Maintenance of the Constant Flow Rate of the Blood from the Ovarian Vein in the Rats

Bull. Univ. Osaka Pref., Ser. B, Vol. 22,
大阪府立大学紀要，農学・生物学， Mar., 1970.

Maintenance of the Constant Flow Rate of the Blood from the Ovarian Vein in the Rats

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On the study of the ovarian function in the rats Eto *et al*[1] measured progestins in the ovarian venous blood. This approach, although it submits the animals to prolonged surgical interference, is valuable because the steroids secreted from the ovary are better indicators of ovarian function than the steroids isolated from the ovarian tissue or from the *in vitro* incubations, and because it has made easy the determination of progestins secreted from the rat ovary owing to the higher concentrations of the steroids in the specimens than those in the peripheral blood. In a number of studies thereafter steroid levels in the ovarian venous blood were measured in the rats. The measurments in these studies were made of concentration[1] or of secretion rate from the ovary.[2–6] In the course of the study on the steroid metabolism in the rat ovary it has been usually observed that the flow rate of the blood from the ovarian vein remarkably decreased during collection. As a result, the differences in period and in time of collection after the beginning of bleeding influenced to a great extent on the concentrations of the steroids in the specimens and it made one hard to evaluate the results obtained.

In the present paper the effects of the continuous injection of glucose solution and whole blood on the flow rate and hematocrit value of the blood from the rat ovary were studied to gain a method for maintenance of a constant flow rate of the blood, so that it can be used for the study of the acute effects of trophic hormones on the ovarian secretion of steroids.

Methods

Sprague-Dawley strain rats bled in this laboratory were used in this study. The adult female rats were mated with vasectomized males. The rats on day 4 of pseudopregnancy were anesthetized with an i.p. injection of nembutal (35 mg./kg. of body weight). The ovarian vein was exposed by a longitudinal incision on the ventral midline. The branch of the vein from the uterus was ligated. After the ligation 200 USP units of sodium heparin were intravenously injected. A polyethylene tubing (Intermedic PE 50) was inserted into the ovarian vein immediately after tieing off the proximal end of the vein. The sites of application of ligatures and tubing are shown in Figure 1. The abdominal incision was closed with wound clips after the cannulation and body temperature was maintained at normal level by warming pads. The test solution was continuously injected through a femoral vein at a constant rate during the blood collection by means of an infusion pump as shown in Figure 2. The blood used for transfusion was collected shortly before the transfusion from the abdominal aorta of several rats which were in the same reproductive state as the recipients and injected with heparin. The collection of the ovarian venous blood and injection of the test solution were commenced simultaneously at the time of cannulation. The blood was collected in a series of graduated tubes immersed in ice water. The blood volume and hematocrit value of the specimens were measured after the collection. Hematocrit value was determined

