cient excitation of FITC involving the light source and the construction and matching of the excitation and barrier filters, and the system antigen-antibody-conjugate.

In the present communication, recent developments in the excitation of FITC in immunofluorescence are touched upon and experiments with laser excitation of FITC in immunofluorescence are reported.

Recently descriptions have been published of interference filters with high transmittance in the region of maximum FITC absorption and with a particularly steep slope toward 500 nm, the transmittance at 525 nm being 0.1–0.001% (KRAFT[2], RYGAARD and OLSEN[3]).

They permit an efficient excitation of FITC with a minimum of unwanted fluorescence by means of a halogen or a tungsten lamp, a fact greatly facillitating the routine work in immunofluorescence.

If visible light is used for FITC excitation, a barrier filter should be used to exclude the strong excitation light and to make fluorescence observable. Usually this is a coloured glass filter or dielectric multilayer filter which opens steep to the emission of FITC. Since the absorption peak at 490 nm and the emission peak of FITC at 520 nm are only 30 nm apart, it is difficult to match the excitation and the barrier filter. Ideal conditions would be obtained if we had an excitation filter whose transmittance fell vertically at 500 nm and a barrier filter which opened vertically at the same wave-length. No such filter system has been designed, but very good results have been obtained with the interference filter combined with Zeiss 50 barrier filter (RYGAARD and OLSEN[3]) and also an interference filter KP 490 combined with a K 510 filter (KRAFT[2]).

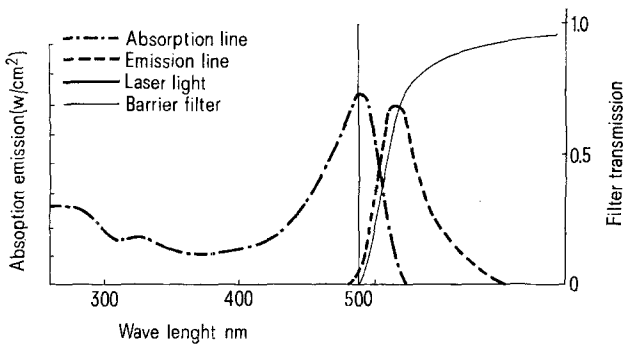

Fig. 1. Spectral distribution of absorption and fluorescence of FITC, laser light and filter transmittance.

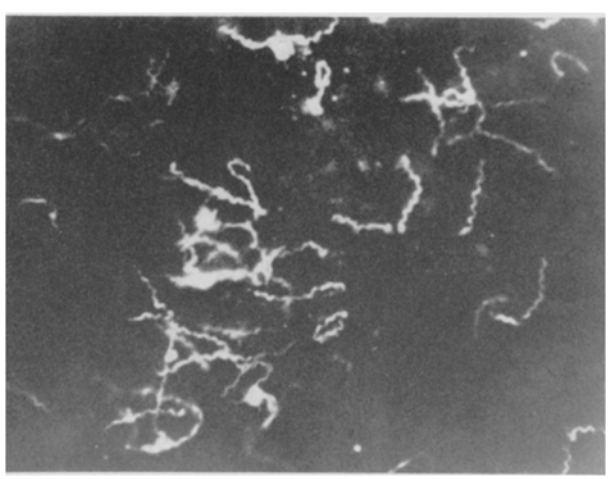

Fig. 2. Fluorescent treponemes in a positive FTA-ABS test.

In our experiments, a monochromatic light beam at 488 nm from an argon laser was used instead of conventional light. The advantages of the system with laser light source are obvious. The wave-length almost coincides with the FITC absorption peak. Since the laser light is monochromatic, there is no need for an excitation filter and the appropriate barrier filter can easily be selected. In our hands the Zeiss 50 barrier filter gave good results.

