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Dorsal aorta catheterization in rainbow trout (*Salmo gairdneri*) I. Its validity in the study of blood gonadotropin patterns

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Summary. The use of a dorsal aorta catheterization technique to study gonadotropin secretion patterns in the raibow trout was tested. Heparin used to flush the cannula between repetitive samplings did not have any effect on plasma GTH levels. Catheterization resulted in a slight short-term change in those levels. The gonadotropin levels returned to their initial values as soon as 30 min to 6 hrs after the operation. From then on, the GTH levels remained close to the initial values in fish exhibiting normal feeding behaviour, whereas they tended to decrease in « stressed » females which did not eat normally. The fish which adapted well to dorsal aortic catheterization did not show any changes in the diurnal pattern of GTH levels or in normal gonadal function and GTH profiles during the processes of oocyte maturation and ovulation. It is concluded that individual catheterized trout can be used advantageously for studying gonadotropin secretion patterns after a 3-day recovery period and the elimination of those fish which neither resume normal feeding nor return to initial, pre-operative GTH levels. Using this technique, it was demonstrated that hypophysial GTH release in trout with oocytes undergoing active vitellogenesis is probably effected by short-term bursts (pulses) of secretion.

Introduction.

It is common practice to catheterize blood vessels in different mammalian species in order to follow the metabolic or hormonal profiles. When studying the temporal patterns of various reproductive hormones in the circulation of rainbow trout, it is desirable to use individually catheterized fish because these parameters vary widely and are not synchronized in different fish of the same population (Crim *et al.*, 1973, 1975 ; Billard *et al.*, 1978 ; Fostier *et al.*, 1978). Also, it is better to sample the blood of cannulated animals than to do repetitive sampling by heart or caudal vessel puncture which may disturb the normal progress of a given physiological process and cause mortality. This is true for any research necessitating frequent sampling of the same fish, such as the determination of short-term hormonal fluctuations or rhythms or assessment of the effects of administered substances on various blood components.

Some studies on teleost species have reported a vascular catheterization technique (Smith and Bell, 1964 ; Garey, 1969 ; Houston, 1971 ; Soivio *et al.*, 1972 ; Soivio and

Nyholm, 1975). Most of these works as well as others (Houston *et al.*, 1969) investigated changes in a variety of hematological parameters or metabolite levels after cannulation. Cannulated fish have only been used once in a study involving the measurement of hypophysial hormone levels (Crim and Cluett, 1974).

In some mammalian species, the plasma levels of several hypophysial hormones have been shown to be significantly affected by the method of blood collection (Neill, 1970 ; Döhler *et al.*, 1977 ; Simms *et al.*, 1978). The aortic cannulation of rats resulted in modified patterns of prolactin and LH secretion during the estrous cycle (Neill, 1972). Also, anesthetization and surgery were shown to affect hypophysial hormone secretion patterns (Ajika *et al.*, 1972 ; Krülich *et al.*, 1974 ; Glass *et al.*, 1978 ; Wang *et al.*, 1978). To our knowledge, no study has considered the possible effects of dorsal aortic catheterization (surgery and the presence of a cannula in a blood vessel) on the circulating levels of any of the hypophysial hormones in fish.

As a consequence, we attempted to determine if a dorsal aorta cannulation technique could be used to investigate blood GTH patterns in the free-swimming trout (*Salmo gairdneri*). A complementary study (Bry and Zohar, 1980) has investigated clinical stress indicators to determine how long it takes the fish to recover from catheter implantation.

Materials and methods.

Animals. — All the rainbow trout used were 3 to 4 years old and weighed 0.8 to 2 kg. They were acclimated to the experimental tanks at least 15 days before being handled and were fed once a day between 10 a.m. and noon. The cannulated fish were kept in individual 100-l rectangular tanks or in 60-l compartments in bigger tanks. The water temperature was maintained at 10 ± 1 °C between December and May and at 14 ± 1 °C between June and November.

Catheterization surgery. — The catheterization procedures were based on those previously described (see Introduction), with some modifications.

The fish were anesthetized in a 300 ppm phenoxy-ethanol solution and placed on the operation table, their gills perfused with refrigerated water containing the anesthesia.

