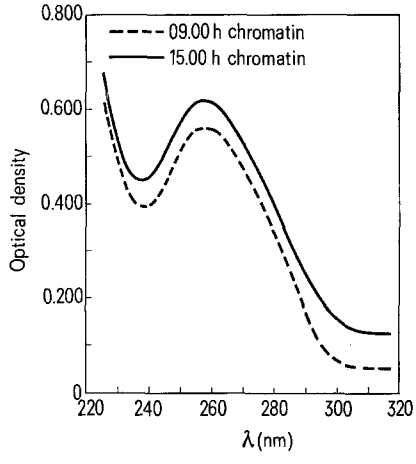
to corresponding functional states of genome previously reported[1,3,4].

Methods. 7-week-old male albino rats of Wistar strain, obtained from the departmental animal house, weighing 200–220 g have been used in these experiments. The rats were housed since weaning in an air-conditioned windowless room with an inverted and displaced lighting schedule in which lights were on from 21.00 h to 09.00 h in a 24 h cycle. The food, a purina chow lab diet, was supplied just before the lights were switched off, and was removed 8 h later according to the '8 + 16' feeding schedule developed by POTTER et al.[2]. Water was supplied ad libitum.

Rat liver chromatin was extracted from the purified nuclei[9] as indicated by BUTTERWORTH et al.[10]. Chromatin resuspended in *Tris*-HCl 1 m*M*, pH 8, was sheared and diluted to 50–100 µg of DNA/ml with the same buffer. Optical measurements were done with a Cary-15 spectrophotometer. DNA was determined by the diphenylamine reaction of BURTON[11], and proteins by the method of LOWRY et al.[12].

Results and discussion. The Figure reports the absorption spectra of liver chromatin extracted at 09.00 h or at 15.00 h from rats under the controlled feeding schedules of POTTER et al.[2]. These 2 times of day have been chosen as representative of low (09.00) or high (15.00) rate of RNA synthesis respectively in our experimental conditions[1,3,4]. There is a marked difference between the UV-absorption spectra of liver chromatin extracted at 09.00 h from that extracted at 15.00 h. This difference is represented by an increase in magnitude of the band at 258 nm in chromatin extracted at 15.00 h. No red- or blue-shifts of the main band are observed.



Absorption spectra of liver chromatin extracted at 2 different times of day from rats maintained under controlled feeding schedules. Measurement conditions were as described in the text. The above experiment was performed with a chromatin solution containing 64 µg DNA/ml.

A control measurement performed on chromatin extracted at 15.00 h from rats starved on the day of the experiment showed that the UV-absorption spectrum was overlapping with the one obtained from rats killed at 09.00 h.

Data reported in the Table show that the food intake is able significantly to increase the band at 258 nm by 12% over the corresponding value obtained from chromatin extracted at 09.00 h or at 15.00 h from rats denied food on the day of the experiment. In the Table data are also reported from previous papers[1,3,4], showing the behaviour of functional parameters obtained in the same experimental conditions. It appears that, at a time of day when there is an increased rate of RNA biosynthesis[1] involving also a modification of the template capacity of chromatin[3,4], measured as capacity of binding actinomycin-D or its homologous enzyme, there is also a corresponding increase of the main band of the UV-spectrum.

It is believed that the difference in UV-absorption spectra is due to different states of chromatin condensation[5,6]. The more flattened spectrum of different chromatins is interpreted as presumably due to local areas of condensed chromatin within the nucleus ('granular chromatin')[6].

Therefore the perturbation of the chromophoric portion of liver chromatin following food intake appears to reflect perturbations in nucleoprotein structure induced by mechanisms related to food intake, which result in an increased rate of RNA synthesis[1,4].

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5-Hydroxytryptamine: Autoradiographic Evidence for Uptake Into Fibroblast Cell Nuclei

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Summary. After incubation with tritiated 5-HT, activity is autoradiographically localized over fibroblast nuclei. This may indicate an effect of 5-HT directly on the nuclei of these cells.

