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.

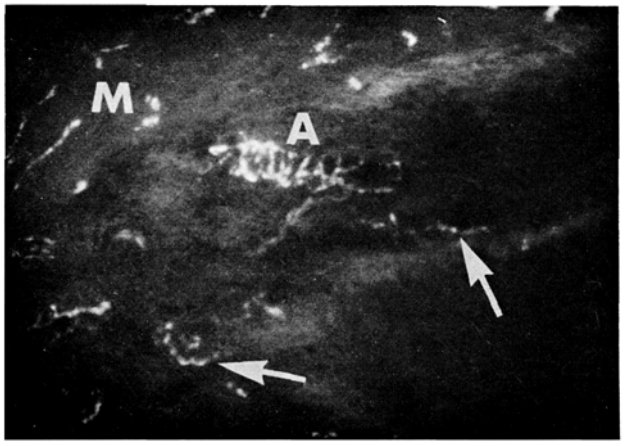

Fig. 2. Oblique section through the rabbit ureter showing nerves related to a submucosal artery (A). Catecholamine-containing nerves are also seen in the ureteric muscle (M) and in the submucosa (arrows) unrelated to vessels.

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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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

dried with gauze. This unit was then suspended over tared beakers and the membranes ruptured, taking special care not to tear blood vessels of the yolk sac membrane. All of the amniotic fluid was thus allowed to drain into the beaker, the embryo being suspended by its umbilical cord. The net weight of the amniotic fluid was then determined and this weight converted to amniotic fluid volume. If any trace of blood in the normally clear fluid was noted, the specimen was considered to be contaminated and was not used. However, in those cases of anencephaly where the amniotic fluid was grossly bloody even when observed in utero the entire sample of amniotic fluid was collected and measured. A few specimens of amniotic fluid were obtained by direct aspiration with a needle and syringe from the amniotic sac. However, it was not possible to aspirate the total volume of amniotic fluid by needle puncture and this method was abandoned. Chemical analyses for total protein, blood urea nitrogen, sodium and potassium in amniotic fluid from 2 normal embryos, 1 exencephalic embryo and 1 embryo with bilateral renal agenesis were done.

All of the fetuses were examined externally for gross malformations and then fixed in Bouin's fluid. Following fixation, the fetuses were dissected for internal malformations.

Results. The data are summarized in the Figure. The embryos were divided into 5 groups: 1. uninjected controls, 2. those injected with sodium arsenate but which did not show any malformations, 3. those injected with sodium arsenate that revealed malformations other than bilateral renal agenesis or anencephaly-exencephaly (rib malformations, other genito-urinary abnormalities, cleft lip/palate, and anophthalmia), 4. those embryos with bilateral renal agenesis with or without other malformations but without anencephaly-exencephaly, and 5. those with anencephaly-exencephaly with or without other malformations but without bilateral renal agenesis. As shown in the Figure, the number (N) of observations for each group as well as the range and mean (M) of the observations is given. Only group 5, those showing anencephaly-exencephaly, revealed a statistically significant increase in amniotic fluid volume over the control groups as well as other groups ($p < 0.00001$). All of the amniotic fluid samples obtained from the ex-anencephalic groups were variously hemorrhagic, ranging from mild to grossly bloody.

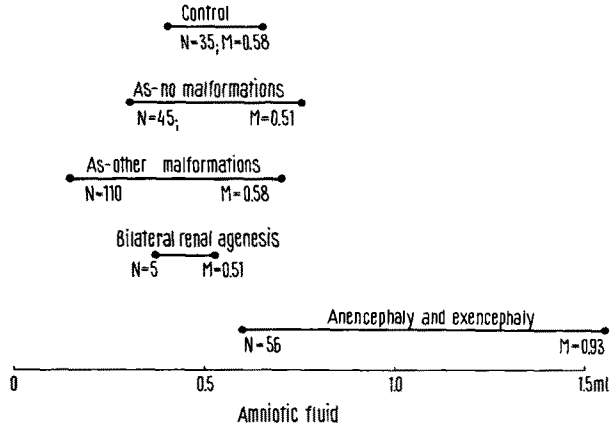
Although chemical determinations were done on insufficient numbers of samples the one amniotic fluid sample from the embryo with bilateral renal agenesis showed a decrease (35 mg/100 ml) in blood urea nitrogen as compared to controls (52 mg/100 ml) and each of the 2 samples from the anencephalic and renal agenesis embryos showed elevated potassium levels (18.4 meq/l) as compared to controls (13.5 meq/l).