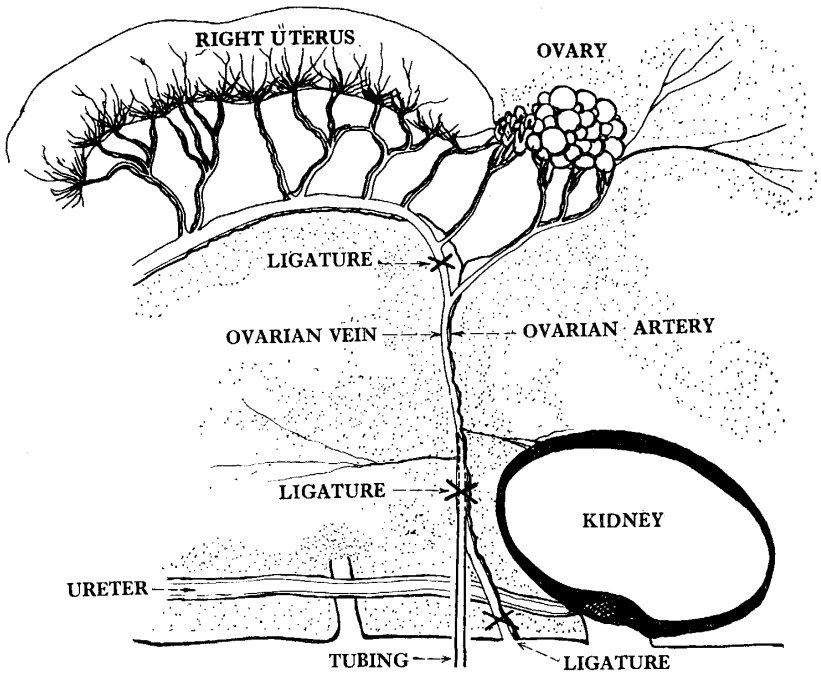

Fig. 1. Schematic representation of the sites of ligation and insertion of a tubing for the collection of the ovarian venous blood in the rats.

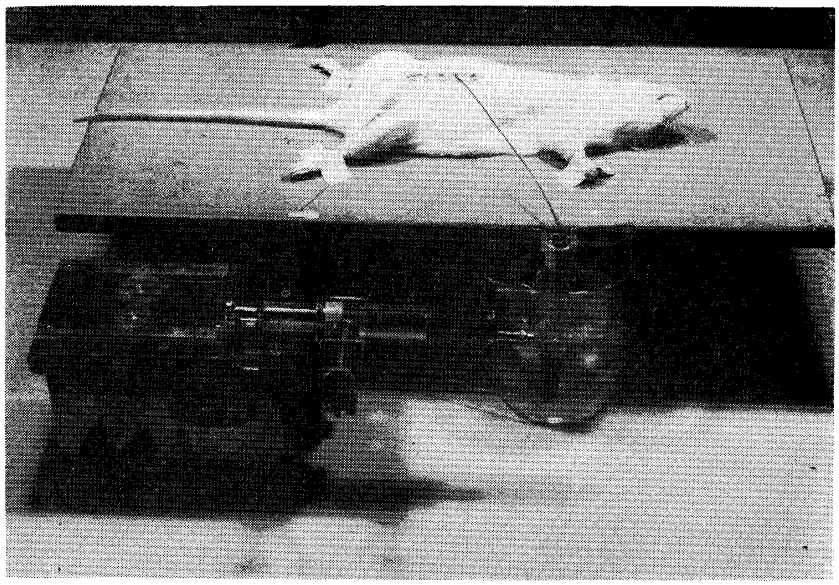

Fig. 2. Collection of the ovarian venous blood in the rats.

by centrifugation of the blood in a capillary tube at 11,000 rpm for 5 minutes, and expressed as percent of cells. Differences among the mean values were analyzed with Duncan's multiple range test.

Results

Three groups of 6 rats were injected with 6% aqueous glucose solution at the rate of 6.2 ml. per hour and with whole blood at the rate of 6.2 ml. and 10.6 ml. per hour respectively. Another six rats were bled without injection. The ovarian venous blood was collected

for four successive 30-minute periods. Changes of the average volumes and hematocrit values of the specimens in each group are shown in Figure 3. The non-injected rats presented symtoms of anemia and fell in low activity within one hour in the course of bleeding. The injection of either glucose solution or whole blood prevented the animals from the loss of physical activity and maintained them in good conditions during the bleeding. However, in the glucose-injected group as well as the non-injected group the flow rate of blood from the ovarian vein decreased less than one half of that in the initial 30–minute specimens and hematocrit value gradually decreased with time. In the group in which the blood was injected at the rate of 10.6 ml. per hour, higher flow rate was maintained and no significant decrease was observed in hematocrit value during the period.