A metal syringe needle, with an inner diameter closely fitting the outer diameter of the cannula (Clay and Adams PE-50), was used for aortic penetration. The 80-cm long catheter, filled with a 0.9 p. 100 saline solution containing 100-150 units/ml of sodium heparin, was placed in the needle which was entered at the midline of the buccal roof, slightly anterior to the first gill bars, and cautiously guided posteriorly at about a 30° angle. Penetration of the dorsal aorta was indicated by blood rushing into the cannula : we then pushed the cannula 8 cm up into the blood vessel, and gently withdrew the guiding needle. When penetration was successful, there was no hemorrhage. The cannula was then attached to the mouth roof, passed through a hole in the snout slightly anterior to the nasal opening, and sutured to the snout. Finally, the dead space of the cannula was filled with an heparin solution, and the cannula was plugged with a blocked syringe needle adapted to a cork floater. It took 8 to 10 min to operate on one fish.

All blood sampling via the catheter was done after a 200-µl sample, corresponding to the cannula dead space, had been eliminated. When sampling was complete, the blood filling the cannula was pushed back by 200 µl of heparinized saline solution.

Experimental procedures for studying :

A. *The effect of heparin on plasma GTH levels.* — At the end of October, we selected 16 males whose testes were beginning spermiation and 13 females having oocytes in the last stages of vitellogenesis. After anesthesia, 500 µl of blood was collected via the caudal vessels, and the fish were given an intracardiac injection of either 500 U/kg of heparin in an isotonic saline solution or of 250 µl/kg of saline alone. The injected dose of heparin was calculated so as to provide 7 to 10 times the amount estimated to be in the circulation after ten successive blood samplings via the aortic cannula. Blood was collected 4, 8 and 24 hrs after injection. The gonadotropin levels in the two experimental groups were compared by an analysis of variance. The F-test was used for testing the linearity of the observed curves and Student's t-test for comparing the curve slopes.

B. *Short and mean-term effects of cannulation on plasma GTH levels.* — The experimental protocol of this experiment has been described in detail by Bry and Zohar (1980). Briefly, 6 fish were acclimated to individual experimental tanks before being operated for dorsal aortic cannula implantation. Blood (600 µl) was withdrawn (i) from the caudal vessels of the non-anesthetized fish prior to the operation (sample — 10 min) and (ii) via the aortic cannula at the end of the operation about 10 min later, then 30 min, 6 hrs and 1, 3 and 5 days after cannulation. The oocytes of one of the females (female A) were in the last stages of vitellogenesis, whereas the other females (B to F) had ovulated and their eggs had been stripped at least 3 weeks prior to cannulation. The pattern of GTH levels during the experimental period was analyzed by an analysis of variance.

C. *Profiles of plasma GTH levels in cannulated and non-cannulated females.* — The experiment was carried out in April. A group of 9 females was acclimated for one month to individual tanks exposed to natural photoperiod. After blood sampling via the caudal vasculature (sample 0), the dorsal aorta was catheterized and the fish were put back into their tanks for an additional 5 days. At the end of that time, 300 µl of blood were sampled via the cannula every 4 hrs for 24 hrs. When the experiment was terminated, all the fish were killed and their ovaries were examined individually under a dissecting microscope.

Four additional groups of 7 females each were acclimated to 600-l tanks exposed to natural photoperiod. After acclimatization, the blood of each group was sampled twice (interval of 48 hrs or more between two samplings of the same group) at different times of the day so as to constitute a sampling pattern parallel to that of the cannulated fish, i.e. every 4 hrs for 24 hrs. Blood (300 µl) was withdrawn from the caudal vessels after rapid 1-min anesthetization. At the end of the experiment, the fish were killed to determine the stage of oocyte development. The two experimental groups were compared by analysis of variance, whereas the pre and post-operative GTH levels in cannulated fish were compared by Student's t-test.

D. *Effect of cannulation on the processes of oocyte maturation and ovulation and on concomitant plasma GTH levels.* — Dorsal aortic catheterization was performed on a group of 5 females having oocytes in the migrating germinal vesicle stage. The developmental stage of the oocyte was determined according to Jalabert *et al.* (1976) after a few oocytes, stripped from the females by abdominal pressure, had been examined under a dissecting microscope. Blood (350 µl) was sampled from the caudal vessels prior to cannulation and via the cannulae every 3 days after cannulation until ovulation occurred. The fish were anesthetized and stripped of a few oocytes for microscopic examination after all post-operative sampling.

