period. 12 rats fed during the 2nd period gave birth to hydrocephalic rats. The average litter numbered 9, 5 of which were hydrocephalic. None of the animals in the 1st and 3rd periods gave birth to hydrocephalic animals.

The 2nd experiment was named the 'single insult' experiment because it consisted of giving 20 g (50 mg tellurium) of the tellurium diet on only 1 day of the experiment to each animal so that 21 such groups of 5 animals represented the 21 days of gestation. Normal food was given on all the other gestative days. 3 animals died during the experiment and 72 animals gave birth to an average of 8 offsprings. There were no hydrocephalic animals.

*Discussion.* It is concluded that 2,500 ppm of metallic tellurium added to a normal diet of a gestating rat every day during the period extending from the 10th to the 15th days of gestation in the rats will result in hydrocephalic offsprings in the majority of the litters. This period of gestation is the target period of most drug-induced congenital malformations in the rat[1,6]. The tellurium absorbed by the mother reaches the fetal brain within minutes, and presumably causes an arrest of maturation of the telencephalic vesicles, which consequently present as hydrocephalus at birth.

*Résumé.* Le terme de «target period», qui peut se traduire par période-cible, signifie la période de grossesse la plus sensible aux effets nocifs d'un agent tératogène. Le présent travail situe chez le rat la période-cible du tellure entre les 10e et 15e jours de la grossesse.

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6 S. Duckett and K. A. O. Ellem, Expl. Neurol., in press (1971).
7 This work is supported by grant No. NS 8891-01 from the U.S. Public Health Service.

## Catecholamine-Containing Nerves in the Submucosa of the Ureter

During an investigation into the arrangement and innervation of smooth muscle in the rabbit renal calix and pelvis[1], nerves containing catecholamine were observed in the submucosa. Histochemical demonstration of monoamines in nerves is regarded as indicative of their effector role; these substances have not been reported as being present in afferent nerves. That monoamine-containing nerves existed in the submucosa of the upper urinary tract suggested the need to investigate their distribution in more detail in the hope that it might prove possible to demonstrate the presence or absence of probable effector target sites in this region. This report presents the results of preliminary studies on catecholamine-containing submucosal nerves in a variety of species.

Adult rabbits, rats and guinea-pigs were killed by a blow to the head; the kidney, together with the proximal half of the ureter were then removed. Each kidney and the attached ureter was placed on a cryostat chuck and plunged into either isopentane or propane previously cooled in liquid nitrogen. Cryostat sections were prepared and processed for tissue catecholamines according to the method of Spriggs et al.[2] using paraformaldehyde at 70.4% relative humidity. Adjacent sections were fixed in formalin and stained using Massons trichrome technique for routine histology. Paraformaldehyde-treated sections were examined using a Zeiss photomicroscope fitted with a Wotan HBO 200W mercury vapour lamp in combination with excitor filter BG12/4 mm and barrier filter GG9/1 mm.

Fluorescent nerves were observed in the submucosa of the renal pelvis and ureter in all the specimens examined. Other than in the submucosa of the calix (where very few nerves were detected) regional differences in submucosal innervation in the pelvis or ureter were not apparent. Relatively large fluorescing nerves were observed adjacent to the muscle coat extending into the submucosa. Finer branches continued towards the epithelium, some of which were closely related to the basal layer (Figure 1). The relative thickness of the tissue sections prevented positive identification of nerves penetrating the epithelial basement membrane. However, catecholamine-containing nerves were not observed in the deeper layers of the epithelium proper. In addition to the nerves described above, others were identified in the submucosa lying adjacent to small arteries (Figure 2). These nerves accompanied the vessels and were arranged in a plexiform fashion on the external aspect of the muscular media. Fluorescent nerve cells or chromaffin cell bodies have not been seen in any of the preparations.

The relationship between catecholamine-containing nerves and small arteries has been well documented in a variety of tissues, including the ureter[3]; the present results endorse this association in the submucosa of the

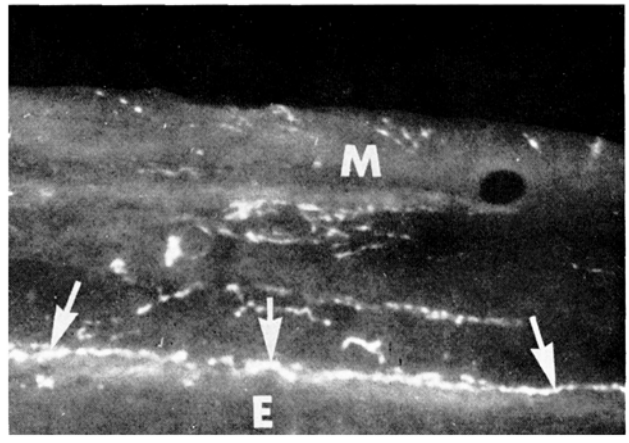


Fig. 1. Longitudinal section through the guinea-pig ureter showing fluorescing nerves (arrows) adjacent to the epithelium (E). Other nerves are observed in the muscle coat (M).

