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.

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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-

[1] I thank Dr. A. M. Jordan, Tsetse Research Laboratory at Bristol, for providing me with pupae of *G. morsitans*, Dr. H. Wetzel, International Atomic Energy Authority, Vienna, for providing antibiotictreated flies and the Gesellschaft für Strahlen und Umweltforschung, München, for financial support.

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niques. Excised mycetomes were either smeared with a needle on a microscope slide and stained with Giemsa fluid, or fixed in Bouin fluid, microtomed into 7 µm sections and stained with Mallory stain for microscopic examination. Mycetomes, dissected under sterile 0.5% NaCl-solution, were incubated in cultures of *Lactobacillus plantarum* and *Streptococcus faecalis*. The test bacteria were inoculated in vitamin assay media containing all necessary growth factors exept patothenic acid and folic acid for *L. plantarum* and *S. faecalis* respectively. By microbiological assay, it was found that the symbionts produce these and other vitamins of the B-group[5].

At the International Atomic Energy Authority, Vienna, tsetse flies were fed on difibrinated horse blood mixed with 25 ppm, 250 ppm and 2500 ppm of oxytetracycline through a silicone-membrane and were sent for further investigation. While there was no difference in longevity after treatment with 25 ppm dosage as compared to the normal flies, higher concentrations always caused increased mortality. LD_{50} for 250 ppm was 18 days, while that for 2500 ppm was 3 days. No offspring were produced in all 3 groups of flies. Microscopical examination proved that the symbionts were severely damaged in all the cases.

A second group of tsetse flies was fed daily on rabbits that were treated with the coccidiostat Sulka (Pharmazeutisches Werk Cuxhaven), containing sulphaquinoxaline in a final concentration of 75 ppm. After 1 and 2 week periods, symbionts in the flies were examined and found to show signs of destruction. Microbiological assay of the mycetomes revealed drastically low amounts of pantothenic acid and folic acid.

In a present research-program on the significance of symbiosis in tsetse flies, lysozyme was applied orally to eliminate the symbionts. It was possible to reduce the dosage of lysozyme so that longevity of the flies was not affected, yet fertility was completely eliminated. Destruction of the symbionts could be proved both by microscopical and microbiological tests.

From these results, it can be concluded that symbionts play an important rôle in tsetse fly reproduction. It is interesting that oxytetracycline does not effect any follicle larger than about 1/4 of its mature size[2]. Recently described rickettsia-like symbionts located in the ovaries of tsetse flies[6], that may also be effected by bactericide drugs, have not been examined in this investigation. Preliminary studies on the significance of endosymbiosis in tsetse flies show that the symbionts provide their hosts with certain vitamins of the B-group[5]. In mosquitoes, where endosymbionts are absent, the reaction of sulphaquinoxaline was antagonized by simultaneous administration of *p*-aminobenzoic acid[7]. In the current experiments with aposymbiotic tsetse flies, it was possible partly to compensate the loss of symbionts by diets supplemented with different B-vitamins.

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Early Development of Gap Junctions Between the Mouse Embryonic Myocardial Cells. A Freeze-Etching Study[1]

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Summary. Using the freeze-etch technique, nexuses have been shown to exist at a very early stage in developing mouse hearts (10 dpc). At this time they are rare, but become more progressively frequent and extensive at 12 and 14 dpc. Special arrangements of particles progressively observed on the fracture faces PF (linear arrays, small associated groups of linear arrays, then hexagonal arrays with 'arms' formed by linear clusters) suggest that in ontogenesis the gap junctions may be built up by successive aggregation of the linear arrays of particles.

Nexus-type junctions or gap junctions, believed to be the sites of low intercellular electrical resistance[3], are common and sometimes extensive in mature mammalian and avian cardiac musculature, particularly in Purkinje fibres. The structure has been described in detail by MCNUTT[4] and MCNUTT and WEINSTEIN[5,6] using both thin sections and freeze-cleave preparations. However, these junctions are rare in early embryonic cardiac muscle, although MUIR[7] observed a few nexuses in the 14-day post coitum (dpc) rat embryonic myocardium. PAGER's[8,9] observations confirm the studies of MUIR[7], and in addition she found 'contact points' (contacts ponctuels) between cardiocyte membranes at the 11th day of the embryonic life in the rat. In embryonic mouse heart, the earliest stage at which gap junctions have thus far been reported is 13-days post fertilization[10,11]. In chick embryonic heart, SPIRA[12] found close oppositions (a 4 nm gap) between cells and freeze-etch studies[13] have also demonstrated small nexuses in chicken myocytes. In addition, gap junctions have been observed in the 9-week human fetal ventricular cardiac muscle[4].

[1] Supported by the Alberta Heart Foundation, National Research Council of Canada and the Délégation Générale à la Recherche Scientifique et Technique (Contrat No. 73.7.1201.01).
[2] One of us, (D. GROS), wishes to thank Dr. W. COSTERTON and Mr. D. COOPER for introducting him to the freeze-etching technique. We wish to thank Miss D. DECOURT and Mr. E. BLANCHARD for assistance in preparing the manuscript and the micrographs.
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