A group of 5 non-cannulated females, showing oocytes with beginning migration of the germinal vesicle, was exposed to similar conditions and served as a control. After anesthetization, the fish in this group were bled through the caudal vessels every 3 days until they had ovulated. The blood was always sampled at the same time of day (10 : 00 to 11 : 00 a.m.) in both experimental groups.

E. *Individual daily GTH profiles in cannulated females.* — The aim of the present experiment is to present a study of blood GTH patterns using individual cannulated females, and to determine the criteria to be used for selecting reliable animals. Six females acclimated to individual tanks exposed to natural photoperiod were cannulated in September. Prior to surgery, 350 µl of blood were sampled from the caudal vasculature of anesthetized fish within 1 min after netting (sample 0). Six days after surgery, 300 µl of blood were sampled via the cannula every hour for a 10-hr period which included the time of the sample 0. Post-mortem examination of the ovaries showed oocytes in active vitellogenesis in all females. The presence of peak GTH levels (outliers) was tested by Dixion's gap test (cited by Bliss, 1967), and the mean of the basal (inliers) GTH levels was calculated. Pre-operative and peak GTH values were compared to this mean using Student's t-test.

Gonadotropin radioimmunoassay (RIA). — All the plasma samples were stored at — 20 °C until assayed. The gonadotropin levels were measured in triplicate according to Breton and Billard (1977). Within-assay variability (the coefficient of variation of the values obtained for three plasma samples, each run several times in the same assay) was 0.10 ($\bar{X} = 1.39$ ng GTH/ml, $n = 4$) and 0.07 ($\bar{X} = 6.53$ ng GTH/ml, $n = 6$ and $\bar{X} = 18.08$ ng GTH/ml, $n = 6$). The existence of peak GTH levels in some samples (figs 2, 3) was verified by assaying the same samples again to eliminate any error due to RIA.

In order to test the putative effect of heparin on the validity of the RIA results, a few standard curves were run : in each test, a different dose of heparin was added to all the tubes. No significant differences were found when the parameters characterizing the different standard curves (slope, intercept, coefficient of regression) were statistically compared, and there was no significant difference between the experimental and the control curves.

Results.

A. *Effect of heparin on plasma GTH levels* (fig. 1). — The mean GTH levels in both sexes were different in the two experimental groups. These divergences were present prior to treatment (sample 0) and were probably accidental.

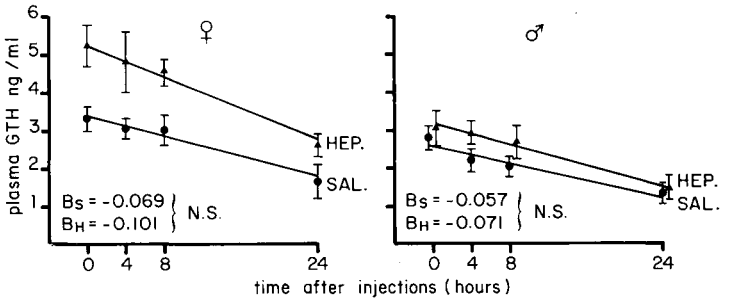

FIG. 1. — *Plasma gonadotropin levels* (mean ± SE) *in male and female rainbow trout injected with either heparin* (500 U/kg) or saline (250 µl/kg). NS : $P > 0.05$; $B_H B_S$ — calculated slopes of curves representing heparin and saline treated fish.

The most clear phenomenon in all the experimental groups was the significant decrease in plasma GTH levels during the experiment (analysis of variance ; $P < 0.01$). The shape of the four curves was not significantly different from linearity (F-test ; $P > 0.05$). The estimated slopes of the curves describing the GTH levels in heparin and saline-treated fish did not differ significantly from each other in either sex (fig. 1 ; Student's t-test ; $P > 0.025$) and the curves were therefore nearly parallel. Moreover, a multifactorial analysis of variance demonstrated that the temporal plasma GTH patterns in both sexes were independent of treatment, saline or heparin injection. Therefore, despite the pre-treatment (sample 0) differences in the plasma GTH levels of saline and heparin-treated fish, neither group in either sex showed any divergence in the temporal hormone pattern.