1 J. A. Gosling and J. S. Dixon, Am. J. Anat. *130*, 393 (1971).
2 T. L. B. Spriggs, J. D. Lever, P. M. Rees and J. D. P. Graham, Stain Tech. *41*, 323 (1966).
3 A. Elbadawi and E. A. Schenk, Am. J. Anat. *126*, 103 (1969).

upper urinary tract. The presence of other fluorescent nerves seemingly unrelated to submucosal vessels requires further consideration. Some nerves have been observed to leave vessels and run for part of their course unassociated with the vascular supply of the region. However, a number of others have been followed in semi-serial sections and these remained unrelated to vessels throughout their submucosal course. Both ELBADAWI and SCHENK[3] and DUARTE-ESCALANTE et al.[4] described adrenergic nerves related to fluorescent ganglion cells and the latter workers noted others ending near chromaffin cells in the epithelium. Based on the results of the present investigation and on those of an earlier study[5], neither ganglion cells nor chromaffin cells can be regarded as possible effector sites since these have not been identified in any of the preparations. As an alternative explanation, some of the nerves presently demonstrated could influence others which were undetected by the present method. In this context it is noteworthy that cholinesterase-containing nerves have been described in the ureteric submucosa[3-5]. Finally, in the absence of any obvious effector site the possibility exists that some catecholamine-containing nerves in the submucosa may perform a sensory function. A detailed light and electron microscopic investigation has been undertaken on the region in the hope of further resolving some of these possibilities.

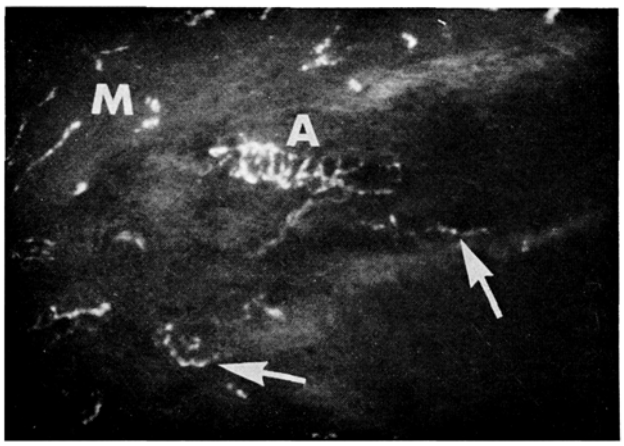


Fig. 2. Oblique section through the rabbit ureter showing nerves related to a submucosal artery (A). Catecholamine-containing nerves are also seen in the ureteric muscle (M) and in the submucosa (arrows) unrelated to vessels.

*Résumé.* Les nerfs de la submuqueuse de l'urêtre des Rongeurs examinés contiennent de la monoamine. Les uns accompagnent les vaisseaux sanguins, d'autres en sont indépendants. Ils ne s'étendent pas au delà de la couche inférieure de l'épithélium. Les cellules ganglionnaires et chromaffines semblent manquer. Le rôle possible des nerfs contenant de la catécholamine sans être reliés à un élément effecteur est discuté.

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[6] Acknowledgments. The authors thank Prof. G. A. G. MITCHELL for his constant interest and Mr. A. N. WAAS for skilful technical assistance.

## Amniotic Fluid Volume in Experimentally Induced Renal Agenesis and Anencephaly

The problem of the source of amniotic fluid has never been adequately settled. Various suggestions as to the origin of this fluid have included: direct secretion from the amniotic epithelium[1], transudation from the fetal pharynx[2], lung[3], umbilical cord[4], or maternal and fetal blood, as well as direct excretion of urine into the amniotic cavity[1]. It is of interest to note that certain abnormalities of pregnancy, as well as fetal congenital malformations, have been associated with changes in amniotic fluid volume and have been used as the basis of hypotheses supporting certain theories of amniotic fluid metabolism. Of the congenital malformation group, the polyhydramnios associated with esophageal atresia is accounted for on the basis of fetal inability to swallow amniotic fluid and its subsequent failure to be absorbed from the gastrointestinal tract[5]. Likewise, the polyhydramnios associated with anencephaly has been attributed not only to the decreased deglutition in this malformation[6] but also to the production of cerebrospinal fluid directly into the amnion from the exposed but intact choroid plexus and meninges[1]. On the other hand, bilateral renal agenesis in the human fetus has often been associated with the virtual absence of amniotic fluid[7] although there is some lingering controversy on this point[8]. These last observations have led to the conclusion that the production of the fetal urine may be an important mechanism in maintaining amniotic fluid volume.

Our interest and attention has been drawn to an experimental model as a method of evaluating the role of the fetal kidneys in the production and maintenance of the amniotic fluid volume, as well as to evaluate the relationship of amniotic fluid volume to other congenital malformations. The experimental induction of renal agenesis in hamster embryos following treatment of pregnant mothers with sodium arsenate[9] provided the basis of this study. Because the method of inducing renal malformations by administration of sodium arsenate also induces other malformations, the study included correlations of amniotic fluid volume with these other developmental abnormalities.

*Materials and methods.* Female hamsters, bred to males in a manner previously described[9], were injected i.v. with 20 mg/kg of sodium arsenate on the 8th day of gestation. The animals were sacrificed on the morning of the 15th day of gestation, 20 h prior to term. The uterus was removed and each embryo was removed from the uterus within its surrounding amniotic yolk sac membranes. If any fluid was lost during this dissection, the fluid volume was not measured nor included in the data. The intact unit, including the fetus within these membranes, was then quickly rinsed in normal saline and