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TEMPORAL EFFECTS OF BARBITUARATES ON PREGNANCY IN ALBINO RATS

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EXTENSIVE investigation on the effects of barbiturates in the field of female reproduction has been carried out. Barbiturates are known to block ovulation by inhibiting the pituitary gonadotrophins and prolactin release in rats[1-2]. Administration of phenobarbital or barbital sodium inhibits the ovarian compensatory hypertrophy and also interrupts pregnancy[3-4]. As pregnancy is maintained by pituitary gonadotrophins during early part and by placental luteotrophins in the later part[5,6], it is of interest to study the temporal effects of these drugs on pregnancy in albino rats.

Nulliparous, female albino rats of Hotzman strain, with regular established estrous cycle, weighing 140–150 g, 80–90 days old, were mated with fertile males at proestrus. The rats showing spermatozoa in the vaginal smears on the subsequent day were selected for experimentation and that day was designated as day 1 of pregnancy. The selected rats were laparotomized under ether anaesthesia on day 8 of pregnancy to note the number of implantations and those having normal number of implantations were selected for further treatment. Earlier tested doses of phenobarbital (7.5 mg/100 g body weight, twice a day) or barbital sodium (20 mg/100 g body weight, twice a day)[4] was injected subcutaneously in 0.5 ml saline from day 8, 10, 13 or 15 through 19 of pregnancy. Saline-treated controls were maintained for these groups. Barbiturate treatment was stopped if the profuse vaginal bleeding was observed. All rats were autopsied on day 20, the number of live foetuses was recorded and the percent foetal survival was calculated. The ovaries were weighed to the nearest mg.

The results suggest that (table 1) the effective period for the action of barbiturates ranges from day 8 to 12 of pregnancy as there is either nil or 11.1% foetal survival with respective phenobarbital or barbital sodium treatment. Administration of phenobarbital from day 10 causes complete foetal resorption, but barbital sodium treatment from day 10 is less effective as 34.5% foetal survival is seen. The drug treatment from day 13 through 19 seems to be less toxic, as the gestation is maintained with 73.8 or 92.7% foetal survival by the administration of phenobarbital or barbital sodium respectively. It is more ineffective when the barbituarates are administered from day 15 onwards, as more than 90.5% foetal survival is seen in both cases. The ovaries of the rats wherein the pregnancy is interrupted are small with many moderate-sized corpora lutea and developing follicles resembling those of nonpregnant rats, as these rats returned to estrus soon after the cessation of vaginal bleeding. But the ovaries of rats where the pregnancy is not interrupted are large, and resemble those of pregnant rats, probably due to the maintenance of large corpora lutea.

It has been demonstrated that hypophysectomy performed prior to day 11 of pregnancy promptly terminates gestation, but pituitary ablation during later half does not disturb the pregnancy in rats[5-6]. Moudgal and co-workers showed the importance of LH in maintaining the pregnancy in hypophysectomized or LH-antiserum treated rats by the administration of proper doses of LH. Studies on pheno- and pentobarbital indicate that these drugs inhibit pituitary surge of LH and also tonic release of LH, FSH and prolactin[1-2]. Therefore, in the present investigation the Interruption of pregnancy by barbiturate administration from day 8 to 10 of pregnancy is probably due to the continued inhibition of pituitary gonadotrophins and prolactin release. As these luteotrophins are essential during early part of pregnancy in rats[7-8] and subnormal production of these hormones after barbiturate treatment may cause low progesterone production resulting in the interruption of pregnancy[9]. These drugs are less effective after day 12, as the placental gonadotrophins take over the function of pituitary gonadotrophins during later half of gestation.

We are thankful to the Department of Zoology, Karnatak University, Dharwar, for providing necessary facilities and one of us (SBP) is grateful to UGC for the award of a fellowship.