Among the many roles of 5-hydroxytryptamine (serotonin; 5-HT) in the organism, an effect on the proliferation of fibroblasts was suggested by the occurrence of fibrosis in the heart and other organs of patients with carcinoid syndrome (see FIORE-DONATI[1] for references). In addition, experiments with injection of 5-HT into subcutis[2] and joint spaces[3] apparently lead to local proliferation of connective tissue. BOUCEK and ALVAREZ[4] have elegantly demonstrated a specific effect of micromolar concentrations of 5-HT on growth and division of cultured fibroblasts, and this effect has been further corroborated recently (BOUCEK and NOBLE[5]).

The present study originated during an attempt to define transmitter substances at certain neuromuscular junctions in the Atlantic hagfish[6]. Tritiated 5-HT is

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[5] R. J. BOUCEK and N. L. NOBLE, Proc. Soc. exp. Biol. Med. *144*, 929 (1973).
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evidently rapidly taken up by fibroblasts, since autoradiography showed silver grains localized over their nuclei shortly after incubation.

Materials and methods. Small 0.5 × 1 mm samples were taken immediately after death from branchial and parietal muscles of Atlantic hagfish (*Myxine glutinosa*, L.) and from subcutaneous tissue and tendons of adult albino rats. The samples from hagfish were incubated in an oxygenated Ringer solution at 4 °C, and those from rats in an oxygenated Krebs solution with glucose at 37 °C. Tritiated 5-HT (5-hydroxytryptamine-^{3}H(G) creatinine sulphate, Amersham; specific activity 500 mCi/mmol) was added to a final concentration of 10^{-5} or 10^{-6} *M*. Incubation lasted 3–5 h, followed by several rinses through $^1/_2$–1 h in the salt solutions before fixation.

Hagfish tissues were fixed in 2% paraformaldehyde + 2.5% glutaraldehyde in 0.5 *M* Na-cacodylate buffer pH 7.3, rinsed, postfixed in 1% OsO_4 in the buffer for 1 h, dehydrated in acetone, and embedded in TAAB Embedding Resin. Ultrathin sections on nickel mesh grids were coated with Ilford L4 emulsion by a loop technique. After exposure for 6 weeks, the emulsion was developed in Kodak D-76 and fixed, and the sections were stained with lead citrate and examined in a Siemens Elmiskop I.

Rat tissues were fixed with the aldehydes in 0.1–0.15 *M* Na-cacodylate buffer pH 7.3, dehydrated, and embedded partly with the resin for semithin sections, partly in paraffin for 10–15 µm sections. The sections on glass slides were covered with Kodak K2 emulsion by a dip method, exposed for 1–4 weeks, developed in Kodak Dektol, and fixed. The semithin sections were examined unstained with dark-field illumination or stained with toluidine blue after development. The paraffin sections were stained with cresyl violet.

Results and discussion. In 2 hagfishes examined, typical silver grains were located over fibroblast nuclei in the electron microscope autoradiograms (Figure 1). The background activity was very low in the preparations, rendering the concentration of grains over fibroblast nuclei striking. Virtually all fibroblast nuclei possessed this labelling, and only very few grains were found over cytoplasm even when the latter was abundant. The labelled fibroblasts generally possessed few cisterns of granular endoplasmic reticulum. Thus, these cells appeared resting and synthetically inactive. No labelling was found over muscle fibres, myosatellite cells, endothelial cells, pericytes, mesothelial cells, various types of blood cells, or mast cells. Practically no grains were found outside the sections, indicating that the grains were due to the radioactivity introduced during incubation. Furthermore, the striking preferential localization of grains also indicated that the grains were due to the intact 5-HT molecules and

