

c) *Possibility of a modification of the pituitary functions.* The administration of oestradiol to intact rats, in a dose of 0.03 µg/kg daily on the first 6 days of pseudogestation, did not interrupt the vaginal dioestrus but resulted – as in our formerly reported experiments – in a marked inhibition of the secretion of progesterone on the 7th day (3.9 ± 0.6 as compared with 15.4 ± 2.0 µg/h). The simultaneous injection of prolactin in a dose of 1 mg/kg daily did not counteract this inhibitory effect, the secretion of progesterone in animals thus treated likewise being markedly diminished (4.7 ± 1.7 µg/h). This absence of action of prolactin under such experimental conditions suggests that oestradiol does not exert its effect on the progesterone secretion by a reduction of the prolactin levels. It must therefore be concluded that oestradiol in a low dose interferes with the secretion of another pituitary hormone. In the rats given LH antiserum together with oestradiol, we observed a significantly higher progesterone value (15.1 ± 1.7 µg/h) than in the animals which received oestradiol alone (3.9 ± 0.6 µg/h). This last observation shows that the reduction in ovarian progesterone production exerted by oestradiol has to be considered as an effect of oestrogen on the regulation of LH secretion.

Discussion and conclusion. Different publications, and especially those of BARRACLOUGH and HALLER⁴, have established that the administration of low doses of oestradiol produce an increase in the secretion of LH. Repeated injections of this pituitary hormone to pseudopregnant rats exert a luteolytic effect which has been measured by morphological (ROTHCHILD⁵) and functional

(YOSHINAGA, GRIEVES and SHORT⁶) criteria. The effective doses of LH are, according to ROTHCHILD⁵, lower than those which induce ovulation.

Considering our observations¹ and the data in the literature, we can conclude that the drop in ovarian progesterone secretion produced by administration of low doses of oestradiol to pseudopregnant rats, is the result of a stimulation of the secretion of LH. This hypophysal hormone apparently exerts a luteolytic action, under our experimental conditions: i.e. in pseudopregnancy.

Résumé. Ce travail propose un mécanisme d'action pour l'effet lutéolytique observé chez des rats pseudogestants, traités pendant 6 jours avec une dose très faible d'oestradiol¹, cet effet lutéolytique doit être rapporté à une sécrétion accrue de LH.

P. BISCHOF, C. KRÄHENBÜHL and
P. A. DESAULLES

*Department of Pharmacology, Ciba-Geigy Ltd.,
CH-4002 Basel (Switzerland), 14 May 1974.*

⁴ C. A. BARRACLOUGH and E. W. HALLER, *Endocrinology* 86, 542 (1970).

⁵ I. ROTHCHILD, *Vit. Horm.* 23, 209 (1965).

⁶ K. YOSHINAGA, S. A. GRIEVES, R. V. SHORT, *J. Endocr.* 38, 423 (1967).

PRO EXPERIMENTIS

Simple Method for the Rapid Collection of Murine Blood Avoiding Death of the Animal

Estimated figures indicate that 30,281,783 mice were used for research purposes in 1971¹. The reasons for this use of mice can be found in their high fertility, easy growth, and overall economy as regards procuring and maintaining. As a result there is a constant demand on the part of many laboratories to obtain blood for several purposes.

The techniques normally utilized for obtaining blood from mice are bleeding from 1. The venous plexus of the orbit,² 2. Intracardiac puncture,³ 3. The inferior vena cava,⁴ Each of these methods suffers from the inconvenience that loss of animals is great. They pose special problems in certain experiments which require that multiple blood samples be collected during the course of treatment, necessitating the survival of the mice. In immunology, for example, it is important to draw blood without killing the animals, so that they can be bled again at the end of the experiment.

It is the purpose of this report to set forth a simple and rapid method by which blood can be obtained over and over again from the same mouse. No special equipment is required and the blood is taken while the animal is immobilized, thus eliminating the use of anesthetic and

ensuring that the blood will be anesthetic-free. Finally, the mice do not appear to be severely stressed by the procedure.