24 January 1983

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TABLE 1

Temporal effects of barbiturates on pregnancy in rats

Phenobarbital: 7.5 mg/100 gm B.W. 2 doses/day

Barbital Sodium: 20 mg/100 gm B.W. 2 doses/day

Treatment	PHENOBARBITAL				BARBITAL SODIUM			
	No. of implantations at laparotomy M ± S.E.	No. of foetuses at autopsy M ± S.E.	foetal survival (%)	Ovarian wt. mg/100 g B.W. M ± S.E.	No. of implantations at laparotomy M ± S.E.	No. of foetuses at autopsy M ± S.E.	foetal survival (%)	Ovarian Wt. mg/100 g B.W. M ± S.E.
Controls	7.60 ± 1.51	7.40 ± 1.51	97.4	39.89 ± 4.19	7.60 ± 1.51	7.40 ± 1.51	97.4	39.84 ± 4.19
Day 8–19	7.50 ± 0.59	-	0.0	31.07* ± 1.68	7.00 ± 0.41	0.78 ± 0.78	11.1	25.59** ± 1.46
Day 10–19	8.00 ± 0.50	-	0.0	31.17 ± 2.53	8.29 ± 0.57	2.86 ± 0.02	34.5	39.57 ± 8.32
Day 13–19	8.40 ± 0.25	6.20 ± 1.35	37.8	44.87 ± 3.49	8.20 ± 0.97	7.66 ± 1.06	92.7	37.45 ± 4.62
Day 15–19	8.40 ± 0.50	7.60 ± 0.81	90.5	40.63 ± 1.84	9.14 ± 0.33	8.43 ± 0.51	92.2	42.44 ± 2.06

Laparotomy is done on day 8 and autopsy on day 20 of pregnancy. M ± S.E. = Mean ± Standard Error. *$P = < 0.05$ **$P = < 0.01$
B.W. = body weight.

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FUNCTIONAL ANALOGY OF PLANT VACUOLES WITH ANIMAL LYSOSOMES

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AFTER the discovery of lysosomes in animal cells by de Duve in early fifties[1], there was a controversy about the existence of lysosomes in plant cells. While Berjak[2] observed lysosome-like organelle in the root cap of *Zea mays* employing an electron microscope, Matile suggeted that the vacuoles represent lysosomes in plant cells[3]. This was later confirmed by the presence of characteristic hydrolases and latency of these enzymes in the vacuoles[4,5]. Also, these criteria are used to detect lysosomes in animal cells[2] which establish the structural analogy of plant vacuoles with animal lysosomes. The present investigation was undertaken to verify the functional analogy of plant vacuoles with animal lysosomes by feeding acridine orange (AO), a foreign substance, to the plant cells and observing the effects on vacuoles.

The secondary roots of *Vicia faba*, while attached to seedlings, were treated with 20 and 50 ppm aqueous AO solution for periods ranging from 6 hr to 7 days. The root tips were then cut and processed for electron microscopy[6].

Ultrathin sections of the control root tip cells reveal typical ultrastructure (figure 1) with nucleolus, nucleus, nuclear membrane, profiles of endoplasmic reticulum, mitochondria, Golgi bodies and amyloplast with electron-transparent starch grains. The vacuoles are generally empty. Presence of prometaphase chromosomes shows that the cells actively divide. In the electron micrograph of cells treated with 20 ppm AO for 6 hr (figure 2) all the cell organelles appear to be normal except the prominant invaginations of the tonoplast (single arrow). In addition, vacuoles contain intravacuolar bodies ranging from 0.2 to 2 μ in diameter. These bodies appear to be of protoplasmic origin owing to their similarity in structure and density with that of protoplasm. As treatment prolonged to 12 hr the amount of intravacuolar bodies increased (figure 3). Some of these bodies contain cytoplasmic organelles like mitochondria (single arrow) and presumably partially digested thin protoplasmic strands (double arrow). The cells, however, appear to be healthly as evident from the presence of metaphase chromosomes which reflects the continuity of cell division. When the cells were treated for longer periods (7 days) the intravacuolar bodies were more complicated and varied (figure 4). The vacuoles displayed numerous intravacuolar bodies ranging from 0.3 to 3 μ in diameter; short protoplasmic strands were also distinct. The cells were, however, still dividing. With the higher concentration (50 ppm) of AO when the cells were treated for 48 hr (figure 5) two types of intravacuolar bodies were discernible (i) similar in structure and density to protoplasm (single arrow) (ii) denser than the protoplasm and appear to be granular abiological mass (double arrow).

AO is a cationic substance and is known to interact with many anionic substances like nucleic acids, proteins, lipoproteins and phospholipids. Some of these substrates are available in the membranes, and as a result AO preferentially binds to membranous structures in the cell. Such attachment of AO leads to general toxicity which is evident from the report that AO inhibits RNA synthesis *in vivo*[7] and is also known to cause structural abnormality in mitochondria and plastids of wheat root meristem[8]. It is possible that in the present system mitochondria and plastids become defective due to excessive binding of the dye and as soon as these protoplasmic regions come in contact with tonoplast, they are recognized and an invagination appears in tonoplast which ultimately engulfs the defective organelles along with surrounding protoplasm. This process is known as autophagy. Numerous cases of autophagy have been reported in the literature[9,10]. The literature on animal cells is rich with reports of autophagy induced by a variety of chemicals like glucagon, actinomycin-D, puromycin and cycloheximide[6].

It is generally agreed that cytoplasmic organelles, once enclosed inside vacuoles, are attacked by vacuolar hydrolytic enzymes and subsequently degraded[3-6]. Likewise in the present study mitochondria can be