B. *Short and mean-term effects of cannulation on plasma GTH levels* (fig. 2). — The GTH levels in six females for 5 days after surgery are shown in figure 2. In females A, C, D and E, we observed a slight but significant increase between the initial levels (sample — 10 min) and those immediately found after cannulation. Gonadotropin levels returned to their original values within 30 min to 6 hrs after cannulation. The GTH levels in females B and F remained constant during the same period. Two different patterns were then evident : in female A (which refused food after cannulation) and females B, C, E and F (exhibiting normal feeding behaviour), the GTH levels remained close to or fluctuated around the initial ones, whereas in female D (refusing food after cannulation), the GTH levels declined continuously during the experimental period.

C. *Profiles of plasma GTH levels in cannulated and non-cannulated females* (fig. 3, table 1). — An analysis of the stage of oocyte development showed that oocytes of both groups were at the onset of vitellogenesis. Oocyte diameter in the cannulated fish was 1 114 ± 135 µm ($\bar{X} \pm SD$), whereas in the non-cannulated trout it was

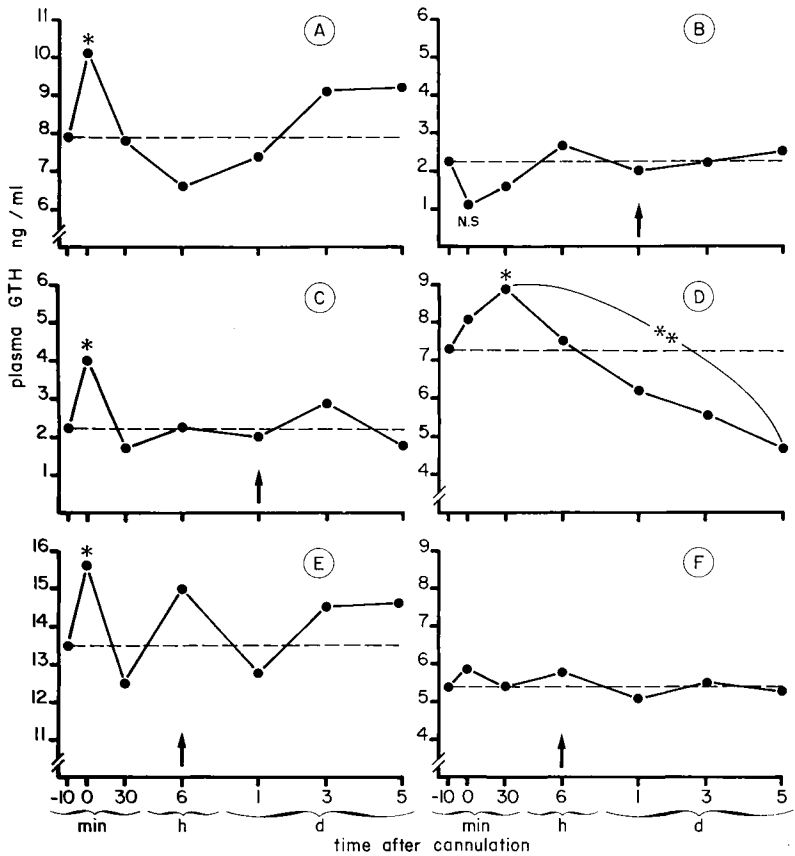

FIG. 2. — *Plasma GTH levels after dorsal aortic cannulation* (time 0) *in 6 individual female trout.* ------ : *represents GTH levels prior to cannulation* (time — 10 min). Arrows indicate resumption of feeding. Statistical analysis was done after calculating a standard deviation value for each GTH level, based on the triplicate assayed * : significantly different ($P < 0.05$) from GTH level prior to cannulation ; min : minutes ; h : hours ; d : day.

1 155 $\pm$ 139 μm. All the females serving in the present experiment exhibited normal feeding behavior when blood sampling started.

