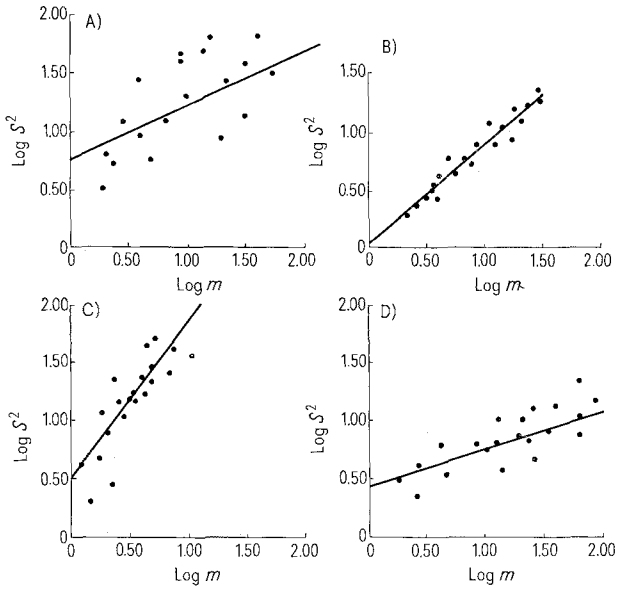
populations are aggregated. But when the males (Figure C), females (Figure B) and immature stadia (Figure D) in the populations are considered separately, b was 0.46 for males, 0.86 for females and 0.30 for immature stadia.



Plot of log variance and log mean for *Trigoniulus lumbricinus* (Gerst) A) entire population; B) female fraction of the population; C) male fraction of the population; D) immature stadia in the population.

For males and immature stadia $b < 1$ suggests the tendency for the individuals to be over dispersed for small means, and underdispersed, i.e. approaching a more regular spatial distribution, for larger means. Only for the females, the distribution is close to random because a and b are both close to 1.00. However, describing randomness on the basis of these two parameters is difficult because every pair of value a, b for which $a\,m^b = m$ holds will indicate randomness. This would be true not only when $a = 1$ and $b = 1$, but for all values satisfying $\log a = (1 - b) \log m$.

Even $b = 1$, $a > 1$ which indicates variance is proportional to the mean over the whole range of observations with the exponent K proportional to the mean m, such that $(1 + K/m) = a$, is a constant, is not a sufficient condition to indicate negative binomial distribution of the individuals. Many other distributions could lead to the same configuration: only certain negative binomial distributions could be described in this way.

Moreover, the distribution patterns of arthropods are known to vary according to the behaviour of the developmental stages of the animals[5], and even seasonally[6,7]. Consequently b as an index cannot remain constant for a species, but will vary according to the temporal distribution of the constituent units of the population, as indeed the males, females and immature stadia of *T. lumbricinus* show.

[5] B. BANERJEE, Sci. Cult. *36*, 236 (1970).
[6] B. BANERJEE, J. Anim. Ecol. *36*, 171 (1967).
[7] S. W. WRATTEN, J. Anim. Ecol. *43*, 191 (1974).

Nucleolar Hypertrophy and Nuclear DNA Replication in Liver[1]

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Summary. Hypertrophy of nucleoli is associated with DNA synthesis in the hepatocytes of untreated rats just as it is in stimulated animals. Nucleolar enlargement is not a sufficient change to ensure that the parenchymal liver cell can make DNA.

In recent years, much attention has been given to defining the changes that endow a resting cell with the ability to replicate its nuclear DNA. One impediment, particularly with systems in vivo, has been the difficulty in distinguishing between the prereplicative alterations that are directly concerned with entry into the S period from those that serve other functions.

Increases in nucleolar size and function are among the early changes that take place in the parenchymal liver cells of rats that have been stimulated to make hepatic DNA by surgical as well as by nonsurgical means[2–4]. We have been interested to learn whether the same nucleolar changes occur in hepatocytes that are making DNA in the untreated rat. Occurrence of the alterations in the replicating cells of the untreated animal would lend support to a relationship between the nucleolus and the control of DNA replication. Absence of the changes would rule out an obligatory relationship between the nucleolar changes and DNA formation.

As a partial test of this question, nucleolar volumes were compared in replicating and resting hepatocyte nuclei from untreated rats. The main purpose of this report is to show that replicating liver nuclei from unstimulated animals contain a much greater volume of nucleolar material than do resting nuclei.

Material and methods. To compare nucleolar sizes in replicating and resting hepatocyte nuclei, rats (female, Fischer 344, Charles River Breeding Laboratories) were labeled with ^{3}H-thymidine and about 150 mg portions of liver were then removed and immediately homogenized in 10 ml of a buffered solution of formaldehyde and glutaraldehyde[5]. After fixation (4 h or more at ambient temperature), a nuclear fraction was prepared by centrifugation in sucrose and the nuclei were affixed to glass microscope slides as previously detailed[4]. Radioautography was with six-fold diluted Kodak Track Emulsion, type NTB 3 (4 day exposure). With diluted emulsion, but not with the undiluted preparation, all the silver grains were at the periphery of labelled nuclei, rather than over

[1] This work was supported by a grant from the National Cancer Institute.
[2] H. SWIFT, L. REBHUN, E. RASCH and J. WOODARD, in *Cellular Mechanisms in Differentiation and Growth* (Ed. D. RUDNICK; Princeton University Press, Princeton, New Jersey 1956), p. 45.
[3] S. CHAUDHURI, O. DOI and I. LIEBERMAN, Biochim. biophys. Acta *134*, 479 (1967).
[4] R. P. BAILEY, W. A. RUDERT, J. SHORT and I. LIEBERMAN, J. biol. Chem. *250*, 4305 (1975).
[5] M. J. KARNOVSKY, J. Cell Biol. *27*, 137A (1965).

