

tinued their development at the site of implantation, *i.e.*, partly in the intraembryonic, partly in the extraembryonic coelom; this location was found to be especially suitable for the further development of the mouse embryos. Fourteen primitive streak stages (7 days) transplanted to the chick by the methods described above developed within 48 hours into typical 9-day embryos which were smaller than normal but looked fairly normal, at least externally. However, a disturbance of the size relationship of the different organs was noticed. The embryos were enclosed in their amnion, had six pairs of somites, a strongly and normally beating heart, cerebral lobes which were well developed but relatively smaller than normal, an irregular slightly winding neural tube and a tail bud. Other embryos, implanted into the extraembryonic coelom in the first somite stages (age about 8 days), continued development up to the stage when maternal circulation becomes of primary importance for the nutrition of the embryos, *i.e.*, at 9 to 10 days. Fur-

ther experiments will have to show whether it will be possible to obtain any development beyond these stages. By observing aseptic precautions, the operations can be made to work successfully without great difficulties in a large percentage of cases.

Thus a method for extended study of post-implantation stages of mammals has been developed which will supplement those already made available by Nicholas and Rudnick<sup>2</sup> and by Törö<sup>3</sup> for rats, and Waddington and Waterman<sup>4</sup> for rabbits.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### AN APPARATUS FOR CONTINUOUS, RAPID AND COMPLETE EXTRACTION OF ESTROGENS

MANY continuous extraction apparatus have been developed for use in estrogen determinations.<sup>1,2,3</sup> In a series of clinical studies now in progress, where multiple determinations are required, we designed an extractor which permits small quantities of liquid to be rapidly extracted as a part of a routine in this series of analyses.

The apparatus schematically illustrated consists of several separate units which are connected by standard ground glass joints. These are boiling flask (*B*), condensing unit and extractor (*A*), which includes an inlet and outlet to facilitate operation, and a stirring device to increase extracting efficiency.

In flask *A* is placed the solution to be extracted to a level about 2 cms below the opening into tube (*a*). In flask *B* is the extracting solvent which is of a lower specific gravity. When heat is applied to flask *B* vapors developed go up (*b*) are condensed, and dropped down through tube (*c*) to pass out at the bottom of flask *A* through perforated bulb (*d*). The stirrer mixes the two liquids into a temporary suspension without disturbing the layers formed at the top. The lighter liquid collects at the top until the hydraulic

head built up in (*c*) is sufficient to cause siphonation at tube (*a*) into flask *B*. From here the whole process repeats itself, continuously. The apparatus is filled through a funnel (*D*) and at the end of the operation is emptied through the stop-cock (*e*) opening at the base of flask *A*.

The inclusion of the stirring device increases extracting efficiency by bringing more liquid into contact with extracting solvent. The inlet funnel (*B*) and outlet stop-cock (*e*) permit filling, separation of layers and emptying without loss of time and danger of breakage in disengaging at the start or conclusion of an operation. This also facilitates cleaning of the apparatus.

This extractor can be adapted for large quantities by substituting a larger extraction flask of relative proportions. The advantage of the stirrer in a larger apparatus becomes more apparent as the peripheral liquid which would ordinarily not be touched by the extracting solvent is brought into direct contact due to stirring action.

Several tests on the efficiency of the apparatus have been made. Specimens used were estrone—estradiol fractions (obtained according to the method of Smith and Smith, 1939, from three separate pooled pregnancy urines, each divided into equal aliquot portions as labeled in Table 1).

<sup>1</sup> T. F. Gallagher, F. C. Koch and R. I. Dorfman, *Proc. Soc. Exp. Biol. and Med.*, 33: 440, 1935.

<sup>2</sup> O. W. Smith, G. Van S. Smith and S. Schiller, *Endocrinology*, 25: 509, 1939.

<sup>3</sup> N. B. Talbot and G. O. Langsroth, *Endocrinology*, 25: 729, 1939.

<sup>2</sup> J. S. Nicholas and Dorothea Rudnick, *P.N.A.S.*, 20: 656-658, 1934; *Jour. Exp. Zool.*, 78: 205-232, 1938.