Figure 3 shows the profiles of plasma GTH levels in both experimental groups. During the 24-hr period, mean hormonal levels were slightly, but not significantly, lower in the cannulated fish then in the non-cannulated fish. This may reflect a GTH level difference between the two experimental groups which existed prior to cannulation, as the GTH level mean before cannulation ($\bar{X}_0$) was lower than or equal to all the mean hormonal levels in the non-cannulated fish (fig. 3). In addition, in none of the cannulated fish did the GTH levels differ significantly before or after cannulation (table 1). The patterns of GTH levels in both groups during the experimental period were similar, as both curves closely fitted a polynomial function of the third order. Mean hormonal levels in both groups tended to decline slightly towards noon, then

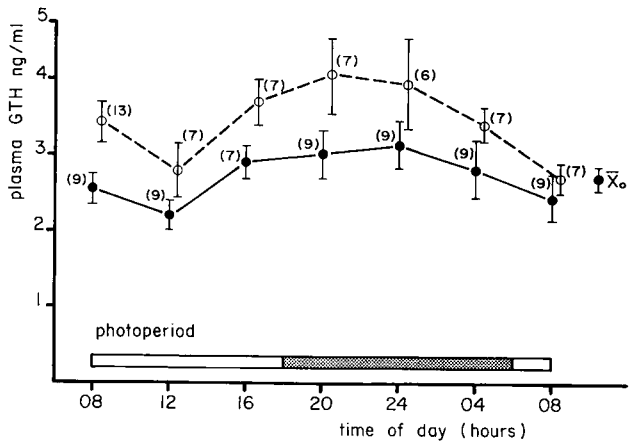

FIG. 3. — *Comparison of mean plasma GTH concentrations in non-catheterized* (o— — —o) *and catheterized* (●———●) *female trout for 24 hrs.* Non-catherized trout were divided into 4 groups, each of which was bled twice at an interval of 48 hrs or more between the two samplings. Vertical lines represent standard errors. $\bar{X}_0$ is the mean of pre-operative plasma GTH levels in the cannulated fish.

TABLE 1

Plasma GTH levels in female trout (April) prior to cannulation and for 24 hours five days later

Fish	GTH level (ng/ml) before cannulation	Mean GTH level 5 days later
1	3.55 (1)	3.26 ± 0.41 (2)
2	2.26	2.54 ± 0.19
3	2.88	3.19 ± 0.21
4	2.58	2.37 ± 0.45
5	3.20	3.49 ± 0.76
6	2.15	2.41 ± 0.16
7	2.38	2.07 ± 0.21
8	3.01	2.77 ± 0.12
9	2.91	3.36 ± 0.78

(1) For statistical comparisons standard deviation values were calculated for each individual GTH level, based on a general coefficient of variation characterizing the corresponding RIA.
(2) Mean ± SE.

to increase during the afternoon, reaching maximal levels between 8 p.m. and midnight, after which they tended to decrease again.

D. *Effect of cannulation on the processes of oocyte maturation and ovulation and on concomitant plasma GTH levels* (fig. 4). — All the fish exhibited normal feeding behaviour during the entire experimental period. The control as well as the cannulated females underwent oocyte maturation and ovulation within the 20 days of experimentation. The plasma GTH levels in all females in both the groups increased progressively during germinal vesicle migration and maturation to reach maximal levels once ovulation had occurred.

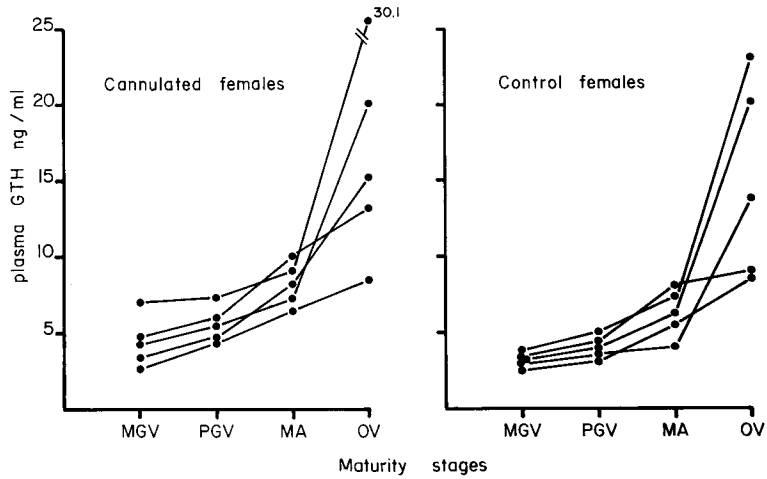

FIG. 4. — *Individual plasma GTH profiles durings different maturity stages in catheterized and non-catheterized female trout.* Maturity stages : MGV : migrating germinal vesicle ; PGV : peripheral germinal vesicle ; MA : maturated ; OV : ovulated.

