

A COMPARATIVE STUDY ON THE SECRETORY ACTIVITY OF THE SUBCOMMISSURAL ORGAN IN THE EUROPEAN GREEN FROGS: *RANA ESCULENTA*, *RANA LESSONAE* AND *RANA RIDIBUNDA*

J. H. B. DIEDEREN and H. G. B. VULLINGS

Section for Histology and Cell Biology of the Zoological Laboratory, State University of Utrecht,
Padualaan 8, 3508 TB Utrecht, The Netherlands

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Abstract—1. The subcommissural organ (SCO), present in all vertebrates, is situated in the roof of the third brain ventricle, and secretes into this ventricle a glycoproteinaceous, fibre-like structure, the liquor fibre (LF).

2. The three forms of European green frogs differ from each other in the three parameters used to measure the secretory activity of the SCO: the amounts within the SCO of stained secretory material and of secretory material labelled by a radioactive precursor, and the growth rate of LF.

3. The observed differences indicate that the secretory activity of the SCO is distinctly higher in *Rana ridibunda* than in *Rana lessonae*, whereas in *Rana esculenta* this activity is intermediate.

4. These findings confirm the hybrid character of *esculenta*, which is in many morphological, physiological and ecological features intermediate between *lessonae* and *ridibunda*.

INTRODUCTION

In Europe three forms of green frogs can be distinguished: the forms *Rana esculenta*, *Rana ridibunda* and *Rana lessonae*. Of these forms *lessonae* and *ridibunda* are supposed to be taxa at species rank, whereas *esculenta* is supposed to be a hybrid originally resulting from interspecific crosses between *lessonae* and *ridibunda* (Berger, 1973; Günther, 1973). The forms *lessonae* and *ridibunda* are easily to distinguish from each other; they differ in many morphological, physiological and ecological features. The form *esculenta* is intermediate between the two other forms (Tunner & Nopp, 1979); its features overlap those of the forms *lessonae* and *ridibunda* considerably.

The green frogs live in mixed populations, mostly of *esculenta* with either *lessonae* or *ridibunda*, whereas mixed populations of all three forms appear to be rare (Wijnands, 1977). As a consequence, a group of European green frogs caught wild surely is a mixture of *esculenta* and either *lessonae* or *ridibunda* and possibly intermediate forms. In such a mixture the variation in morphological and physiological features can be expected to be considerable.

In the past green frogs have been used in studies on the secretory activity of the subcommissural organ (SCO) (Diederén, 1973, 1975a,b; Hess *et al.*, 1977). This organ, which is present in all vertebrates, is situated in the roof of the third brain ventricle at the transition from diencephalon to mesencephalon; it mainly consists of ependymal cells. From the secretory substance (a glycoprotein) being released into the brain ventricle by the SCO cells, a fibre-like structure originates, the liquor fibre (LF), formerly called Reissner's fibre. This fibre is moved slowly through the brain ventricle and the central canal of the spinal cord as far as the caudal end of the central canal in

the filum terminale, where it leaves the central nervous system and disappears. The functional significance of the SCO and the LF is still a matter of speculation.

Most of the results of previous experimental studies on the SCO of green frogs showed fairly great quantitative variations which might be due to the hybrid nature of the animals used. For this reason it was decided to compare the secretory activity of the SCO in pure specimens of *lessonae*, *ridibunda* and *esculenta*. The different forms of green frogs can be distinguished from each other by using serological and biometrical characteristics (Wijnands & Van Gelder, 1976). To measure the secretory activity of the SCO three parameters were used: the amount of secretory material within the SCO as visualized by a modified staining method after Bock & Ockenfels (1970); the amount of radioactive secretory material within the SCO labelled by ^3H -cystine, and the growth rate of the LF.

MATERIALS AND METHODS

The green frogs used were caught wild during the summer at several places in the Netherlands. The identification of the individual frogs as belonging to one of the three forms of green frogs was based on biometrical and serological characteristics as described by Wijnands & Van Gelder (1976).

During an adaptation period the frogs were kept under laboratory conditions at 18°C with free access to water and daily exposed to a photoperiod of 18 hr and a 6 hr period of darkness starting each day at 22.00 hr. The frogs were of both sexes. In previous experiments on the SCO of green frogs similar conditions were applied; no differences in secretory activity of the SCO between male and female frogs were observed (Hess *et al.*, 1977).