In the next experiment two groups of 6 rats were injected with blood at the rate of 6.2 ml. and 10.6 ml. per hour respectively. Ovarian venous blood were fractionated in 5–minute specimens for 60 minutes. Changes in the mean blood volumes in the two groups are given in Figure 4. The analysis of variance for the mean flow rates is depicted in Table 1. In the

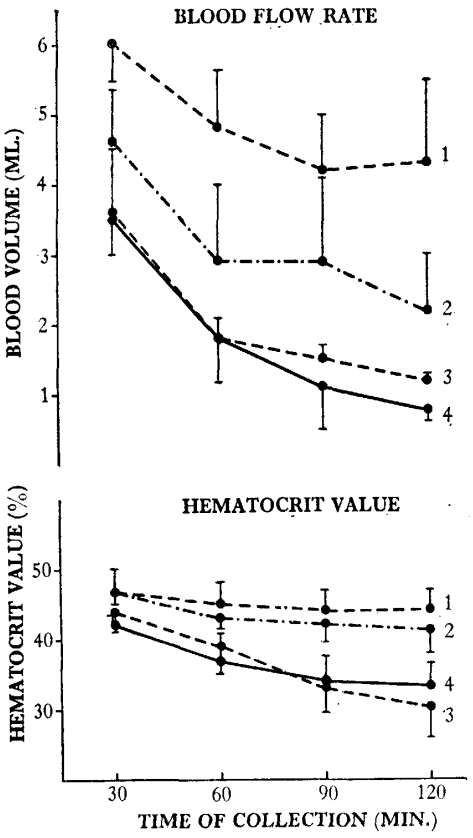

Fig. 3

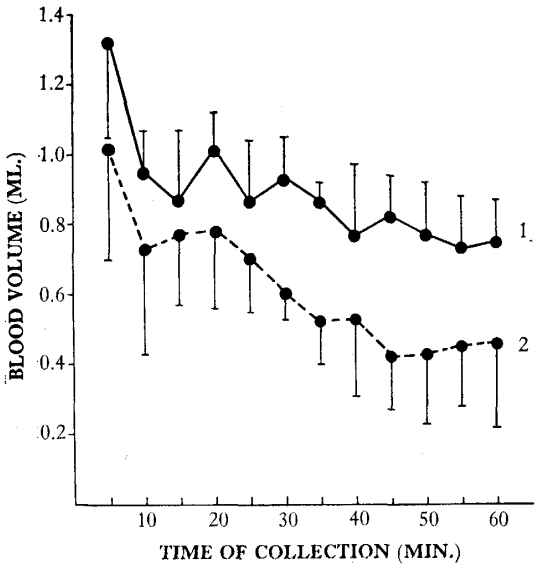

Fig. 4

Fig. 3. Changes in flow rate and hematocrit value of the ovarian venous blood in the rats. The animals were continuously injected with whole blood and 6% aqueous glucose solution at a constant rate. The rate of injection: Graph 1, 10.6 ml. of blood/hr.; Graph 2, 6.2 ml. of blood/hr.; Graph 3, 6.2 ml. of glucose sol./hr.; Graph 4, no injection. The values are given as the means (points) and one standard error of the means (bars) in volume and hematocrit value of the blood samples taken every 30 minutes from 6 rats.

Fig. 4. Changes in flow rate of the ovarian venous blood in the rats injected with blood at the rate of 10.6 ml./hr. (Graph 1) and 6.2 ml./hr. (Graph 2), respectively. The values are given as the means (points) and one standard error of means (bars) of the blood samples taken every 5 minutes from 6 rats.

Table 1. Multiple range test for the data of Figure 4. Any two means not underscored by the same line are significantly different at the level of 5%. Any two means underscored by the same line are not significantly different.