E. *Individual daily GTH profiles in cannulated females* (fig. 5). — Plasma GTH levels in vitellogenetic females prior to cannulation, and during a 10-hr period 6 days after cannulation, are shown in figure 5. We observed a fluctuating GTH level profile in females G, H, I and J, all exhibiting normal feeding behaviour. Peak levels occurred once or twice during the sampling period. Hormonal levels close to the pre-peak ones were reached 1 to 2 hrs after the peak values appeared. In females K (normal feeding) and L (refusing food), no fluctuations were observed and the GTH levels were relatively constant. The mean of the basal GTH levels was calculated for every female after eliminating the peak (outliers) values (Dixion's gap test ; fig. 5). In none of the females G to K was the pre-cannulation plasma GTH level significantly different from the mean of the basal levels calculated for the same females (Dixion's gap test, Student's t-test : $P > 0.05$). In female L, the only one refusing food, the GTH level before cannulation was significantly higher than the mean of the basal values (all values in this case) in that fish once it was cannulated and serially sampled.

Discussion.

The blood sampling of unanesthetized, free-swimming fish is advantageous in studying certain physiological functions. However, dorsal aortic catheterization involves a traumatic surgical intervention and a long-term implanted cannula in the blood vessel, both of which may disturb the normal profile of a given physiological process and induce a transient or chronic stress situation. Repetitive blood sampling via an implanted cannula may have the same effects, as it causes changes in blood volume and the possible introduction of a certain amount of heparin into the circulation. Therefore, when cannulating fish, the advantages and limitations of the technique must be considered in relation to the function to be studied.