Material and methods. 1. A plunger of a 60 ml plastic disposable syringe (A) and a plastic container (B); 2. A sterile plastic tube 5 mm in diameter and 5 cm long (C); 3. a rubber stopper with a hole 4 mm in diameter (D); 4. a graduated sterile test tube (E); 5. a 20 gauge needle (G) connected to a vacuum pump (I); 6. a 100 watts lamp (J); (Figure).

The mouse must be immobilized as shown in the Figure. The plunger may be used to adjust the plastic container to the size of the individual mouse. (It may be more

¹ *Ilar News* (Eds. R. H. YAGER and C. B. FRANK; Institute of Laboratory Animal Resources, Washington, D.C. 1972), vol. 16, p. 1.

² S. SCHERMER, *The Blood Morphology of Laboratory Animals*, 3rd edn. (F. A. Davis Company, Philadelphia 1967), p. 61.

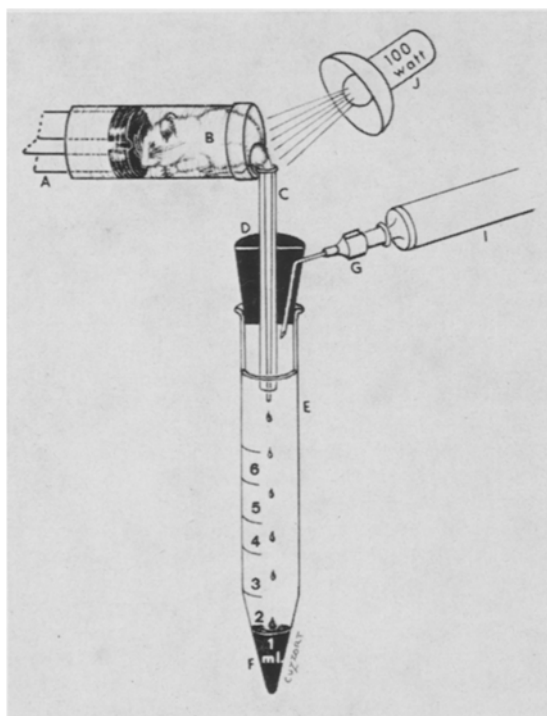
³ A. J. CRESKOFF, T. FITZ-HUGH and E. T. FABBIS. *The Rat in Laboratory Investigation*. 2nd edn. (Eds E. T. FABBIS and J. Q. GRIFFITH; J. B. Lippincott Co., Philadelphia 1949), p. 406.

⁴ J. D. BROOME, personal communication.

C3H/HeJax mice	Weight	No. of mice	Volume of blood obtained in (ml)	Death	Sterile on culture
	26–32 g	28	0.81 ± 0.30	2	17

practical in some cases to immobilize the mouse by taping its feet and hands to a piece of cork). Then the tail must be washed with surgical soap, rinsed and dried. Next the tip of the tail is cut with a sterile scalpel and then introduced into the sterile tube. The tube, the stopper and the graduated test tube are assembled as shown in the Figure.

A needle connected to a vacuum pump is then introduced into the test tube through the rubber stopper so that the suction pulling down the tail will effect a complete closure of the tube. The vacuum thus created will prevent air from passing into the test tube and enable the blood to be sucked into it.



A) Plunger; B) plastic container; C) sterile plastic tube; D) rubber stopper; E) graduated sterile test tube; G) 20 Gauge needle; I) vacuum; J) 100 Watts lamp.

If bleeding is slow, it is helpful to apply the heat of a 100 watts lamp to the mouse from a distance of a few cm for 2–3 min, producing vasodilatation. In this way, one can usually draw from 0.75 to 1 ml of blood from each mouse weighing approximately 26–32 g. If the animal appears weak after bleeding, it is useful to administer 2 ml of 5% dextrose in saline i.p.

Blood cultures. Blood, obtained with the method described, is transferred immediately to a tube of thioglycollate medium, incubated at 37°C, and examined throughout 2 weeks.

