Frozen pellets were resuspended in 20 ml of deionized water, left at 22 °C for 20 min, and then centrifuged at 50,000 × g for 20 min; this cycle was repeated twice more, and the final pellets were weighed and resuspended in 3.2 ml of Na^+-free, Tris-citrate medium (50 mM; pH 7.1). Aliquots (100 µl) of tissue suspension plus 100 µl of Tris-citrate medium, either free of added substance or containing a final concentration of 10^{-3} M unlabelled GABA (to estimate 'specific' binding), were pipetted into small centrifuge tubes, mixed, and allowed to stand for 10 min. Then 250 µl of Tris-citrate medium containing 5.8–57.7 nM of [methylene-^{3}H(N)]-3-hydroxy-5-aminomethyl-isoxazole ([^{3}H] muscimol; 13.68 Ci/mmole and 62.6 - 626 nM of [^{14}C (U)] sucrose (final concentrations) were added. Samples were mixed, kept on ice for 20 min, and then centrifuged at 79,000 × g for 5 min to obtain final pellet and supernatant fractions. Radioactivity and protein were determined[22,23]. The [^{14}C]sucrose provided estimates of the amounts of supernatant fluid trapped in the pellets[22]. Radioactive products were purchased from New England Nuclear.

The table shows that the body weight of dwarf mice was only about 23% that of the controls and that their brain weight was only about 70% that of controls. Although pellet weights reflected the difference in brain weight, both the pellet protein content and the [^{14}C]sucrose pellet/supernatant distribution ratio did not differ between the two groups of mice (table). A Lineweaver-Burk plot of the 'specific' binding of [^{3}H]muscimol is shown in the figure. No difference existed between dwarf and control mice. Although their body and brain weights were markedly decreased, in comparison to control mice[4], cerebral membrane particles of dwarf mice bound [^{3}H]muscimol to a similar extent to those of controls. Preliminary experiments in our laboratory have revealed that the specific binding of [^{3}H]GABA also does not differ between dwarf and control mice. Hence, the marked decrease in brain weight that occurs under the abnormal hormonal control in dwarf mice does not appear to produce any change in the characteristics of high-affinity [^{3}H]muscimol (or [^{3}H]GABA) binding which appears to be associated with cerebral GABA-receptors.

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Arteriovenous anastomoses in the juxtamedullary cortex of human kidney

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Summary. In the juxtamedullary cortex of normal human kidney the existence of arteriovenous anastomotic channels is demonstrated by means of microscopic observation. A clear arteriovenous anastomosis occurring at the level of an afferent arteriole is also presented.

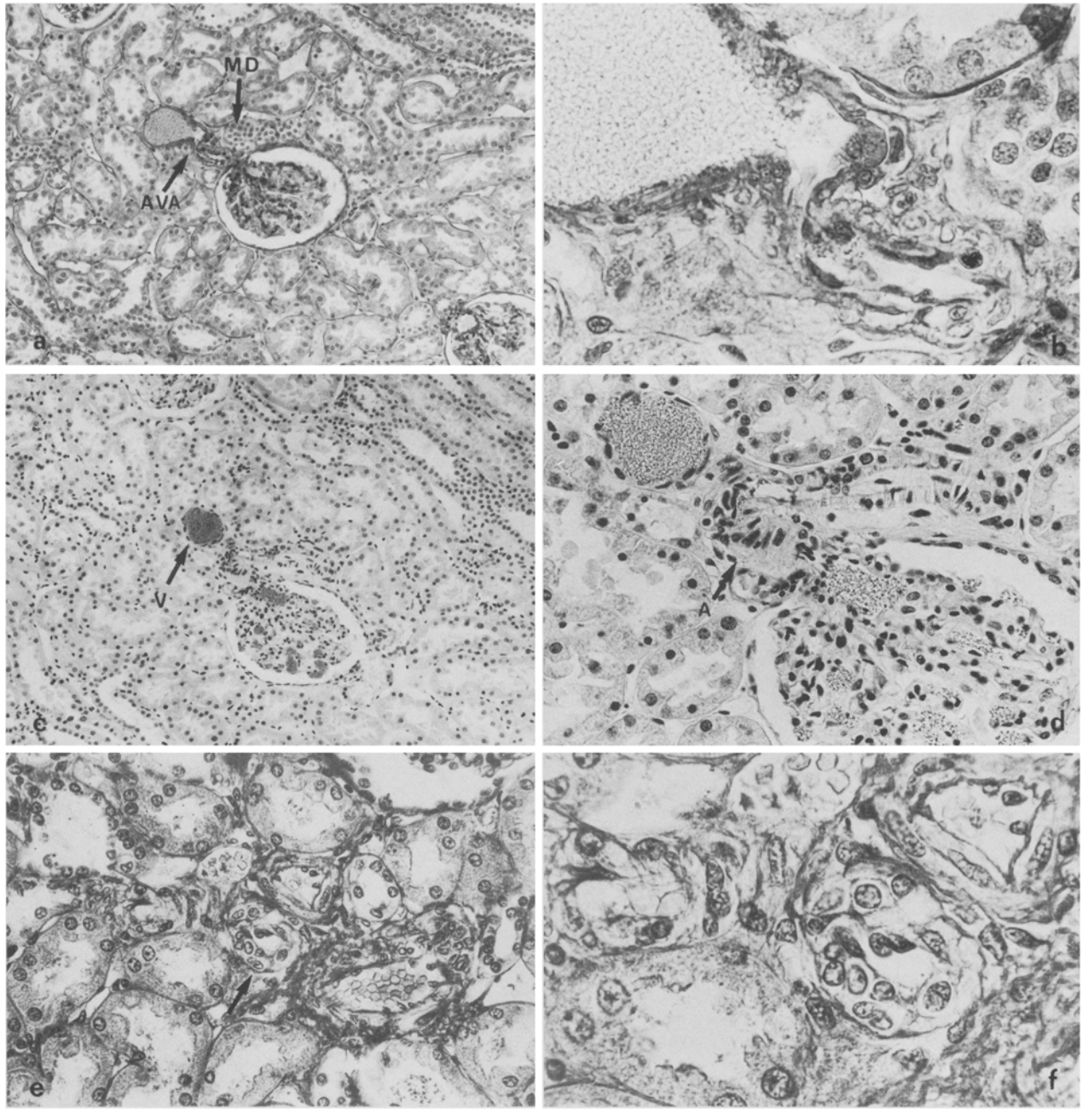
The existence of arteriovenous anastomoses in the normal human kidney is still disputed[1-3]. They have been postulated mostly on the basis of physiological experiments[4,5], but have never been shown by direct histological observation. By using a special technique of injection, Spanner[6] showed that arteriovenous anastomoses are always present in the renal capsule, cortex and sinus. On the other hand Clara[7], after reviewing the literature, suggested that only a patient histological study could objectively demonstrate the presence in the kidney of arteriovenous anastomoses, acting as regulating structures under physiological conditions. Aukland[3], referring to previous studies by other investigators, asserted that "... if arteriovenous shunts exist, they must be rare (or have diameters less than those of the afferent arterioles)".