The argon laser we worked with was made by E. VRENKO and I. RAJVER of the Department of Technical Optics, Iskra, Ljubljana. The energy of 100 mW was sufficient to excite bright fluorescene. We used the standard Reichert Zetopan microscope with a dark-field condenser from which the lamp, lenses and filters were removed. Since the laser beam was too narrow, an objective of 40× magnification was inserted between the laser and the condenser to enlarge the beam. In this way enough light reached the object through the annular construction of the dark-field condenser. The microscope image obtained in this way was clear and sharp.

We employed this system in the FTA-ABS test for syphilis. In negative specimens neither treponemes nor any other tissue or material were detectable. In positive sera, the titers were always much higher than those obtained by standard method. Very little autofluorescence was observed. The specificity and sensitivity of the laser induced FTA-ABS test for syphilis is now being investigated.

Zusammenfassung. Methode für die Anregung des FITC in der Immunofluoreszenz mit Argon-Laser-Licht, die eine sehr starke Fluoreszenz ermöglicht. Diese Methode wird in dem FTA-ABS-Test für Syphilis benutzt.

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The Induction of Ovarian Dysfunctions in *Thermobia domestica* by the *Cecropia* Juvenile Hormones

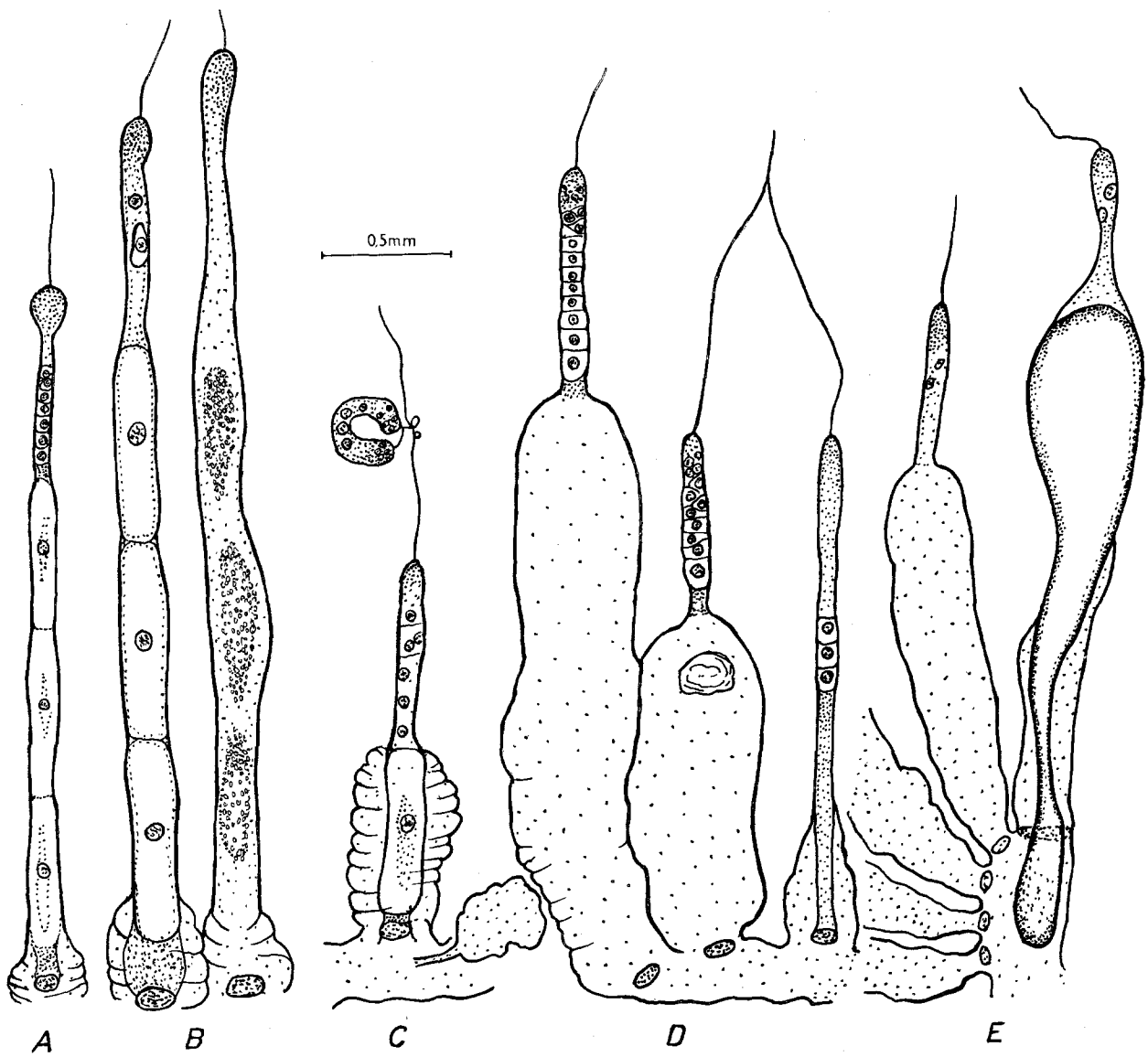
The corpora allata, source of the juvenile hormone, have long been known to stimulate insect reproduction by supporting the yolk deposition into the growing oocytes[1]. The juvenile hormones isolated from *Hyalophora cecropia* were also shown to possess this stimulatory activity[2,3]. We have found, however, that administration of 1–100 µg of either of these 2 hormones[4] into an adult female of the firebrat *Thermobia domestica* Packard, may, in a long run,