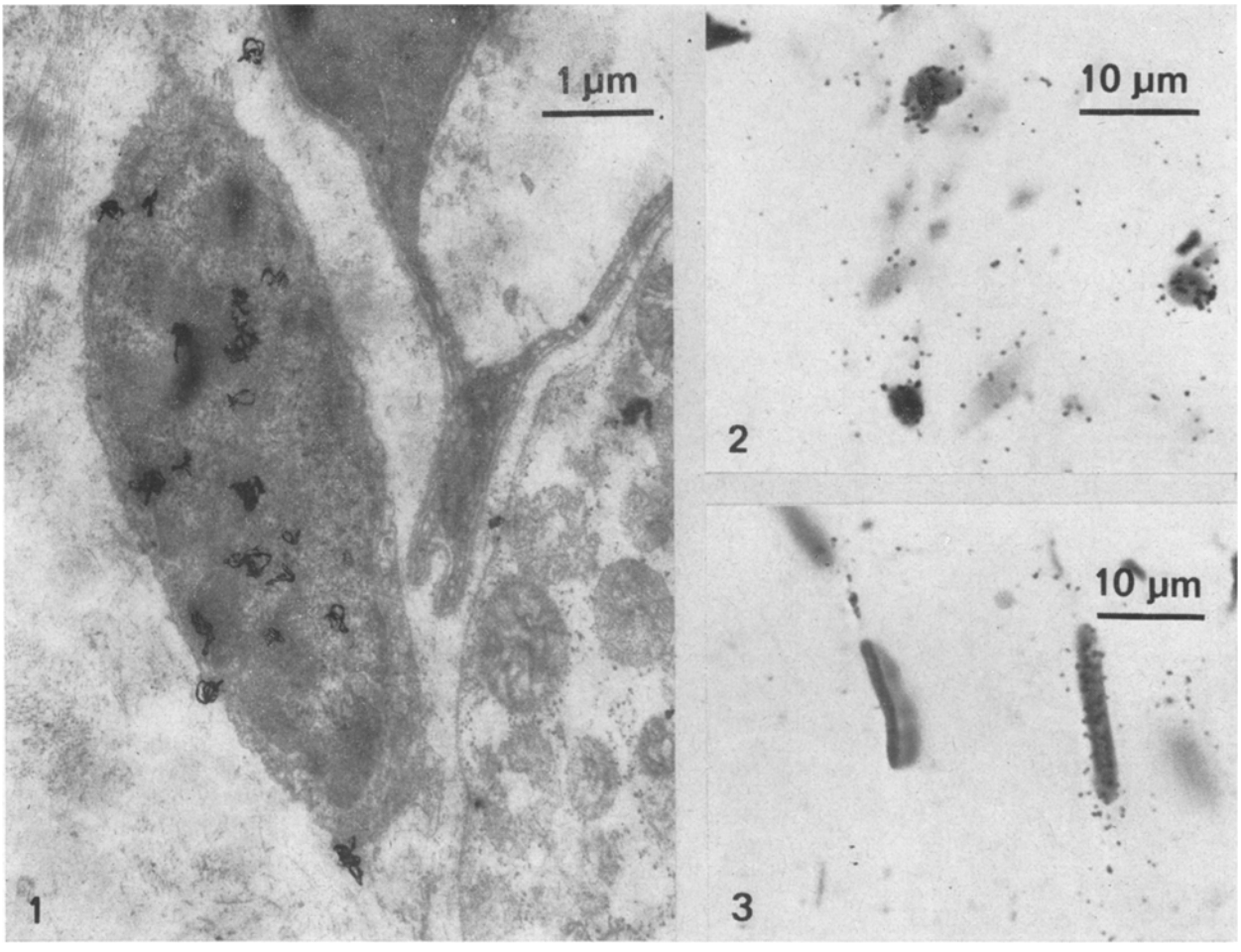

Fig. 1. Electron microscope autoradiogram of Atlantic hagfish fibroblast after incubation in 10^{-6} *M* tritiated 5-HT for 3 h. Silver grains are located over the nucleus.

Figs. 2 and 3. Light microscope autoradiograms of fibroblasts from rat subcutis (Figure 2) and tendon (Figure 3) after incubation in 10^{-6}*M* tritiated 5-HT for 4 h. Silver grains are located over 3 fibroblast nuclei in Figure 2 and over one of two fibroblast nuclei in Figure 3.

not to fragments after chemical degradation of the tritiated molecules.

The findings in the rat material may appear less conclusive. Also here very few grains were located outside the tissue in the sections. The background label over the sections, however, was significant in all experiments, probably indicating binding of 5-HT to tissue components. The fibroblasts were easily identified. However, only ca. 3–5% of the fibroblasts possessed increased numbers of grains, and then mostly over the nuclei. Figure 2 shows labelled fibroblasts from subcutaneous tissue, and Figure 3 one from a tendon.

