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

Table 1. Hypothesis of genetic control of γ-globulin isoantigenic sites by an allelic pair of autosomal genes tested by examining progeny from 81 litters of domestic rabbits resulting from all six possible matings among the three known phenotypes, namely those with only RGG-I, only RGG-II, and both RGG-I and RGG-II in their sera.

RGG group of parents		Litters (No.)	Progeny (No.)	No. of progeny in each RGG group						Probability
				Experimental			Theoretical			
Dam	Sire			I	II	I/II	I	II	I/II	
I	I	2	9	9	0	0	9	0	0	
II	II	27	125	0	125	0	0	125	0	
I	II	4	12	0	0	12				
II	I	5	17	0	0	17				
Totals		9	29	0	0	29	0	0	29	
I	I/II	6	25	4	0	21				
I/II	I	5	10	7	0	3				
Totals		11	35	11	0	24	17.5	0	17.5	.02–.05
II	I/II	16	69	0	37	32				
I/II	II	3	13	0	5	8				
Totals		19	82	0	42	40	0	41	41	.8–.9
I/II	I/II	13	55	14	15	26	13.7	13.7	27.5	.8–.9
Test for no association with sex										
180 bucks				17	98	65	18.3	97.8	64	
155 does				17	84	54	15.7	84.2	55	.90–.95
335 progeny				34	182	119				

to RGG-I rabbits could have occurred by chance alone but might be related to the fact that four of the litters, comprising 17 rabbits, were offspring of the same parents. Only one of these offspring was an RGG-I phenotype, while the other 16 were RGG-I/RGG-II phenotypes.

A study of litters of the sire and dam with other mates might lead to possible explanations of the deviations observed. The RGG-I/RGG-II sire had given rise to five litters with three RGG-II dams to yield 11 RGG-II and 12 RGG-I/RGG-II phenotypes, in close agreement with the hypothesis. Unfortunately, the sire is no longer available for other matings, but heterozygote buck and doe offspring are available which have the γ^I allele derived from the sire. A pair of such heterozygotes gave rise to two litters with a phenotype distribution of 1 RGG-I, 7 RGG-II, and 10 RGG-I/RGG-II (probability, .1 to .2), again suggesting some difficulty in yielding RGG-I phenotypes. The RGG-I dam of the four deviant litters is still available, and perhaps phenotypes of her offspring will give additional clues for investigation of factors, possibly lethal or semilethal, which might be associated with the less frequently occurring allele, γ^I.

As of now, 1006 domestic rabbits (*Oryctolagus cuniculus*) of more than 16 breeds have been typed according to their γ-globulin isoantigenic sites: 61 were type RGG-I, 749 were RGG-II, and 196 were RGG-I/RGG-II. In addition, four San Juan rabbits (*Oryctolagus cuniculus*), obtained from the San Juan Islands, Washington (derived from European stock prior to the myxoma epizootic and living in the wild state) were typed RGG-II (*9*). Only a few of the colonies tested showed the presence of the γ^I gene, beyond that of an occasional heterozygote. The fact that no domestic rabbits have been found with neither RGG-I nor RGG-II is further corroboration of the genetic hypothesis but does not exclude the possibility of additional alleles at the same locus present at low frequency (*10*). Also, it must be emphasized that γ-globulin is a heterogeneous mixture of proteins and that the genetic conclusions pertain only to the isoantigenic portion of those molecules which have the site in question.

The isoantigens should be useful in the study of differentiation of species. The sera of other lagomorphs, namely, 28 cottontail rabbits (three or four species of *Sylvilagus*), 24 jack rabbits (*Lepus californicus deserticola*), 2 snowshoe hares (*Lepus americanus*), and 2 pikas (*Ochotona collaris*), were tested for RGG-I and RGG-II with isoprecipitins of the domestic rabbits, and none were found to have either RGG-I or RGG-II (*11*). Neither did the sera of guinea pig, rat, mouse, hamster, chicken, sheep, horse, cat, dog, monkey, or man show any RGG-I or RGG-II. Thus it would appear that these isoantigenic sites are genus-specific.

In the heterozygotes, the allelic genes are most likely to produce two distinct γ-globulins, each indistinguishable from the γ-globulin of the corresponding homozygote. Nevertheless, hybrid substances are known to occur, and this question is under investigation (*12*).

The molecules having isoantigenic sites under the genetic control postulated in this report (*13*) are soluable proteins, namely γ-globulins. Some of these molecules may also react as antibodies. As antigens, precipitable by antibodies, they should be subject to quantitative estimation and cytological studies. Moreover, these γ-globulins may be expected to pass through maternal-fetal barriers. Thus, this immunogenetic system may be uniquely suited for investigation of some basic problems in genetics, embryology, immunology, and protein chemistry. Investigations of other protein isoantigens in serum and of such isoantigens in other tissues and species should also be encouraged (*14*, *15*).

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7. We express our gratitude to Samuel M. Poiley and Robert D. Dettman of the animal production section of NIH for their helpful advice and assistance throughout the course of this work.
8. Thompson's Rabbit Farm, Reisterstown, Md.
9. We thank Murray L. Johnson, Tacoma, Wash., for the sera of San Juan rabbits.
10. Subsequent to our submitting this report for publication, S. Dubiski (Institute of Haematology, Warsaw, Poland) kindly sent us a sample of serum from one rabbit (No. 394) which has neither RGG-I nor RGG-II, suggesting that an additional allele may be present at the same locus in some rabbit populations.
11. Our thanks are expressed for gifts of lagomorph sera from Richard E. Shope, Samuel B. Salvin, Robert Rausch, Murray L. Johnson, Herbert T. Dalmat, and Griffith E. Quinby.
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13. This paper was presented at the annual meeting of the Genetics Society of America, Pennsylvania State University, 31 Aug. 1959.
14. We have recently identified a third γ-globulin isoantigenic site, RGG-III, in the sera of domestic rabbits. The genetic and immunologic relationships of RGG-III to RGG-I and RGG-II are being investigated.
15. Comparison of our isoimmune sera with isoimmune sera received recently from Dr. Dubiski corroborates his personal communication to the effect that he and his collaborators have found still more γ-globulin isoantigens.

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