<sup>3</sup> Emeric Törö, *Jour. Exp. Zool.*, 79: 213-236, 1938.

<sup>4</sup> C. H. Waddington and A. J. Waterman, *Jour. Anat.*, 67: 355-370, 1933.

TABLE 1

Purpose of experiment	Specimen identification	Time of extraction	Bio-assay* $\gamma$ /cc of extract
Reproducibility of results	P.U. 1A	6 hrs.	63
	P.U. 1B	6 "	63
Time of complete extraction	P.U. 2	24 "	42
	P.U. 2A	4 "	42
	P.U. 2B	8 "	42
	P.U. 2C	16 "	42
	P.U. 3AI.	4 "	42
	P.U. 3AII.	2 "	28
	P.U. 3AV.	1 "	28

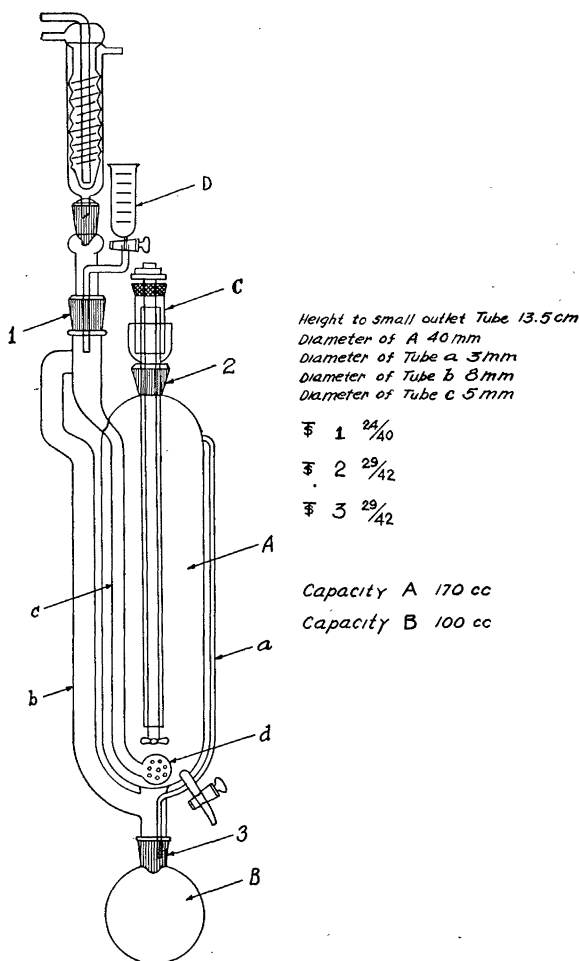
\* Rat Unit =  $1\frac{1}{2}$   $\gamma$  estrone

FIG. 1

Results indicate both reproducibility and relatively great efficiency.

This apparatus has given complete extraction of identical quantities requiring less time than with the use of other extractors.

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## OBTAINING BLOOD FROM MICE

WHILE testing the sera of individual mice for antibodies several years ago, the writer obtained blood more easily and in larger quantities by severing the axillary artery than by cardiac puncture. Since a number of papers have appeared recently in which the investigators reported using cardiac puncture in bleeding mice, the method employed by the writer is hereby described with the hope that it will be useful to others.

The mouse is anesthetized, placed on its back on a dissecting board and its extended legs fastened with spring clips. The hair is thoroughly moistened and, with scissors, a mid-line incision is made through the skin from the abdomen to the neck. The skin of the right side is grasped with small hemostatic forceps and reflected by pulling until the muscles of the right foreleg are exposed. A pocket will be formed between the skin and body wall in the axilla. When the mouse shows signs of regaining consciousness, the axillary artery is severed by cutting deeply with sterile scissors through the center of the pectoral muscle into the axilla. A pool of blood immediately forms in the pocket and can be removed easily with a sterile pipette. A greater yield is obtained if the mouse almost recovers from the anesthesia before the artery is severed than if it is cut during deep anesthesia. The bleeding is so profuse that the animal rapidly loses consciousness. This method also has been used successfully on rats and may be useful in bleeding small birds.

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