacked both γ-globulin specificities. Of 500 rabbit sera tested, there were 24 with only RGG-I, 379 with only RGG-II, and 97 with both RGG-I and RGG-II (*4*).

The simplest genetic hypothesis for the control of the three phenotypes is that the two isoantigenic sites RGG-I and RGG-II are controlled by a single allelic pair of autosomal genes with both specificities exhibited by the heterozygote. The three genotypes may be designated γ^I/γ^I, γ^{II}/γ^{II}, and γ^I/γ^{II} and correspond to the phenotypes RGG-I, RGG-II, and RGG-I/RGG-II. Of the 500 rabbits, 162 were from a small closed colony of Flemish giants (4 sires, 20 dams) at the National Institutes of Health which are bred according to a plan to minimize inbreeding and thus possibly approach the conditions of the Hardy-Weinberg law (*5*). The distribution of RGG groups among this population was as follows: 19 RGG-I, 68 RGG-II, and 75 RGG-I/RGG-II (*6*). According to the hypothesis, the gene frequencies would be $.35\gamma^I$ and $.65\gamma^{II}$. When the Hardy-Weinberg formula is applied for these gene frequencies in a random-bred population of 162, the expected distribution of phenotypes is calculated to be 19.8 RGG-I, 68.4 RGG-II, and 73.8 RGG-I/RGG-II, in close agreement with the experimental findings (probability, .98 to .99).

The purpose of this investigation was to test the above genetic hypothesis directly by analysis of the progeny of 81 litters of domestic rabbits obtained from all six possible matings of the three groups for the presence of RGG-I and RGG-II in their sera.

The γ-globulin isoantigenic sites RGG-I and RGG-II were identified in the sera by the agar gel methods described previously (*2, 4*). The sera were obtained from 8- to 9-week-old rabbits. As a control for the absence of maternal γ-globulin, many of the offspring were tested again several months to a year after the initial test, and such tests always confirmed the original typing of the sera obtained at 8 to 9 weeks. Of 335 progeny tested, 208 were produced by the animal production section of NIH (*7*), 90 by a commercial breeder (*8*), and 37 by our own laboratory. Only in our own laboratory was breeding selective on the basis of known γ-globulin phenotypes.

Table 1 presents the γ-globulin phenotypes of the 335 offspring. The experimentally determined distribution of progeny among the three γ-globulin groups is generally in accord with the genetic hypothesis.

The unexpected deviation of the experimentally determined and theoretically expected RGG groups of the offspring resulting from the backcross