exhibit an adverse effect on the reproduction by eliciting severe disorders in the development and function of the ovaries.

The females, which were kept in constant darkness at 37°C and 85% RH, were treated with 1 µl of acetone containing a known amount of one of the hormones. The insects were then kept in Petri dishes in groups of 5 treated females and 10 normal males and their fertility was recorded for 40–60 days after the treatment[5]. The subsequent examination of ovaries revealed severe defects concerning primilarily the differentiation of oocytes and follicular cells but also the egg deposition.

The derangements of differentiation were reflected in all parts of the ovariole. The germarium was reduced or, on the contrary, hypertrophied (Figure 1 A) and the previtellarium contained only a few or no oocytes (Figure B). Lack of well-formed egg chambers (Figure C), failure of the ovarioles to initiate yolk deposition into the new batch of oocytes (Figure D) as well as the resorption of partly grown oocytes, appeared to be consequences either of an incomplete number or of a dysfunction of follicular cells. The supression of differentiation processes in the germarium often led to a great reduction of the whole ovariole. In extreme cases, the ovariole became very thin and torn into 2 parts (Figure C). The germarium remained attached to the terminal filament but lost any connection with the rest of the ovariole which shrank into a tiny rudiment at the oviduct.

The following defects seemed to result from disordered oviposition. The mature eggs were occasionally seen to be



The ovarioles of females treated with *Cecropia* juvenile hormones (A and B – treated with 100 µg of C_{18}-JH; C – 1 µg of C_{18}-JH; D – 1 µg of C_{17}-JH; E – 100 µg of C_{17}-JH) and dissected 41 (D, E) or 53 days (A, B, C) later: A) a hypertrophied germarium of a female dissected just after ecdysis; B) ovarioles of a freshly ecdysed female: the numbers of oocytes in the previtellaria is reduced to one or zero and the yolk deposition in the right ovariol is abnormal; C) an ovariole showing precocious shrinkage of the vitellarium and lacking egg chambers in the previtellarium; the other ovariole is torn into germarium, which is attached to the terminal filament, and the rest of the ovariole, which is adjacent to the oviduct; D) ovarioles dissected from a freshly ecdysed female: 2 ovarioles with sack-like vitellaria and no vitellogenesis and 1 ovariole (to the right) containing just 3 non-developing oocytes; E) a mature egg is trapped in the ovariole when passing out into the oviduct (female dissected just before ecdysis).

trapped in the outlet of the ovariole (Figure E) being unable to pass out. In other instances, the eggs had apparently been dispatched but the vitellarium failed to shrink and remained sack-like (Figure D). On the other hand, the shrinkage of the vitellarium sometimes began before the terminal oocyte was mature enough to be deposited (Figure C).