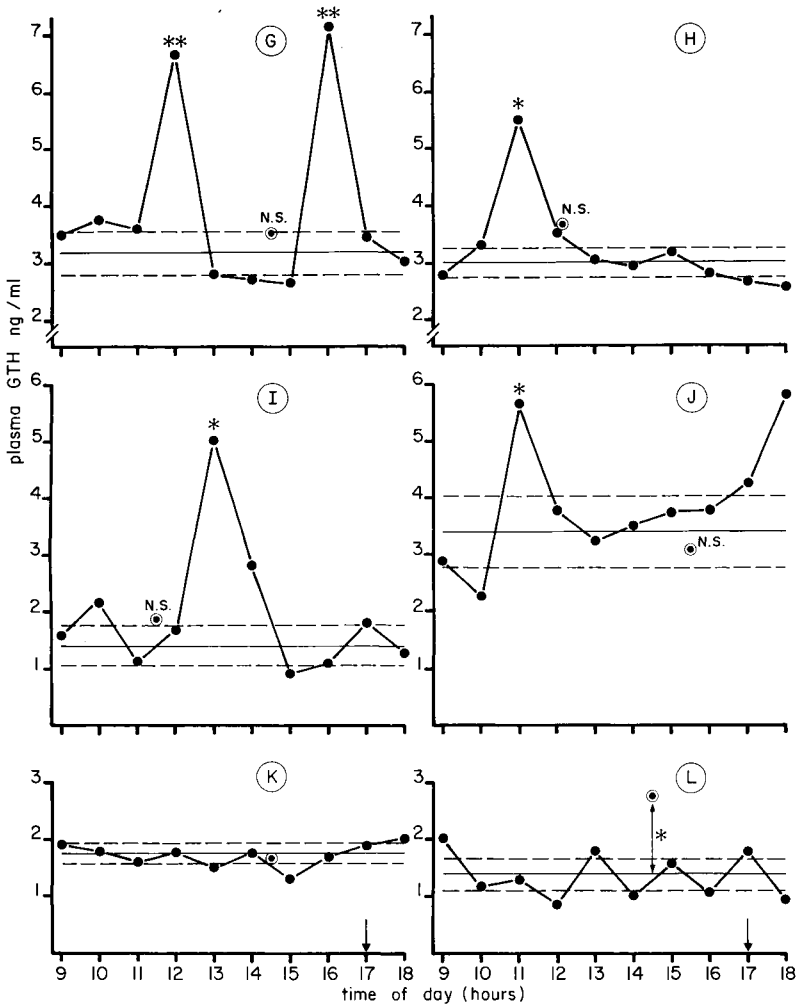

FIG. 5. — *Individual plasma GTH profiles in catheterized female trout during a* 10-*hr sampling period.* ⊙ : plasma GTH level prior to cannulation (sample 0) ; ▪▪▪▪▪▪▪▪ mean ± SE of basal GTH levels (calculated after eliminating peak values by Dixion's gap test). Arrows indicate the beginning of the dark period. For statistical analysis, the standard deviation values, based on a general coefficient of variation characterizing the corresponding RIA, were calculated for the individual GTH levels. * : $P < 0.05$, ** : $P < 0.025$. NS : Not significantly different ($P > 0.05$) from the mean of basal GTH levels.

During the operation for catheter implantation, the fish are exposed to several stressing factors, i.e. anesthetization, surgery and hypoxia : the first two have both been found to influence plasma gonadotropin levels in different mammalian species (see Introduction). In the present study, there was a slight rapid increase in GTH levels after cannulation in 4 of the operated fish. That change was concomitant with an important increase in the hematocrit and the glucocorticoid levels in the same females

(Bry and Zohar, 1980), and might reflect either hemoconcentration and/or a possible relation between a stress situation and the hypophysial secretory mechanims. The two different patterns of GTH level response (increased or steady levels), observed in the different fish may represent individual variations in the temporal pattern of the same phenomenon, as a biphasic LH response to acute anesthetization stress, consisting of an initial stimulatory phase and an ensuing inhibitory one, was observed in the rat (Krüliche *et al.*, 1974).

Recovery to pre-operative GTH level was complete within 30 min to 6 hrs after surgery. Two females, A and D (fig. 2), did not return to normal feeding after cannulation ; this was probably due to chronic stress as implied by the abnormal glucocorticoid profiles in those females during the entire period (Bry and Zohar, 1980). In one (female A), we could not detect any abnormal GTH profile, whereas the GTH levels decreased progressively in the other female (D). A similar phenomenon was observed in female L (also refusing to eat after cannulation), i.e. the pre-operative GTH level was higher than all the levels observed 6 days later (fig. 5). Thus, a decline in GTH level was found in two out of three « stressed » females. That decline could have been induced by chronic stress either directly or indirectly as a result of food deprivation, as starving decreases gonadal activity and hypophysial and plasma GTH levels in the goldfish (Clemens and Reed, 1967 ; Gillet *et al.*, 1980). In all cannulated females which resumed normal feeding, the plasma GTH levels observed in repetitive samples at 6 post-operative days did not differ from the pre-operative levels. Thus, the resumption normal feeding behaviour and the return to initial gonadotropin levels may be used as routine criteria to test the state of recovery of cannulated fish and the validity of their use for studying gonadotropin secretion patterns. The present work as well as that by Bry and Zohar (1980), describing the development of glucocorticoid levels, hematocrit, leucocrit and feeding behaviour after cannulation, suggest that minimally stressed fish, adapted to a cannula, can be available as soon as 3 days after the operation.