In the Atlantic hagfish, most fibroblasts evidently actively concentrate 5-HT, and the 5-HT rapidly accumulates in their nuclei. In the rat, however, only comparatively few fibroblasts (and nuclei) appear to take up 5-HT. This may be due to real species differences. Another possibility is that the ability to accumulate 5-HT is associated with one certain phase of the cell cycle, which is shorter in rat fibroblasts than in those of hagfish. Still another explanation is that a postulated transport mechanism of 5-HT into fibroblasts and their nuclei in the rat material has remained largely saturated with non-radioactive 5-HT during the incubation.

Although many aspects thus need further analysis, the present preliminary findings indicate that fibroblast nuclei may take up 5-HT. Also steroid hormones (see O'MALLEY and MEANS[7]) appear to mediate their effect on proliferation and division of target cells by direct action on nuclei. A similar mechanism for the action of 5-HT on fibroblasts may explain the observed effect on the growth of cultured fibroblasts[4,5]. Such a mechanism of action of 5-HT in the organism may attribute an important additional role to the large amounts of 5-HT being present normally. In addition, it points at possible roles for 5-HT from mast cells and blood platelets, for instance during hemostasis and in healing of wounds and scar formation.

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Sterility and Lethality in Crosses Involving Two Translocation Heterozygotes of the German Cockroach, *Blattella germanica* (L.)[1]

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Summary. Productivity in crosses involving two independent reciprocal translocations in *Blattella germanica* are reported. Lethal effects alone could not account for the reductions in hatch since completely unproductive crosses occurred frequently. The latter are attributed to the inability of reduced numbers of viable embryos to force open the egg case. The implications for genetic control of the joint dominant effects from embryonic trapping and translocation semisterility are discussed.

The possibility of using reciprocal translocations for control of the German cockroach, *Blattella germanica* (L.), is under investigation in our laboratories[2]. Introduction of a single translocation into laboratory populations effectively retards population growth[3,4]. However, a much greater inhibition is expected from the use of double translocation stocks[5]. A first measure of productivity in crosses involving two translocations has been obtained using T(2;11) *Cu* in combination with 2 other stocks: T(3;12) and that identified previously as T(9;11)[3,6]. The latter is referred to throughout this paper as 'T(9;?)', for reasons explained below. High lethality was associated with the double translocations and resulted in striking reductions in the numbers of progeny. The combined lethal effects of 2 translocations could not account for the sterility (non-hatch), which reached 94% in one set of crosses, and an additional mortality-causing factor is reported here.

Each of the translocations noted above has somewhat different characteristics. Briefly, metaphase I chromosome disjunction is random in heterozygous T(2;11) *Cu* males and, presumably, in (T9;?) females since hatch averages in crosses to wild type are close to the expected 50%[6]. T(2;11)*Cu* females are sterile. Alternate disjunction occurs in about 60% of the cells from T(9;?) males[6]. This agrees with an average hatch of 58%. Hatch in T(3;12) males and females is estimated at 60–62%, but disjunction is alternate in more than 70% of the cells in heterozygous males[7]. These differences account for some of the results presented from crosses involving 2 translocations.

T(9;?) and T(3;12) are maintained in backcross systems to the closely-linked markers ruby eye (*ru*) and hooded pronotum (*hd*), respectively[6,7]. In these systems, > 98% of the phenotypically normal progeny are translocation heterozygotes (T/+). T(2;11)*Cu* is identified by its curly-wing phenotype[8]. In order to obtain phenotypically distinct double heterozygotes, 2 stocks of T(2;11)*Cu*, one homozygous for *ru* and the other for *hd*, were developed. From the former, *Cu* males were selected and crossed to T(9;?) females (T +/+ *ru*). Progeny with normal eye color and curly wings were assumed to be double translocation heterozygotes. T(2;11)*Cu*; T(3;12) double heterozygotes were developed similarly, using the T(2;11)*Cu*, *hd*/*hd* stock.

Cytological examination of male progeny was made to verify the presence of both translocations. As expected, T(2;11)*Cu*; T(3;12) pachytene cells showed 2 separate

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