Graph 1 (10.6 *ml. blood per hour*)												
Time of collection	5	20	10	30	15	25	35	45	40	50	60	55
Mean volume	1.32	1.02	0.95	0.93	0.87	0.87	0.87	0.82	0.77	0.77	0.75	0.73
Graph 2 (6.2 *ml. of blood per hour*)												
Time of collection	5	20	15	10	25	30	40	35	60	55	50	45
Mean volume	1.02	0.78	0.77	0.73	0.70	0.60	0.53	0.52	0.47	0.45	0.43	0.42

rats which were injected with blood at the rate of 6.2 ml. per hour the flow rate of the ovarian venous blood significantly decreased with time ($P<0.01$). However, in the rats which were injected with blood at the rate of 10.6 ml. per hour no significant difference was observed between any two mean volumes of the 5–minute specimens collected for 60 minutes, except the initial 5–minute fraction.

Discussion

The flow rate of blood from the ovarian vein in the rats which were not supplied with physiological fluid decreased rapidly with time during collection. Fajer and Barraclough[4] on the collection of the ovarian venous blood infused saline to keep near the flow rate at the beginning of collection. In the present study the infusion of 6% aqueous glucose solution at the rate of 6.2 ml. per hour had no amending effect on the decrease of both flow rate and cell volume. Injection of whole blood at the rate of 10.6 ml. per hour maintained the flow rate of blood from the ovarian vein in almost constant for at least one hour. In this group the flow rate at the beginning of collection was still higher than the rate of the succeeding blood flow. If the initial 5–minute fraction would be excluded, the blood specimens will be obtained at a constant flow rate thereafter and it will make one possible to evaluate the changes in secretion rate of steroids from the ovary, whether they were expressed in concentration or in secretory rate.

In the present method the small branches of the ovarian vein were not ligated. Nevertheless it can be considered that effluent of ovarian tissue is predominant component of the specimens, since the diameters of these tributaries were less than one sixth of the ovarian vein's.

To our knowledge no report has appeared in the literature which showed incompatibilities of the blood groups in the rats. In this study the recipients and donors were taken from the same colony bled in this laboratory and no visible disorder has been observed in the recipient animals during the experiments.

Summary

The effects of continuous injection of 6% aqueous glucose solution and whole blood on the flow rate and cell volume of the blood taken from the ovarian vein were studied in the rats to gain a method for a maintenance of a constant flow rate of the blood. Flow rate and hematocrit value of the blood taken from the rats without supply of fluid decreased rapidly with time. The injection of glucose solution had no amending effect on the decrease of both flow rate and hematocrit value. By transfusion of whole blood at the rate of 10.6 ml. per hour

an almost constant flow rate and normal cell volume of the ovarian venous blood were maintained at least for one hour during the collection.

References

1) ETO, T., H. MASUDA, Y. SUZUKI and T. HOSHI. Progesterone and Pregn-4-ene-20α-ol-3-one in rat ovarian venous blood at different stages in reproductive cycle. *Jap. J. Animal Reproduct.*, **8**: 34–40, 1962.
2) PORTER, J.C., P.K. SIITERI and C.W. YATES JR. Secretion of progesterone by the ovary of the rat. *Fed. Proc.*, **26**: 533, 1967.
3) YOSHINAGA, K., S.A. GRIEVES and R.V. SHORT. Steroidogenic effects of luteinizing hormone and prolactin on the rat ovary in vivo. *J. Endocr.* **38**: 423–430, 1967.
4) FAJER, A.B. and C.A. BARRACLOUGH. Ovarian secretion of progesterone and 20α-hydroxypregn-4-en-3-one during pseudopregnancy and pregnancy in rats. *Endocrinology*, **81**: 617–622, 1967.
5) HASHIMOTO, I., D.M. HENDRICKS, L.L. ANDERSON and R.M. MELAMPY. Progesterone and pregn-4-en-20α-ol-3-one in ovarian venous blood during various reproductive states in the rat. *Endocrinology*, **82**: 333–341, 1968.
6) UCHIDA, K., M. KADOWAKI and T. MIYAKE. Ovarian secretion of progesterone and 20α-hydroxypregn-4-en-3-one during rat estrus cycle in chronological relation to pituitary release of luteinizing hormone. *Endocr. Japon.*, **16**: 227–237, 1969.