Aortic cannulation was shown to modify blood prolactin and LH levels in rats, although the presence of such a cyclic phenomenon as the proestrous secretory surge of both hormones was undisturbed (Neill, 1972). One of the possible reasons for such changes is the presence of heparin in the circulation. This anticoagulant was shown to interfere with LH binding to gonadal receptors (Salomon and Amsterdam, 1977 ; Fox and Wisner, 1979). Nevertheless, our study demonstrates an absence of exogenous heparin effect on plasma GTH levels in male and female trout. The decline in gonadotropin levels in heparin and saline-treated fish was independent of the treatment and probably resulted from the handling stress to which the fish were exposed during the entire experimental period. Moreover, the pattern of GTH level for 24 hrs, in fish sampled via a cannula, was parallel to that observed in non-cannulated fish blood sampled by caudal vessel puncture. The cannulation of females with oocytes beginning germinal vesicle migration did not affect the normal continuation of oocyte maturation and ovulation ; gonadotropin levels accompanying these processes were not modified in cannulated animals and exhibited the typical previously described increase (Jalabert *et al.*, 1976 ; Fostier *et al.*, 1978). Thus, cannulated female trout, well adapted to the cannula, showed gonadotropin secretion patterns and gonadal activities similar to those seen in non-cannulated females.

Some of the experiments in the present study confirm the individual heterogeneity of plasma GTH levels in fish representing the same ovarian stage. This fact necessitates the use of the cannulation technique for the study of plasma gonadotropin patterns. An example of the importance of this technique is included in figure 5. When cannulated females having oocytes in active vitellogenesis were bled hourly for 10 hrs to determine the gonadotropin levels, very different patterns were seen. The GTH levels in most « unstressed » females showed short-term fluctuations, whereas they remained constant in one female. The pattern of plasma GTH level was not synchronized in the different females. These observations suggest a possible pulsatile secretion of gonadotropin in females with oocytes in active vitellogenesis. Hontela and Peter (1978) reported significant daily fluctuations in the serum GTH levels in female goldfish undergoing ovarian recrudescence. In their study, a group of fish was bled and killed at different times so as to constitute a 24-hr period in which sampling was effected every 4 hrs ; their results suggest a relative synchronization in GTH secretion events among the different females. The trout model, as shown in our study, might be different as the individual GTH profiles were not synchronized, indicating that short-term GTH secretion events were distributed distinctively during the day according to the female. When individual female trout undergoing vitellogenesis were bled (via cannulae) every 4 hrs for 32 hrs, daily fluctuations were observed in some, but again no profile synchronization was found (Zohar, unpublished data), thus confirming the above hypothesis. Other GTH secretion profiles were observed in cannulated female trout at different stages of ovarian development (Zohar, unpublished data).

In conclusion, unstressed, cannulated fish must be used to study the short-term temporal profiles of any of the circulating hormones when those profiles, as in the case of female trout GTH patterns, are not synchronized. The present study suggests a short-term transient effect of acute surgical stress on plasma GTH levels in most cannulated fish, and a long-term effect of surgery or the cannula in non-adapted specimens. Nevertheless, following a recovery period of at least 3 days and after elimination of chronically stressed females, individual cannulated fish can be advantageous models for studying gonadotropin secretion patterns as reflected by the blood hormone levels.

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Résumé. L'utilisation de la technique de cathétérisation de l'aorte dorsale pour l'étude des profils de sécrétion gonadotrope chez la truite arc-en-ciel a été testée. L'héparine utilisée pour rincer la canule lors des différents prélèvements n'a aucun effet sur les niveaux plasmatiques de GTH. La cathétérisation entraîne une variation faible et de courte durée de ces niveaux. Les niveaux gonadotropes reviennent à leurs valeurs initiales 30 min à

6 h après l'opération. Les concentrations de GTH demeurent alors, proches de leurs valeurs initiales chez les poissons présentant un comportement alimentaire normal ; cependant, chez les femelles « stressées » ne se nourrissant pas normalement, ces niveaux ont tendance à diminuer.

Chez les poissons bien adaptés, la cathétérisation de l'aorte dorsale n'a d'effet ni sur le profil diurne des niveaux plasmatiques de GTH ni sur la fonction gonadique et le profil de GTH pendant le processus de maturation ovocytaire et d'ovulation. En conclusion, des truites cathétérisées peuvent être utilisées avec avantage pour étudier les profils de sécrétion gonadotrope, ceci après un délai de trois jours de récupération et en éliminant les poissons qui ne retrouvent ni leur comportement alimentaire normal ni leurs niveaux initiaux de GTH pré-opératoire. L'utilisation de cette technique nous a permis de montrer que, chez la truite en vitellogenèse active, la secrétion de GTH hypophysaire s'effectue probablement sous forme de pulses de courte durée.

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