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Effect of Reserpine on Adrenalectomized Rats.* (25774)

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Gaunt *et al.*(1) noted that doses of reserpine ranging upwards from 50 μg/kg/day reduced survival time of adrenalectomized rats. This might be due to an increased requirement for cortical hormones or to release of serotonin from tissues(2). Jaques, Mogenson and Fisher(3) showed that hemorrhage occurred readily when adrenalectomized rats were given dicumarol, all dying of hemorrhage while controls all survived. This was explained by the observation(4,5) that interference with any 2 of the 3 major hemostatic lines of defense (blood coagulation, platelet plugging, vascular integrity and response) present an overwhelming hemorrhagic defect. The work of Correll *et al.*(6) and Zucker and Borrelli(7) indicated that platelet serotonin is involved in normal hemostatic mechanisms, and Jaques and Fisher(4) showed that reserpine produced hemorrhage in rabbits subjected to stress. The experiments reported were undertaken to compare hemorrhagic death in adrenalectomized rats treated with reserpine, with that already studied in other experiments using these treatments.

Methods. Adrenalectomized rats were maintained on 0.9% saline and received hydrocortisone (1 mg/100 g/day) for 3 days post-operatively. Treatments were started one week after operation, as follows—reserpine 2 mg/kg intraperitoneally, desoxycorticosterone acetate (DOCA) 2.5 mg/kg daily subcutaneously. The animals were examined daily. Mortality was recorded as number of deaths to the tenth day. A careful autopsy was made.

Results. Groups of approximately 25 rats (adrenalectomized and sham-operated) were divided into subgroups receiving reserpine and no reserpine. The experiment was then repeated with DOCA given at various time intervals. Number of animals, respective treatments, and mortality are shown in Table I.

Adrenalectomy + Reserpine. There was a profound diarrhea and intense gastrointestinal discomfort in animals of Group 1. Half of them died within 6 hours, others died within 24 hours to give a mortality of 80% (16 rats). In every case diarrhea was present. Feces were eliminated and thin mucus kept issuing from the anus. The presence of gross blood in feces and/or intestinal lumen was observed in 25% of the post mortem examinations.

TABLE I. Mortality of Adrenalectomized Rats Treated with Reserpine.

	No.	Died	% mortality
Adrenalectomized + reserpine	20	16	80
Adrenalectomized only	18	3	17
Sham operated + reserpine	25	1	4
Sham operated only	21	1	5
Adrenalectomized DOCA for 8 days, reserpine on 6th day	10	1	10
Adrenalectomized DOCA for 4 days, reserpine on 6th day	10	4	40
Adrenalectomized DOCA for 4 days, control	10	1	10
Adrenalectomized DOCA for 8 days, control	9	2	22
Sham operated DOCA for 8 days, control	7	0	0
Sham operated DOCA for 8 days, reserpine on 6th day	10	1	10

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The lungs were clear indicating that death following reserpine was not due to pulmonary complications. Heart and liver appeared normal but with some engorgement. In almost every animal, the testes were found withdrawn into the abdominal cavity, giving a picture of distress. All animals receiving this dose of reserpine were tranquilized but adrenalectomized animals alone showed the extreme intestinal symptoms. The sham-operated animals receiving reserpine (Group 3) showed far less severe diarrhea than Group 1 and there was no obvious blood in the stools. Only one animal died. It showed a badly hemorrhaged intestine with melena. The lungs, heart, and liver were normal. In control animals (Groups 2 and 4), there was no diarrhea. Two adrenalectomized animals died between 24-48 hours. One sham-operated animal died on the ninth day of apparently self inflicted wounds and/or bleeding from the tail and forefoot. The post mortem appearance was quite different from the reserpine-treated animals. Nasal bleeding and lung hemorrhage were observed suggesting respiratory involvement. The third adrenalectomized animal which died showed nothing unusual.