Discussion. The technique for collection of amniotic fluid volume in these experiments is the same as that used by PURDY and HILLEMANN[10] in the golden hamster. Data for comparable stages in gestation showed a slightly greater volume of amniotic fluid for our control embryos (0.58 ml: 0.46 ml). Our data are derived from individual measurements of each of 58 separate fetuses, while their data are the average for each litter. The method of averaging may explain the lower values, since any slight error in collection would tend to diminish the average value.

Bilateral renal agenesis is an uncommon event in human development. Although few, if any, quantitative measurements have been made on amniotic fluid volumes in human new-borns affected with this malformation, it is the almost unanimous clinical impression that the amniotic fluid volume is considerably reduced. Oligohydramnios has also been reported in the cases of obstruction of the urinary tract outflow. These observations form the basis of the hypothesis that fetal urine is an important component in making up amniotic fluid volume.

GIROUD and MARTINET[11] have reported an excess of amniotic fluid in rat fetuses with anencephaly induced by hypervitaminosis A. Also, GULIENETTI et al.[12], utilizing a radio-isotope dilution method for determining amniotic fluid volume, have reported an increase in amniotic fluid volume in rat fetuses with sodium salicylate-induced anencephaly. It is of interest to note that two cases of human new-borns with bilateral renal agenesis and iniencephaly were reported to have normal or increased amounts of amniotic fluid[13]. In addition to the possibility that the increased amniotic fluid found in anencephaly might be due to continuous secretion of cerebrospinal fluid directly into the amniotic sac[1], it might also be considered to be a result of increased oncotic pressure because of the presence of serum proteins.

The mechanism of the formation of the amniotic cavity in those vertebrates possessing such membranes (amniotes) differs among the various species. Amnion formation



A comparison of the volume of amniotic fluid in m. of 15-day-old hamster embryos following i.v. injection of sodium arsenate on the 8th day of pregnancy. For a description of the animals in each group see text. N = Total number of embryos in each group. M = mean volume per group.

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is the result of either a process of sudden cavitation of the inner cell mass or a folding process involving the lateral edges of the embryonic disc[14]. Fluid has been noted in the amniotic sacs of some blighted human ova, and fluid is also present in very early human pregnancies before organ differentiation has progressed very far[15]. It is possible that the actual production and metabolism of amniotic fluid varies from one species to another, and given the variety of differences in mammalian extra-embryonic membranes, it would be difficult to extrapolate data from one species to another. However, the fundamental nature of the evolution of this membrane suggests some common denominator of function and purpose in all of the amniotes. From the data in our experiments, however, it is clear that in the hamster, the fetal urine plays little or no role in the maintenance of amniotic fluid volume[16].

Zusammenfassung. Experimentelle Prüfung beim Goldhamster, *Mesocricetus auratus*, ob Nieren- oder Gehirnmissbildungen einen Einfluss auf die Quantität der Amnionflüssigkeit ausüben, was nur für die Gehirnmissbildungen bejaht werden konnte.

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Ultrastructural Changes in Rat Liver Cells After Intramuscular Implantation of a Walker Tumor

Rats bearing Walker tumors exhibit an increase in liver weight with a decrease in hepatic drug-metabolizing enzyme activity and cytochrome content[1-3]. Experiments in this Institute have shown that, 3–4 weeks after i.m. implantation of this tumor, there is a slight diminution of P-450 content, ethylmorphine demethylation and aniline *p*-hydroxylation as well as an increase of microsomal proteins in the liver cells[4]. Administration of Walker tumor extract produces similar biochemical changes, indicating that a 'toxohormone' is released from the tumor tissue which affects the liver and leads to reduced activity in the hepatic microsomal system[5]. The ultrastructural equivalent of this hepatic microsomal hypofunction was studied in the present work.