Since the morphological foundation of arteriovenous shunts has not been clearly demonstrated in the human kidney, we examined samples of kidneys obtained from subjects in whom the case history and clinical-pathological study excluded renal damage.

Our research has been carried out on 10 cases post mortem: 6 men and 4 women, aged between 24 and 36 years, with

severe cranial traumas. In all cases the causa mortis permitted samples of the kidneys to be fixed within 3–6 h of death. Case histories and the necropsy observation revealed that none of the subjects had a kidney disease. Series of 20–30 sections prepared according to the usual histological techniques have been examined for every case. The sections were 5 µm thick.

Within the juxtamedullary cortex of one kidney we found the whole section of an arteriovenous anastomosis showing in the same plane the arteriole, the anastomotic channel and the venule (fig. a and b). An afferent arteriole immediately before entering the renal corpuscle appeared to bifurcate into a T shape with one branch directed into the glomerulus and the other into a larger thin-walled vessel.



a Section revealing an arteriovenous anastomosis (AVA) between a branch of the afferent arteriole and a venule. MD, macula densa, touched by the cutting plane; fix. Bouin, stain Azan, × 100. *b* Higher magnification of the AVA shown in *a*. The short anastomotic channel forms an S-bend and appears dilated at the outlet into the wider vessel. Its wall contains epithelioid cells protruding into the lumen covered only by endothelium. Note the progressive decrease in thickness of the muscular layer from the arteriole to the venule. × 615. *c* Micrograph of the slice adjoining that illustrated in *a*. The section does not include the anastomotic channel any more, but allows the afferent arteriole to be well recognized. This appears superficially cut both in its part directed towards the renal corpuscle and in that directed towards the venule (V); fix. Bouin, stain H & E, × 100. *d* Higher magnification of *c*. The bifurcation in T shape of the arteriole (A) can be clearly seen. Note the thin muscular layer of the venule. × 250. *e* Cross-section of a vascular segment (arrow) whose wall consists of epithelioid cells separated from the lumen only by endothelium; fix. Zenker, stain Azan, × 260. *f* Enlargement of *e*. Note the difference in wall structure between the vascular segment previously described and the arteriole seen up on the right. In particular, the former does not display an internal elastic lamella, which is clearly visible in the latter, × 615.

This communication was established by means of a short anastomotic channel containing epithelioid cells in its wall. The channel formed an S-bend and dilated; then, at the outlet into the larger vessel, it formed a pad of epithelioid cells which protruded, covered by endothelium, into the lumen. As the vessel with the wider lumen left its origin, its muscular supply rapidly decreased in thickness. Regarding the musculature, it must be added that epithelioid cells could be clearly distinguished in the afferent arteriole, whereas the vessel with the wider lumen had thin, obliquely running muscle cells, with the muscular layer virtually reduced to 1 cell in thickness. In subsequent sections, this larger vessel assumed the characteristic features of a venule, confirming this as an arteriovenous anastomosis occurring at the level of an afferent arteriole (fig. c and d). The very careful observation – also by means of serial sections – of the tissue zone in which this anastomosis is located excluded the presence of pathologic alterations.