After 4 weeks all animals were injected into the thigh muscles with 6.5 μCi per g body weight of L-(3,3'- ^3H)-cystine hydrochloride (spec.act. 1.6 Ci/mmol) dissolved in

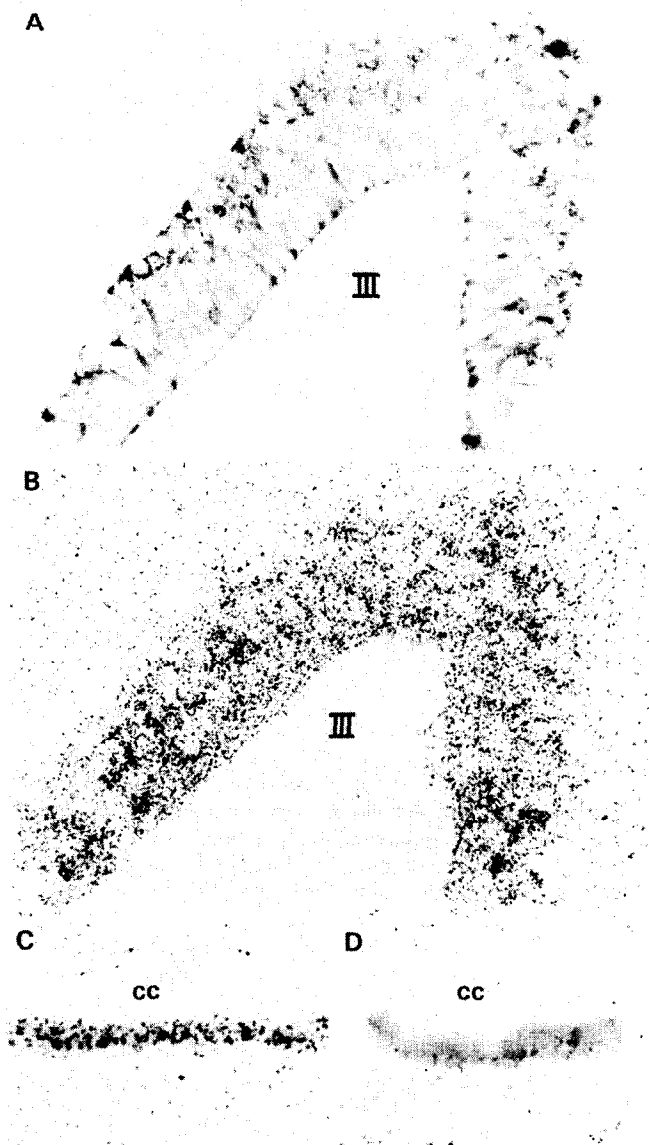


Fig. 1A-D. Two successive frontal sections of the SCO showing the localization of the stained secretory material (A) and the localization of the radioactive-labelled secretory material (B), and a sagittal section of the spinal cord showing a radioactive-labelled portion of LF (C) and an unlabelled portion of LF (D).
 III = third brain ventricle; CC = central canal of spinal cord.

0.02 ml of amphibian Ringer's solution. After having been reduced to L-³H-cysteine in the body, this amino acid is incorporated into the secretory substance of the SCO.

Thirty-five hours later all animals were injected in the same way with a high dose (0.15 mg/g body weight) of unlabelled L-cysteine in order to diminish strongly the specific activity of the free radioactive precursor still present, thereby suppressing the radioactive labelling of the secretory substance synthesized by the SCO cells from that moment on. Six hours after this injection the frogs were quickly anaesthetized with ether and the brains and spinal cords were isolated as a whole and fixed in Bouin's fluid. The fixed material was dehydrated, embedded in paraffin after the spinal cords had been separated from the brains, and cut into either frontal (SCO region) or sagittal (spinal cord) 5 µm serial sections.

Sections from five, equally spaced levels along the length of the SCO were placed on slides in such a way that sections of similar regions of the SCO of animals belonging to the three different forms of green frogs were present on the same slide.

To demonstrate differences in the secretory activity of the SCO between the three forms of green frogs, in each frog three parameters were measured:

The amount of stained secretory material in the SCO

Sections from each of the five regions into which the SCO was divided were stained with p-rosanilin-crotonaldehyde according to Bock & Ockenfels (1970; modified: p-rosanilin instead of diaminobenzophenon) as identically as possible; no counterstain was employed. By this method the secretory material within the SCO (see

Fig. 1A) is stained purplish blue. In one section from each region the light transmission in a rectangle containing the entire SCO area was measured by computer-controlled scanning cytophotometry (wave length of light 570 nm) by means of a Zeiss Mikroskopphotometer 01 coupled to a Hewlett-Packard 9825A calculator. The transmission outside the tissue section was set at 100%. Scanning was carried out in a meander mode, measuring adjacent square spots of $16 \mu\text{m}^2$. Likewise the light transmission in a rectangle only containing tissue outside but close to the SCO, the so-called background area, was measured; from this the mean transmission value per measured spot of background area was calculated.

