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.

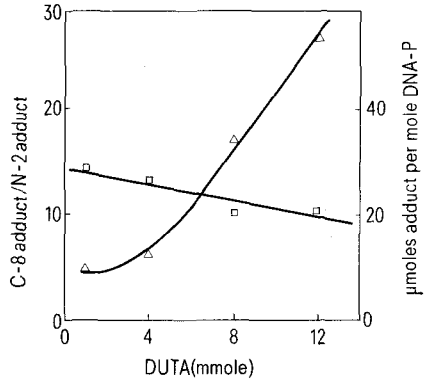
Materials and methods. Ring labeled ^{3}H-AAAF (878 mCi/mmole) in methylenechloride was obtained from the Midwest Research Institute, Kansas City, MO, USA. Before use, the methylenechloride was evaporated and the ^{3}H-AAAF dissolved in a small amount of anhydrous dimethylsulfoxide (Pierce Chemical, 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

Yamamoto et al.[8], and the DNA was extracted by standard procedures with phenol/$CHCl_3$/isoamylalcohol (50/50/1). The specific activity of T7 DNA was approximately 5200 dpm per OD_{260} unit. Aliquots of 60 µg of ^{14}C-thymidine labeled T7 DNA were reacted with 0.006 µmoles ^{3}H-AAAF in 0.1 ml of 0.2 M potassium phosphate buffer pH 6.8 for 150 min at ambient temperature in the presence or absence of the reagents to be tested. The reaction was stopped by the addition of 3 volumes of cold 95% ethanol. After an additional ethanol precipitation the precipitates were taken up in 0.5 ml 1 × sodium chloride-sodium citrate and extracted 6 times with 0.5 ml ether. The DNA was recovered by ethanol precipitation, dissolved in 0.01 M tris buffer pH 7.9 and denatured by heating for 3 min to 100 °C and rapid cooling in an icewater bath. Aliquots were withdrawn for the determination of total AAF-adduct concentrations from the ^{3}H over ^{14}C ratios while the rest of the samples were enzymatically degraded according to Yamasaki et al.[9] after adjusting the tris buffer to 0.1 M in NaCl and 0.001 M in $MgCl_2$. The resulting deoxynucleoside mixtures were chromatographed on Sephadex LH 20 columns as described previously for the separation of covalent adducts of benzo(a)pyrene[10]. From the chromatograms the concentrations of the guanine C-8 adduct and the guanine N-2 adducts were determined.

Effect of spin traps, radical scavengers and stable nitroxide radicals on adduct formation between AAAF and DNA

	% total AAF-adducts*	C-8 adduct/ N-2 adduct**
Control	100	5.8
0.01 M 4-POBN	100	6.2
0.01 M PBN	106	5.8
0.01 M acetone	102	5.7
0.01 M L-cysteine	25	4.7
0.01 M DUGT	43	7.3
0.025 M DUGT	25	17.5
0.001 M DUTA	28	5.3
0.012 M DUTA	21	27.0

* Total adduct concentration in control DNA treated with AAAF in the absence of additional reagent was 200 µmoles per mole DNA-P and this concentration was defined as 100%. ** Ratio of the concentrations of AAF guanine C-8 adduct to AAF guanine N-2 adduct from Sephadex LH20 chromatograms of enzymatic DNA hydrolysates.



Effect of stable nitroxide radical DUTA on the formation of covalent adducts between AAAF and bacteriophage T-7 DNA in vitro. △, ratio of AAF-guanine C-8 adduct over AAF-guanine N-2 adduct as a function of DUTA concentration (ordinate to the left); □, total AAF-DNA adducts formed as a function of DUTA concentration (ordinate to the right).

Results and discussion. The effect of 3 types of reagents, i.e. spin traps, general radical scavengers and stable nitroxide radical derivatives of deoxyuridine, on the formation of total and individual covalent adducts of AAAF to bacteriophage T7 DNA in vitro was investigated. These reagents are expected to influence the reaction of AAAF with DNA if radical intermediates are indeed formed as has been postulated. The table lists the amounts of total adduct formed and the ratios of AAF guanine C-8 adduct over guanine N-2 adduct. It is evident that the spin traps 4-POBN and PBN had no effect on adduct formation. While acetone was also inactive, 0.001 M L-cysteine suppressed the reaction to 25% of untreated controls. The stable nitroxide radicals DUGT and, more pronouncedly, DUTA not only suppressed the overall reaction but strongly influenced the adduct composition. From the ratios of guanine C-8 adduct over guanine N-2 adduct of 17.5 for 0.025 M DUGT and of 27 for 0.012 M DUTA it is evident that these reagents inhibited the formation of the minor AAF adduct to the exocylic amino group of guanine preferentially. DNA adduct formation in the presence of DUTA, i.e. the most active reagent, was further studied as a function of concentration. The data is shown in the figure. In the concentration range from 1 to 12 mM DUTA only a small additional decrease from 28 to 21 µmoles total adduct per mole DNA-P was observed while the ratio of the AAF-guanine C-8 adduct over the AAF-guanine N-2 adduct increased drastically from 5.3 to 27.

Our results support the notion that AAF radical intermediates play a role in the reaction of AAAF with DNA in vitro. However, the AAF radical intermediates are apparently not able to interact with the spin traps 4-POBN and PBN. It is conceivable that the trapping rate with these spin traps is too slow to compete with other pathways for the AAF radical cations. The 3 reagents found to be active, i.e. cysteine, DUGT and DUTA, inhibited the formation of both AAF guanine adducts suggesting that the same active intermediate of AAAF is reacting with the C-8 portion and the exocyclic amino-group of guanine in DNA. For the case of DUTA which was investigated in more detail a strong effect on adduct composition was only observed when the concentrations were raised above approximately 4 mM. The preferential inhibition of the formation of the AAF guanine N-2 adduct occurring at high DUTA concentration may be due to an effect of DUTA on DNA conformation. It has been observed previously that the reaction of AAAF with heat denatured DNA leads almost exclusively to the formation of the AAF-guanine C-8 adduct[11]. The mechanism of the effects of DUTA and DUGT on the formation of AAF-DNA adducts is under further investigation.

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