TABLE 2

Sensitivity limits (μg) of metal ions with various spray reagents

Sl. No.	Metal Ions	Reagent I		Reagent II		Reagent III	Reagent IV	Reagent V	Reagent VI	Reagent VII
		Visible	UV	Visible	UV	Vis	Vis	Vis	Vis	Vis
1.	Ce(III)	20	8.0	—	—	—	—	—	—	—
2.	Co(II)	10	2.0	10	8	—	—	0.03	—	—
3.	Cu(II)	15	5.0	10	8	—	0.4	0.006	—	—
4.	Fe(II)	1	0.5	—	—	—	—	—	—	—
5.	Fe(III)	1	0.6	10	4	3	10.0	—	—	—
6.	Mn(II)	10	5.0	10	6	—	—	—	—	—
7.	Ni(II)	10	2.0	10	6	—	—	0.012	—	—
8.	Ti(IV)	5	1.0	10	6	—	—	—	0.01	0.5
9.	U(VI)	8	4	10	6	3	10.0	3	—	—
10.	V(V)	30	10	—	—	—	—	—	—	—

Reagents: I = β-resorcylaldehyde; II = o-vanillin oxime; III = quercetin; IV = 8-hydroxyquinoline; V = rubeanic acid; VI = morin; VII = chromatropic acid.

All the metal ions showed coloured spots on spraying with the reagent. The transition metal ions gave intense colour reactions.

The results of the sensitivity limits for the ten metal ions reveal that in the UV light the reagent is quite effective for concentrations ranging from 0.5 μg to 10 μg. For comparison, the corresponding limits are given for the reagents o-vanillin oxime[6], quercetin[7], 8-hydroxyquinoline[7], rubeanic acid[7], morin[7] and chromatropic acid[7].

The compound can, therefore, be used as a useful spray reagent for the identification and distinction of metal ions by paper chromatography using proper solvent systems. The possible use of the reagent as a spectrophotometric and gravimetric reagent for the determination of metal ions and the nature of the coloured complexes formed by the reagent is under investigation.

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THE OCCURRENCE OF FIBROUS ATRETIC FOLLICLES IN THE OVARIES OF *RANA CYANOPHLYCTIS* AND *RANA TIGERINA*

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FOLLICULAR atresia in the vertebrate ovary has recently been reviewed[1,2]. While it is a common feature of vertebrate ovaries the mode of follicular atresia differs[2]. The common type of atresia involves the hypertrophy and hyperplasia of granulosa cells; they become phagocytic, invade the egg/oocyte and digest its contents leaving behind pigment matter and thecal elements[2]. Occasionally, 'bursting atresia' has been observed in some non-mammalian vertebrates other

than in amphibians[2]. The bursting takes place before the yolk has been completely resorbed resulting in ectopic yolk masses which are later resorbed by the phagocytic cells. The present paper describes a new type of atresia *i.e.* fibrous atresia not reported hitherto for any non-mammalian vertebrate. Several such atretic follicles were observed during the course of our study of histology of the ovaries of two species of *Rana*, *R. cyanophlyctis* and *R. tigerina*. In such atretic follicles, the theca hypertrophies and several fibrous layers are seen around the oocytes (figure 1). It is interesting to note that fibrous atresia is seen only in vitellogenic follicles. It is not known whether previtellogenic follicles also undergo fibrous atresia. The cause and consequences of fibrous atresia are not known. Recently, several other types of atretic follicles have been described in the ovary of the toad, *Bufo melanostictus*[3].