In the rectangle containing the SCO area the measured spots with transmission values smaller than the mean background transmission value -1 SD , were considered to belong to the SCO. The transmission values of these spots were converted into extinction values, from which the mean background extinction value $+1 \text{ SD}$ was subtracted. The sum of the remaining extinction values was calculated.

The mean value of the sums of extinction from the five measured sections was multiplied by the total number of sections containing SCO, resulting in the value of the sum of extinction in the entire SCO. This sum can be expected to be proportional to the amount of stained material and thereby to the amount of secretory substance within the SCO.

The amount of radioactive-labelled secretory material in the SCO

Unstained sections from each of the five regions of the SCO were covered with Ilford K2 liquid nuclear emulsion diluted 1:1 with distilled water. After an exposure time of about 3 weeks the slides were developed in Kodak D19 developer (see Fig. 1B). The amount of silver grains, which is proportional to the amount of radioactive-labelled material, was measured by scanning cytophotometry. Scanning cytophotometry was carried out as described for the stained material with the exception of the wave length of light, which was set at 480 nm. The same quantities were calculated as for the stained sections; however, from the extinction values of the measured spots belonging to the SCO the mean background extinction value $+1 \text{ SD}$ was not subtracted. The corresponding mean background transmission value -1 SD was only used to distinguish between measured spots which presumably belonged to the SCO and those which did not.

The growth rate of LF

All sagittal sections of the spinal cord containing LF were stained with aldehyde-fuchsin according to Gomori-Gabe (Gabe, 1968) and covered with Ilford K2 liquid nuclear emulsion in the same way as the SCO sections. The aldehyde-fuchsin staining method clearly demonstrates the LF. After an exposure time of about 8 weeks the slides were developed in Kodak D19 developer (see Fig. 1C and D). The length of the radioactive portion of LF present in the spinal cord expressed as percentage of the length of the spinal cord until the last vertebra was used as a measure for the growth rate of LF. The transition of the labelled to the unlabelled portion of LF was established by light microscopical observation. Results from previous experiments (Diederer, 1973) indicate that in green frogs the production of radioactive-labelled LF by the SCO starts within quite a short time after injection of a radioactive precursor under conditions similar to those applied in the present experiment.

RESULTS

The amount of stained secretory material is significantly larger in *Rana lessonae* than in *ridibunda* (Fig. 2A). The amount of this material in *esculenta*

tends to be smaller than in *lessonae*, but is clearly larger than in *ridibunda*. All three forms of green frogs significantly differ from each other in amount of radioactive-labelled secretory material (Fig. 2B). The largest amount of this material is present in *lessonae*, the smallest in *ridibunda*, whereas in *esculenta* this amount is intermediate. The growth rate of LF is significantly lower in *lessonae* than in *ridibunda*. In *esculenta* again this parameter is intermediate.

DISCUSSION

The differences in the growth rate of LF indicate that the production of this fibre and, therefore, the secretory activity of the SCO is higher in *ridibunda* than in *lessonae*, whereas in *esculenta* this activity is intermediate. The differences in the amount of radioactive-labelled secretory material and especially the differences in the amount of stained secretory material are less easily interpretable. Comparing the Figs 2A, 2B and 2C one may already conclude that the larger the amounts of stained and of radioactive-labelled secretory material, the lower the growth rate of LF in a particular group of frogs. This conclusion is confirmed by statistical analysis of the individual values of the three parameters (calculation of the regression coefficients). This analysis indicates a significant negative linear correlation ($P < 0.05$) between the growth rate of LF and the amount of radioactive-labelled secretory material as well as the amount of stained secretory material within the SCO of each frog, whereas a significant positive linear correlation ($P < 0.05$) exists between the two latter parameters. These findings may be explained as follows. In the SCO of *ridibunda* the secretory material is rather rapidly released after its synthesis keeping the storage of secretory material and thereby the radioactive-labelling within the SCO low. In *lessonae* on the other hand, it takes more time on the average before the newly synthesized secretory material is released by the SCO; it is partly kept in storage for some time causing a larger accumulation of secretory material and thereby a stronger radioactive-labelling within the SCO. In *esculenta* the situation is intermediate.