In other cases, vascular segments were less frequently (3–6/cm^2) encountered and showed a complete lining of epithelioid cells separated from the lumen only by endothelium (fig. e and f). These segments also were always located in the juxtamedullary cortex. Their epithelioid cells were very closely apposed with only a sparse and delicate connective tissue network between. Such segments were 30–40 µm thick and appeared to lack both circumferential smooth muscle cells and an internal elastic lamella. However, even if they presented the distinctive features of arteriovenous anastomotic channels, the above described vascular segments could not be proved – perhaps owing to their length – to empty into venules.

The difficulties met with in demonstrating arteriovenous anastomoses even by close histological survey make any estimate of their actual frequency quite inconclusive. In fact, small arteriolovenular anastomoses can be microscopically detected only when the section is oriented so that an arteriole, a venule and the anastomotic channel between them fall on the same plane; furthermore, the anastomosis must be open. For these reasons we think that the frequency of observation of such anastomoses, rather low in our experience, cannot reflect their actual distribution in the kidney. However, the existence of such vascular formations in the juxtamedullary renal cortex suggests a possible role in the local microcirculation with resulting influences upon renal function.

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Mechanism of reaction of carcinogen N-acetoxy-2-acetylaminofluorene with DNA[1]

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Summary. Scavenging studies are supporting the notion that triplet radical cations are formed during the binding of the carcinogen N-acetoxy-2-acetylamino-fluorene to native DNA in vitro.

The carcinogen N-acetoxy-2-acetylamino-fluorene (AAAF) reacts with native DNA in vitro to form approximately 80% of the N-(deoxyguanosin-8-yl)-AAF (guanine C-8 adduct) and 20% of the 3-(deoxyguanosin-N^2-yl)-AAF (guanine N-2 adduct)[3,4]. AAAF in aqueous solution is believed to undergo rapid ionization to the N-2-fluorenyl-N-acetylnitrenium ion which then degenerates to the energetically more favored triplet radical cation ground state[5]. It has been proposed that the reactivity of AAAF toward the stable free radical 2,2-diphenyl-1-picryl-hydrazyl is due to the presence of a high percentage of radical cations among the ions formed upon decomposition of AAAF. In contrast, the comparatively low reactivity of N-hydroxy-2-acetamidophenanthrene toward this stable radical may reflect the formation of mostly nonradical N-aryl-N-acetyl-nitrenium ions[5]. The same authors suggested that the relative reactivity of these compounds with this stable radical might be paralleled by their abilities to form DNA adducts.

We have investigated the mechanism of the in vitro reaction of AAAF with DNA in studies of the effect of the addition of nitroxide labeled deoxyuridine derivatives, spin traps and the radical scavengers L-cysteine and acetone. Of these reagents the stable nitroxide radical DUTA (N-[1-oxyl-2,2,6,6,-tetramethyl-4-piperidinyl]-N-[β-D-2′-deoxyribofuranosyluracil-5-yl]-amine) was the most potent inhibitor of DNA adduct formation. Both DUTA and the structurally related DUGT (N-[1-oxyl-2,2,6,6,-tetramethyl-4-piperidinyl]-O-[β-D-2′-deoxyribofuranosyluracil-5-yl]-glycolamide) inhibited the formation of the guanine N-2 adduct more efficiently than the formation of the major guanine C-8 adduct. Our results support the notion that radical cations represent important intermediates in the reaction of AAAF with DNA.

Materials and methods. Ring labeled ^{3}H-AAAF (878 mCi/mmole) in methylenechloride was obtained from the Midwest Research Institute, Kansas City, MO, USA. Before use, the methylenechloride was evaporated and the ^{3}H-AAAF dissolved in a small amount of anhydrous dimethylsulfoxide (Pierce Chemical, Rockford, Ill., USA). [2-^{14}C]-thymidine (59 mCi/mmole) was bought from Amersham, Zürich, Switzerland. DUGT (N-[1-oxyl-2,2,6,6,-tetramethyl-4-piperidinyl]-O-[β-D-2′-deoxyribofuranosyluracil-5-yl]-glycolamide), a nitroxide labeled deoxyuridine derivative, was synthesized according to published procedures[6]. The structure of DUTA, an analog of DUGT, has been discussed elsewhere[7]. The spin traps α-4-pyridyl-1-oxide-N-t-butyl-nitrone (4-POBN) and α-phenyl-N-t-butyl-nitrone (PBN) were purchased from Aldrich Chemical Co., Chicago, Ill., USA. Bacteriophage T7 was grown in *Escherichia coli* B thy$^-$ in M9 medium which was supplemented with 1.5–2.0 µg/ml of unlabeled thymidine and 5 µCi/l of [2-^{14}C]-thymidine. T7 phage was purified as described by