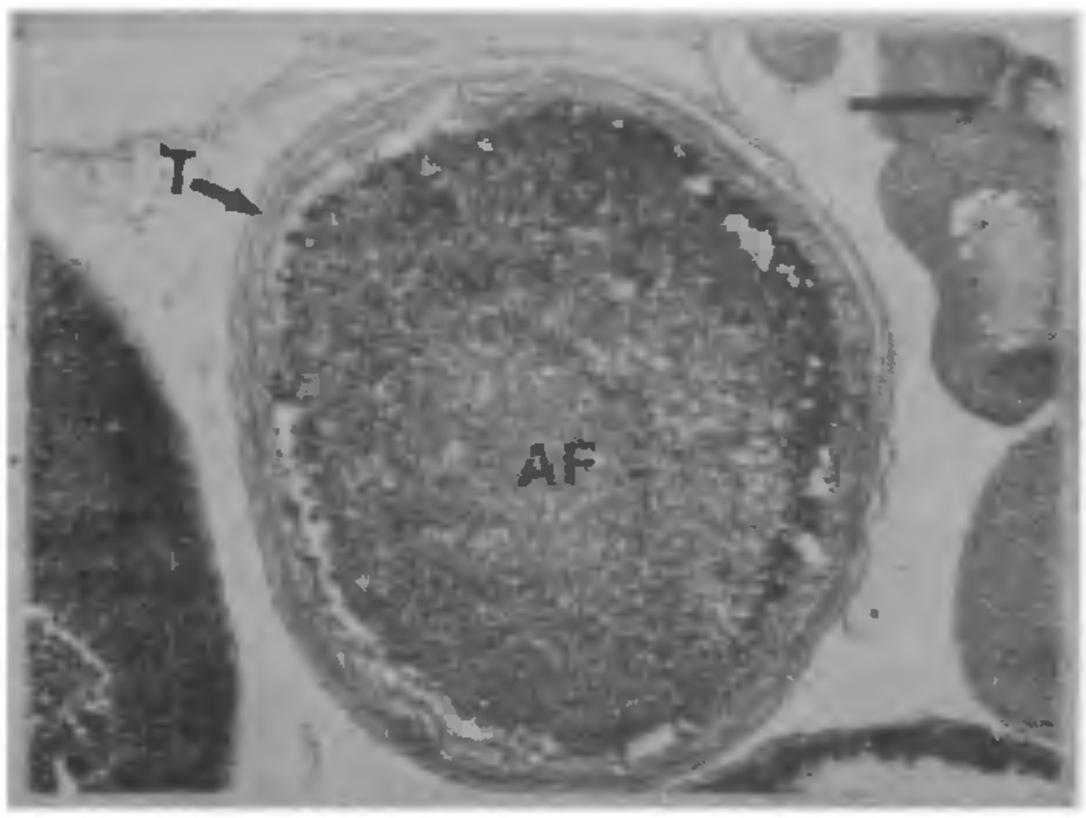

Figure 1. Cross section of *R. cyanophlyctis* ovary showing a fibrous type of atretic follicle (AF). Note the abnormal hypertrophy of theca (T). Scale line = 100 μm.

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OVULATION INDUCTION IN THE MUSK SHREW BY CLOMIPHENE CITRATE ADMINISTRATION

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RECENT studies[1] indicate that ovulation can be readily and dependably induced in several members of the Order Insectivora by administration of exogenous gonadotrophins. Normal ovulation results in the musk shrew (*Suncus murinus*) following injections of PMSG, HCG, LH or FSH[1,2]. Clomiphene citrate is known to induce ovulation in anovulatory women[3-5] and rats[3]. In the present study, the ability of this drug to induce ovulation in the musk shrew was evaluated.

Twenty one adult female shrews were divided into seven groups of three individuals each. Shrews in Group I, II, III, IV, V and VI respectively received subcutaneous injections of clomiphene citrate, 100 μg, 200 μg, 300 μg, 500 μg, 1000 μg and 1500 μg/female, for 3 days. Shrews in Group VII were given injections of 0.9% NaCl, 0.05 ml/female/day, for 3 days (see table 1). All females were sacrificed 24 hr after the last injection and their reproductive tracts were flushed with 0.9% NaCl for recovery of ova. The numbers of corpora lutea in the right and left ovaries were also recorded.

The results (table 1) indicate that clomiphene citrate induces dose-related ovulatory response in the musk shrew which normally ovulates only after coitus. An absolute ovulatory response was obtained only when the drug was given at a dose of 1000 μg or above, for 3 days. Eggs were found in the genital tracts and newly formed everted corpora lutea in the ovaries of ovulated females. A dose of 500 μg induced ovulation in two females. Lower doses (300 μg or less) were ineffective in inducing ovulations; however, the presence of well developed haemorrhagic follicles[1] in the ovaries of nonovulated females indicated that either the dose given or the duration of treatment or both were not sufficient for induction of ovulation. Exploratory studies revealed that clomiphene citrate therapy for less than 3 days is ineffective in inducing ovulation in the musk shrew. This delayed ovulatory response to clomiphene citrate administration in this species contrasts with the ovulatory response within 24 hr of single injections of FSH[2], HCG[6] and prostaglandin F^{2} [7].

It is generally believed that clomiphene citrate induces ovulation by causing the release of hypophysial gonadotrophins[8-11]. Investigations on hypophysectomized women and those with hypopituitarism indicate that clomiphene citrate acts through the hypothalamo-hypophysial axis[12,13]. It is suggested