

Effects of Carbon Tetrachloride on Mitosis

WE have observed that carbon tetrachloride produces striking effects on *Allium cepa*, *Allium sativum* and *Zaphranthus* sp. root tips. Root tips were treated for five minutes to a half-hour in carbon tetrachloride and later transferred to normal nutrient medium and fixed at various intervals from half an hour to six hours. Among the prominent changes taking place in the resting nucleus is a dissolution of the nuclear membrane and an outflow of its contents. Similar observations have been made in root tips of *Allium cepa* treated with chloroform¹ and potassium cyanide². It would appear that the lipid layer of the nuclear membrane is either dissolved or rendered weak by the chemicals, in consequence of which the nuclear contents flow out. After treatment for 15 min. with carbon tetrachloride, resting and premitotic nuclei break up. A number of chemicals are known to produce this effect, for example, urethane³ and nitrogen mustard⁴. Perhaps the most characteristic changes brought about by carbon tetrachloride are dissolution of chromatin in the resting nucleus and disorganization of the chromosome arms in the premitotic stages, resulting in their progressively negative reaction to the Feulgen reagent.

Mitotic stages show even more striking effects. One of the commonest is the occurrence of sticky bridges in anaphase where, due to decrease in viscosity of the matrix, the distal ends of chromosomes fail to separate⁵. Bridge formation has been reported as a result of the action of chloroform¹, ethylene glycol⁶ and nitrogen mustard⁷, among others. Metaphase chromosomes tend to become more or less despiralized, acquire side loops and resemble the 'lampbrush' chromosomes. Levan and Wangenheim² have observed a similar phenomenon in onion root tips treated with potassium cyanide. Evidently the chemical affects spiralization and induces the material of the chromosome to flow out and form loops. Delayed separation of chromatids as well as chromosome fragmentation are also common in material treated with carbon tetrachloride. The spindle, too, is affected, and results in irregular and unequal segregation of chromosomes and in polyploid nuclei.

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Urinary Oestrogens in the Buffalo as measured Chemically

A KNOWLEDGE of the level and the rate at which hormones are excreted is of considerable academic value for diagnostic and therapeutic purposes. The determination of oestrogen activity in the excreta is limited by the fact that these hormones are eliminated from the body in biologically active and inactive forms, thus necessitating chemical methods of

measurement. The present work, consisting of two experiments, was carried out to ascertain the level and the rate at which urinary oestrogens are excreted in the buffalo and their chemical form.

Clean twenty-four hour specimens of urine were collected from four buffaloes during the fourth month of pregnancy, acidified with concentrated hydrochloric acid and boiled under reflux for 15 min., then extracted four times with one-fourth volume peroxide-free ethyl ether. The extract was evaporated and the residue taken up in a minimal amount of ethanol. Each alcoholic extract was added to 300 ml. of toluene and then fractionated into neutral and phenolic fractions. The phenolic fraction was chromatographed and further fractionated by the use of Girard's reagent T and phthalic anhydride^{1,2}.

To determine the rate at which administered oestrogens are excreted in the urine, an ovariectomized buffalo was injected intramuscularly with 10 mgm. of oestradiol monobenzoate in microcrystalline suspension, and 10 mgm. and 60 mgm. of oestradiol dipropionate dissolved in oil. Periods of seven days elapsed between the three different treatments, and urine samples were collected every six hours during the following 24 hr. Estimations of oestrogen were based on a physicochemical colorimetry³.

When the urinary oestrogens were fractionated, oestradiol and oestrone were found whereas oestriol was absent. The titres found for urinary oestrogens (Table 1) were too low to have any quantitative significance. The pregnanediol titres, although incomplete, were of a very low order.

Table 1. OESTROGEN AND PREGNANEDIOL TITRES EXCRETED PER LITRE OF URINE BY PREGNANT BUFFALOES

Animal No.	Days pregnant	Oestrone (μ gm.)	Oestradiol (μ gm.)	Oestriol (μ gm.)	Pregnanediol (μ gm.)
1	95	6	12	0	226
2	96	4	10	0	—
3	105	2	0	0	—
4	102	2	0	0	324

In the second experiment, only 0.5 per cent of the oestrogen injected in the third treatment was recovered in the urine specimen collected during the first six hours; while the first and second injections were not followed by any excretion of urinary oestrogens. Oestrous manifestations appeared after the dipropionate injections only.

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