Results. The method described has been used many times over a period of 2 years without accurate records being kept. For the purpose of having an exact report, it was used on 28 C3H/He mice obtained from the Jackson Laboratories, Bar Harbor, Main. The results are summarized in the Table.

We conclude that: 1. 1 ml of blood can safely be taken from healthy mice, weighing more than 26 g but not more than this (for example 1 mouse weighing 28 g, died after 1.30 ml of blood was obtained from it, another weighing 27 g died after 1.25 ml). 2. With mice weighing less than 26 g the safe limit is 0.55 ± 0.25 ml. 3. Blood can be collected by cutting about 1 mm of the tip of the tail at intervals of 30 days. 4. The technique results in generally 'clean' blood samples, and in the majority of occasions there are bacteriologically sterile.

Riassunto. Viene descritto un semplice metodo che permette il prelievo di sangue dai topi senza che questi vengano sacrificati. Il metodo è raccomandato in tutti quei casi in cui si richiede il minimo stress da parte dell'animale ed in quei casi in cui, come in immunologia, si vogliono ottenere campioni di sangue dallo stesso animale in varie riprese.

A. COPPOLA^{5,6}

Department of Pathology, College of Medicine,
Downstate Medical Center, State University of New York,
450 Clarkson Avenue, Brooklyn
(New York 11203, USA), 13 February 1974.

⁵ Supported by U.S.P.H.S. Research Grant No. CA-06801.

⁶ We acknowledge the valuable technical assistance of Mr. M. BIRNBAUM of Bacteriology Department and Miss E. CUZZORT from Medical Illustration.

Synthetic Coating: An Improvement in Ultracryotomy

The principal advantages of using semi-thin and ultra-thin frozen sections, instead of sections made of embedded material, have been described repeatedly^{1–7}. With the development of commercial equipment for ultracryotomy (Sorvall MT-2B, FTS LTG2; LKB CryoKit; Reichert OMU3, FC2) and the description of several methodical variants^{8–13}, the interest in this procedure has continued to grow. Since not all technical difficulties involved in ultracryotomy have been overcome, however, we attempted to develop a procedure which not only allows better cutting of the frozen tissue but also facilitates manipulation of even ultra-thin sections and enables determination of section thickness based on the occurrence of interference colours.

Method. By immersion in liquid nitrogen unfixed kidney and liver tissue was frozen onto a silver (Reichert) or else onto a copper slide (Sorvall) and then attached to

¹ W. BERNHARD and E. H. LEDUC, *J. Cell Biol.* 34, 757 (1967).

² F. L. DOLLHOPE, G. WERNER and E. MORGENSTERN, *Mikroskopie* 25, 33 (1969).

³ L. SEVÉUS, *Process Biochem.*, Lond. 4, 35 (1969).

⁴ A. K. CHRISTENSEN, in *Autoradiography of Diffusible Substances* (Ed. L. J. ROTH and W. E. STUMPF; Academic Press, New York 1969).

⁵ S. HODSON and J. MARSHALL, *J. Microsc.*, Lond. 91, 105 (1970).

⁶ A. K. CHRISTENSEN, *J. Cell Biol.* 51, 772 (1971).

⁷ E. MORGENSTERN, K. NEUMANN and G. WERNER, *Mikroskopie* 29, 163 (1973).

⁸ T. KOLLER, *J. Cell Biol.* 27, 441 (1965).

⁹ S. HODSON and J. MARSHALL, *J. Microsc.*, Lond. 89, 373 (1969).

¹⁰ S. A. HODSON and J. MARSHALL, *J. Physiol.*, Lond. 201, 63P (1969).

¹¹ J. R. IGLESIAS, R. BERNIER and R. SIMARD, *J. Ultrastruct. Res.* 36, 271 (1971).

¹² W. BERNHARD and A. VIRON, *J. Cell Biol.* 49, 731 (1971).

¹³ G. WERNER and K. NEUMANN, *Mikroskopie* 28, 34 (1972).