Table I. Nucleolar volumes in replicating and nonreplicating hepatocyte nuclei of growing untreated rats

Labeled nuclei (%)	Volume of nuclei (μm^3) per nucleus	
	Unlabeled	Labeled
1.6	4.3 ± 0.78	7.0 ± 1.0
2.0	4.3 ± 0.84	6.7 ± 0.78

Each of 2 rats (55 g) was given 100 μCi of ^{3}H-thymidine in the tail vein and portions of liver were removed after 1 h. Nucleolar volumes were estimated after radioautography from measurements of 200 unlabeled and 30 labeled nuclei and standard deviations are shown.

Table II. Nucleolar volumes in replicating and nonreplicating hepatocyte nuclei of stimulated rats

Infusate	Labeled nuclei (%)	Volume of nucleoli (μm^3) per nucleus	
		Unlabeled	Labeled
NaCl	0.1	4.3 ± 0.83	
TK solution	16	6.6 ± 1.0	8.4 ± 1.6
	24	6.8 ± 1.3	8.6 ± 1.1

2 rats (150 g) were infused in the tail vein for 3 h (3.3 ml/h) with the TK solution[6] (100 μg of 3,3′,5-triiodo-L-thyronine, 5 μmol of dibutyryl cyclic AMP, 10 mg of theophylline, 300 mg of amino acids, and 100 U.S.P. units of heparin). At 22 h from the start of infusion, each animal was given 200 μCi of ^{3}H-thymidine and liver samples were taken 1 h later. The control animal was infused with 0.15 *M* NaCl. Nucleolar volumes were estimated after radioautography from measurements of 200 unlabeled and 100 labeled nuclei and standard deviations are shown.

[6] J. Short, K. Tsukada, W. A. Rudert and I. Lieberman, J. biol. Chem. *250*, 3602 (1975).

the nuclei, and visualization of the nucleoli was not obstructed. The number of grains around labeled nuclei ranged from 12 to 27 and unlabeled nuclei had no grains. Nucleoli were stained after radioautography by flooding the slides with 0.005% azure B in 0.01 *M* sodium citrate–0.01 *M* sodium phosphate buffer, pH 5.5, and allowing the stain to evaporate at 55 °C. Nucleolar measurements were made at a magnification of 2500 diameters as described before[4].

Results and discussion. Table I shows the results that were obtained with 2 untreated, growing rats. About 2% of the hepatocyte nuclei were labeled with ^{3}H-thymidine and the volume of nucleolar material in the labeled nuclei was 1.5-times greater than in the unlabeled nuclei (representing about a 20% increase in diameter). The Table does not show that the average number of nucleoli per labeled and unlabeled nucleus was the same, 2.5. These observations are consistent with a relationship between the regulation of some nucleolar function and the control of liver DNA formation.

The results of Table I do not exclude the possibility that nucleolar enlargement in the untreated animals took place, not prereplicatively, but only after the nuclei had entered the S period. This, however, would seem to be unlikely since hypertrophy begins immediately and is already maximal at the time of DNA synthesis in partially hepatectomized animals[2] and in unoperated rats that have been given biochemicals to induce hepatic DNA synthesis[4].

The question was also asked whether nucleolar enlargement is a sufficient change to ensure that the parenchymal liver cell can form DNA. Mature rats (150 g) were infused with a mixture of biochemicals (TK solution[6]) that causes nucleolar enlargement in the liver[4] and induces hepatic DNA synthesis[4,6]. The animals were then labeled with ^{3}H-thymidine and nucleolar volumes were compared in replicating and nonreplicating hepatocyte nuclei. It can be seen from Table II that even nonreplicating nuclei contained hypertrophied nucleoli although enlargement was more pronounced in the replicating nuclei.

Sterility in Tsetse Flies (*Glossina morsitans* Westwood) Caused by Loss of Symbionts

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Summary. Tsetse flies fed on blood containing oxytetracycline, sulphaquinoxaline or lysozyme do not reproduce. It could be proved that primarily the symbionts in flies are damaged, which secondarily leads to sterility.

Since certain bacterial infections cause high mortality in tsetse fly colonies (*G. morsitans* Westw.), when fed through artificial membranes, different antibiotics were applied. Oxytetracycline was found to be most effective. However, fecundity of the colonies also dropped drastically[2]. Similarly, *G. austeni* Newst. fed upon rabbits, whose diet contained a coccidiostat, sulphaquinoxaline and pyrimethamine, showed a marked fall in fecundity[3].

In both cases, it can be suggested that primarily the symbionts in flies are damaged which secondarily leads to sterility. This assumption is also supported by earlier investigations, where the production of symbiont-free *G. morsitans* was associated with loss of female fertility[4]. Since the antibiotics were applied through i.m. injection to rabbits, nothing could be said about the precise uptake of antibiotics by the flies.

To elucidate the problem, tsetse flies were treated with oxytetracycline, sulphaquinoxaline and lysozyme in 3 different experiments. The symbionts, located in a mycetome in the anterior part of the midgut were controlled with histological and microbiological tech-

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[2] H. Wetzel and B. Bauer, Symposium on the Sterility Principle for Insect Control, Innsbruck, 22.–26. 7. 1974.

[3] A. M. Jordan and M. A. Trewern, Nature, Lond. *245*, 462 (1973).

[4] P. Hill, D. S. Saunders and J. A. Campbell, Trans. R. Soc. trop. Med. Hyg. *67*, 727 (1973).