Walker tumors were implanted i.m. into the hind limb of 16 female ARS/Sprague-Dawley rats (100 g). The livers of these animals and of 8 controls were examined with an electron microscope 1, 2, 3 or 4 weeks later, using standard techniques (fixation in osmium, dehydration in graded ethanol, embedding in Epon resin).

As compared with the controls (Figure 1), the tumor-bearing rats displayed progressive dilatation and ballooning of the rough-surfaced endoplasmic reticulum (RER) in hepatocytes (Figure 2). The granular membranes were irregularly-shaped and were broken up into smaller units, from the external surfaces of which the ribosomes had gradually detached themselves. These changes were conspicuous although they varied considerably among the animals of the same group and in the different areas of the same liver. The alterations were generally mild 2 weeks after implantation, became pronounced after 3–4 weeks, and were usually observed along with progressive smooth-surfaced endoplasmic reticulum (SER) hypertrophy (Figure 3).

In previous studies[6-8], with other types of tumors, mitochondrial abnormalities and lysosomal accumulation were the principal changes found in the hepatocytes. In the present work, however, conspicuous endoplasmic reticulum alterations were detected.

RER injury might represent the ultrastructural equivalent of hepatic microsomal hypofunction in rats bearing Walker tumors. SER proliferation, which is generally equated with enhanced drug detoxication by liver microsomes[9-11], is reminiscent of the hypoactive, hypertrophic

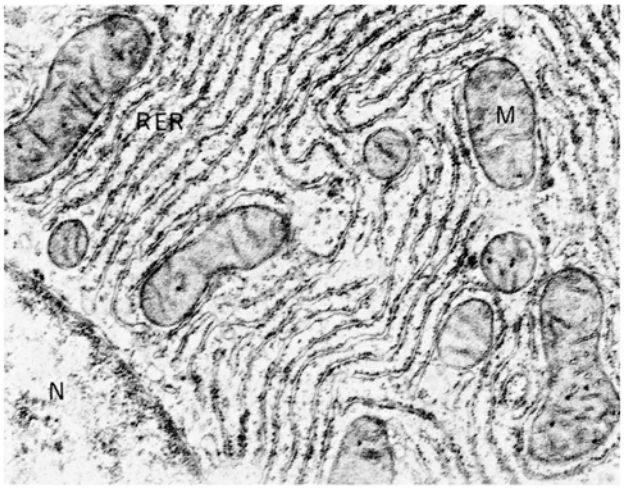

Fig. 1. Portion of a hepatocyte in control rat showing characteristic features. M, mitochondrion; RER, rough-surfaced endoplasmic reticulum; N, nucleus. ×14,133.

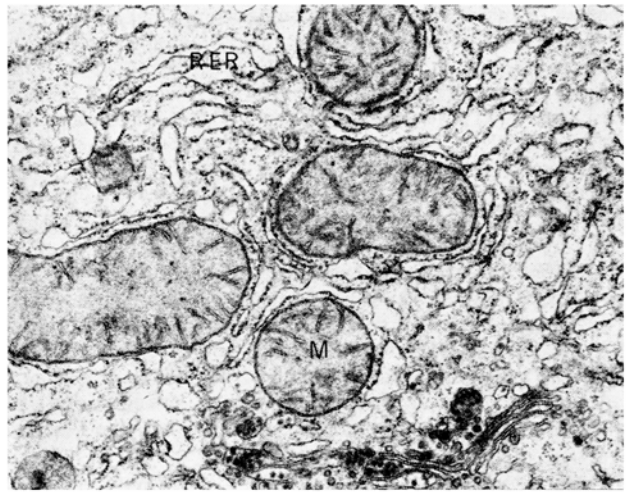

Fig. 2. RER dilatation, disorganization, disruption and degranulation in a rat hepatocyte 3 weeks after Walker tumor implantation. ×13,733.