The effects of juvenile hormone on the differentiation processes in the ovaries of *Thermobia* resemble the morphogenetic action exerted by the hormone during metamorphosis. The hormone influences only the tissues that have not yet passed a certain point of developmental determination[6]. The differentiation processes in the gonads of pterygote insects seem to be determined and often also accomplished prior to the imaginal ecdysis and are therefore affected only if the hormone or its analogues are administered to larvae[7–9] or pupae[10]. In the firebrat, the differentiation of oogonia and prefollicular cells continues in the adult stage[11] and, as shown in the present study, may therefore be fully impeded by the juvenile hormone. The defects in egg deposition are difficult to interpret. Their possible cause may be either malformation of the follicular epithelium (incomplete number of follicular cells etc.) or disturbances in the hormonal interplay that controls the individual phases of reproduction.

Zusammenfassung. Applikation von 1–100 µg irgendeines der *2 Cecropia*-Juvenilhormone auf die erwachsenen Weibchen von *Thermobia domestica* verursacht Störungen des Differenzierungs- und Eiablageprozesses in den Ovarien und erniedrigt demzufolge die Vermehrungsfähigkeit der Tiere.

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RNA Metabolism in Brain of Suckling Normal and Hypothyroid Rats[1]

It is well established that in the rat, neonatal thyroid deficiency markedly affects cerebral protein synthesis[2,3]. Although this alteration has been considered as the underlying factor of the biochemical, histological and behavioural alterations accompanying neonatal hypothyroidism[4], the mechanisms by which thyroid hormone would stimulate protein synthesis in developing brain have not yet been clearly elucidated.

It is known that RNA metabolism plays a key role in the control of processes of growth and development, and thyroid hormone regulates such processes by stimulating RNA synthesis in its target tissues[5]; it has also been demonstrated that RNA synthesis is very high in the immature brain, decreasing rapidly during early postnatal maturation[6,7]. Considering that in the rat brain, the maturational effects of thyroid hormone take place during the first 10–12 postnatal days[8], it seemed interesting to study the effects of neonatal thyroidectomy in the rat upon brain RNA metabolism, at 10 days of age, when brain morphogenesis is very active.

Materials and methods. Rats from a highly inbred Wistar strain were used throughout, normal controls and neonataly thyroidectomized animals being prepared and kept as previously reported[4]. 5-(^{3}H)-orotic acid (sp. act. 15 Ci/mmole) was obtained from the Commissariat à l'Energie Atomique (France), and all other chemicals were reagent grade.

At 10 days of age, each animal was injected s.c. with a labelled orotic acid solution (1 µC/g body weight), and killed by decapitation 1, 3 or 24 h after the injection. The cerebral hemispheres from 3 brains were pooled in each experiment and slightly homogenized in 10 volumes of cold 0.32 *M* sucrose, containing 1 m*M* $MgCl_2$ and 0.4 m*M* potassium phosphate buffer (pH 6.7). The homogenate (HT) was used to isolated crude nuclear (CN), crude mitochondrial (MIT), microsomal (MIC), and supernatant (S) fractions according to SEMINARIO et al.[9], except that pellets were washed with homogenizing solution and crude nuclei were washed 3 times. Purified nuclei (PN) were obtained from CN following the procedure of BALAZS and COCKS[10]. The final pellets were resuspended in homogenizing solution; aliquots of these suspensions, as well as one of S and HT, were used to determine RNA according to MUNRO and FLECK[11]. Radioactivity incorporated in RNA was measured in each fraction as follows: cold trichloroacetic (TCA) solution was added to give a final TCA concentration of 10%; after centrifugation in the cold, the precipitate was washed once with cold 10% TCA and

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