One may perhaps generalize the meaning of the observations in the present study by stating that the occurrence in the SCO of green frogs of a larger amount of stained secretory material or of a larger amount of secretory material labelled by a radioactive precursor is an expression of a lower LF producing secretory activity.

The results of the present study indicate that the secretory activity of the SCO is higher in *ridibunda* than in *lessonae*, whereas in *esculenta* this activity is lower than in *ridibunda* but higher than in *lessonae*. This observation is in good agreement with the hybrid character of the form *esculenta*. Just as it is in many other features (Tunner & Nopp, 1979), this form of green frogs is also intermediate between the forms *lessonae* and *ridibunda* in the secretory activity of the SCO.

The results of this study once more indicate that using European green frogs in experimental research one has to select very carefully pure specimens of one or another of the three forms in order to minimize the

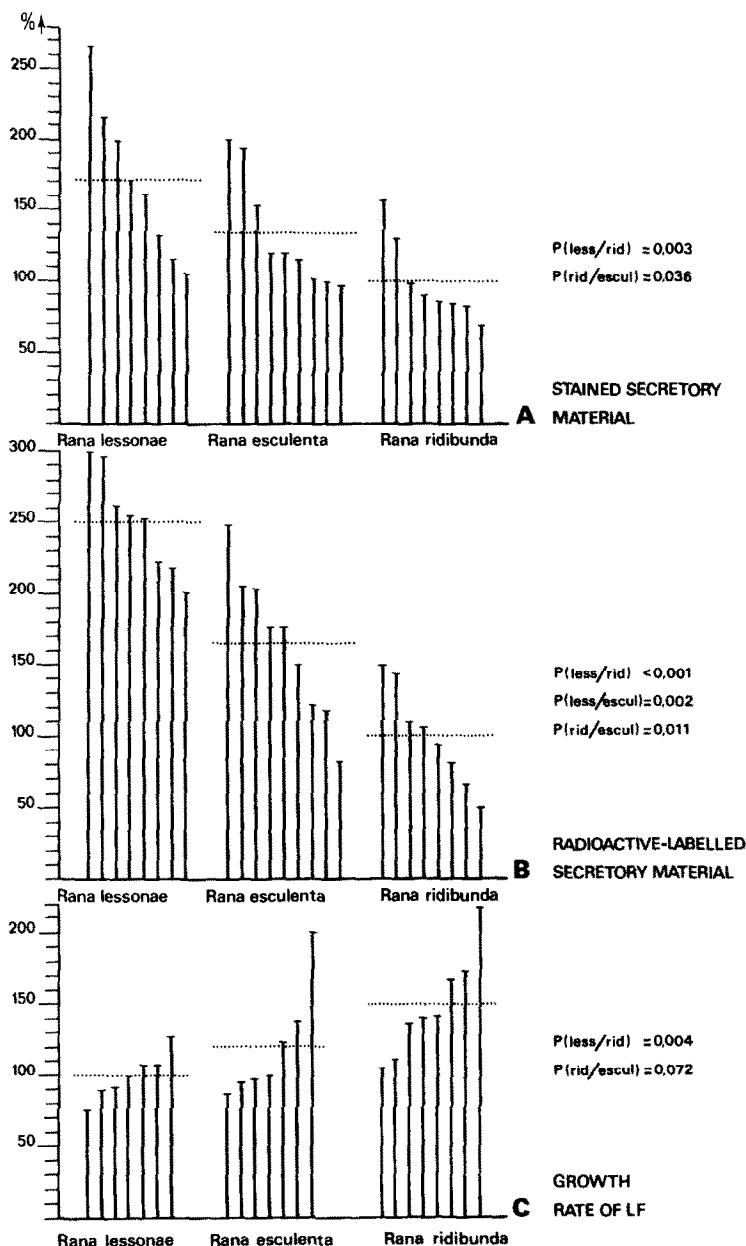


Fig. 2A-C. Amount of stained secretory material (A), amount of radioactive-labelled secretory material (B), and growth rate of LF (C), in the individual animals of the three forms of green frogs. In A and B the values represent the light extinction relative to the corresponding mean values in *Rana ridibunda* which are fixed at 100%. In C the values represent the growth rate of LF relative to the mean value in *Rana lessonae* which is fixed at 100%. Mean values are indicated by dotted lines. Error probabilities were calculated by Wilcoxon's test.

biological variation within the group of experimental animals.

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