Adrenalectomy + DOCA + Reserpine. When adrenalectomized rats were maintained on DOCA throughout, (Group 5) there was only one death, indicating that DOCA afforded considerable protection to such animals receiving reserpine. Withdrawal of DOCA 2 days before reserpine (Group 6) resulted in greater mortality. Gross pathology was similar to that (Group 1) of adrenalectomized animals dying after reserpine. Two deaths occurred within 6 hours and 2 occurred at 16 and 40 hours. Diarrhea was evident and on testing the feces or intestinal contents for occult fecal blood three strong positives were obtained. None showed grossly hemorrhagic lungs although one or 2 petechiae were noticed, which could not have been the cause of death. A few deaths were observed in the control groups (Groups 7, 8, 9). Gastrointestinal symptoms and stools positive for blood were not observed in these animals. In those that died there was pronounced lung congestion and hemorrhage so that pulmonary complications were evidently the cause of death. Sham-operated rats (Group 10) receiving reserpine and DOCA showed nothing unusual. One animal died 16 hours after reserpine and showed an intense loss of blood via intestine, lung hemorrhages and withdrawn testes. The other 9 animals exhibited no signs of gastrointestinal distress.

Discussion. Diarrhea and intestinal hemorrhage appear responsible for death following this dose of reserpine in adrenalectomized rats. Plummer *et al.*(8) describes activity of reserpine on the gastrointestinal tract as augmenting both secretory and motor activity, presumably due to release of serotonin from gastrointestinal mucosa. Van Cauwenberge and Jaques(5) showed that when dicumarol was given to adrenalectomized rats there was considerable mortality (40%). Hemorrhage was not prominent but adrenalectomized rats maintained on DOCA, when given dicumarol, all died of spontaneous hemorrhage; maintained on cortisone all survived anticoagulant treatment. Normal rats receiving both reserpine and dicumarol showed a high mortality (50%) from hemorrhage. In all these experiments, spontaneous hemorrhage was quite widespread through the body and was produced by a double defect in hemostasis—coagulation affected by dicumarol, capillary resistance by adrenalectomy(9), platelets by reserpine. Cortisone and DOCA respectively corrected and increased the vascular defect in adrenalectomized animals.

Adrenalectomy and reserpine caused 80% mortality. The post mortem revealed much loss of fluid and blood *via* intestine. Hemorrhage was always in close association with intestinal tract, and was not found elsewhere probably due to the short survival time. Fluid loss was reduced following administration of DOCA. The difference in observations between reserpine-dicumarol-normal rat and reserpine-adrenalectomized rat suggests that the intestine is more sensitive to reserpine (serotonin) in the adrenalectomized rat. Selye and Heuser(10) mention that an inadequate adrenal defense mechanism appears to predispose the tissues of the gastrointestinal tract to ulceration. The known defects in water and electrolyte balance in the adrenalectomized rat and correction of these by DOCA

could also explain the present results with sudden loss of mucosal serotonin causing intraintestinal loss of water. Cause of death in adrenalectomized animals receiving reserpine therefore is not as simple as that of normal rats receiving dicumarol and reserpine, or adrenalectomized rats receiving dicumarol, where there is a clear interference with 2 mechanisms of hemostasis simultaneously. However, it seems reasonable to accept that part of the situations which are common—increased capillary fragility and serotonin displacement, contribute to the hemorrhage. The marked gastrointestinal activity of reserpine in the adrenalectomized animal will mean this must be main site of hemorrhage, and the fact that platelet physiology has been disturbed may possibly mean that when intraintestinal hemorrhage occurs, the platelet plugging required for hemostasis cannot follow.

Summary. Reserpine given to adrenalectomized rats produced diarrhea and gastrointestinal hemorrhage with high mortality. This was not observed in sham-operated animals. Maintenance on desoxycorticosterone protected the animals from this action of reserpine.

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Cytostatic Activities of Steroidal Estrogens against Zebra-Fish Embryos. (25775)

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To find a superior estrogen for chemotherapy, we have studied a large list of steroidal estrogens for their cytostatic effects on the egg of the zebra-fish (*Brachydanio rerio, Hamilton*). Of the 14 steroidal estrogens studied, we have also tested the 3-methyl and 3-ethyl ethers of each. Some of these ethers are reported here for the first time.

Materials and methods. The method was a modification of one previously described(1). Our present practice is to use 10 embryos (8-64 cell stages) in 50 ml of culture solution exposed at 26°C for 24 hrs. Each test culture is replicated and at least 3 replicated tests are used to determine minimum concentration (in parts per million) of the steroid which affects 50% of the embryos (ED/50/24). Thus, a minimum of 50-60 embryos was used in testing each concentration.

Results. The results are summarized in Table I.

Discussion. As measured in the restraint of cell division in the freshly-fertilized zebra-fish egg, 17-ketosteroids are in general less cytostatic than 17-hydroxysteroids. The one exception is equilenin, which produced a curious type of cytostasis. The equine estrogens

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