Table II. Cyanide (CN) sensitive alkaline phosphatase activity in the basal and luminal area of rat renal proximal tubular segments

Structure	Without CN	With 10 m*M* CN	CN sensitive phosphatase	CN-sensitive activity total activity
Basal area from PTC	41.9±11.1[a] (15)	20.4±14.8 (7)	21.5	0.51
from PTR	11.7± 5.5 (7)	3.8± 1.4 (6)	7.9	0.68
Luminal area from PTC	137.8±79.2 (12)	3.6± 2.8 (5)	134.2	0.97
from PTR	171.0±70.5 (10)	4.2± 2.5 (6)	166.8	0.97

PTC, proximal tubular convoluted portion; PTR, proximal tubular straight portion. [a] MKH = moles *p*-nitrophenol/kg dry weight/h at 37°C ± SDM. Number of analyses in brackets.

As yet it is not clear whether mitochondrial membrane contains a. P'tase[13]. Another intracellular site of a. P'tase is the Golgi apparatus. Morphometric data in rat renal tubule reveal a much higher amount of Golgi membranes in the PTR than in the PTC epithelia[14]. However, a. P'tase in basal area demonstrates an inverse activity pattern (Table I). The conclusion can be drawn that a. P'tase activity (Table I and II) in the basal area of PTC and PTR belongs to the basal labyrinth membrane.

Two thirds of a. P'tase in rat renal tubular epithelia consist of an insoluble desmoenzyme and $^1/_3$ of a soluble lyoenzyme[15]. The lyoenzyme is localized in the basal labyrinth membrane as shown by electron microscopic technique[15]. This might explain the lack of a. P'tase in basal labyrinth membrane fractions[16].

Our results show that a. P'tase is localized in the whole surface membrane of the renal proximal tubular cell, including the basal infoldings. This fact excludes a. P'tase to serve as a marker in differentiating brush border from basal labyrinth. As a proof for the purity of the brush border fraction, we propose the lack of Na K ATPase activity as such a marker. Na K ATPase is localized only in the basal labyrinth membrane and is missing in the brush border[8].

Zusammenfassung. Mit Hilfe einer Ultramikrotechnik konnte an isolierten gewundenen und geraden Hauptstücken der Rattenniere gezeigt werden, dass die alkalische Phosphatase nicht nur in der Bürstensaum–, sondern auch in der basalen Labyrinthmembran lokalisiert ist. Das Enzym ist infolgedessen kein geeignetes Leit-Enzym zur Erkennung des Bürstensaumes aus einem Plasmamembrangemisch. Es wird vorgeschlagen, das Fehlen der Na K ATPase-Aktivität als Mass für die Reinheit der Bürstensaumfraktion zu benutzen.

U. SCHMIDT and U.C. DUBACH[17]
with technical assistance
of I. BIEDER and B. FUNK

Enzyme Laboratory of the Medical Policlinic,
University of Basel, Hebelstrasse 1,
CH-4056 Basel (Switzerland), and
Institute of Pathology,
University of Tübingen (Germany), 20 December 1971.

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Reaction of DNA with Phosphoric Acid Esters: Gasoline Additive and Insecticides

Trimethyl phosphate (TMP), a gasoline additive and the insecticides dichlorvos (DDVP, 0, 0-dimethyl-2, 2-dichlorovinyl phosphate) and dipterex (dylox, 0, 0-dimethyl-2, 2, 2-trichloro-l-hydroxyethyl phosphonate) have recently been reported to exhibit 'suspicious' biologic properties suggesting that they may have adverse effects on health. Thus TMP is mutagenic in mice[1] and DDVP in *E. coli*[2]. Dipterex has been stated to be weakly carcinogenic for rats[3] and DDVP to cause chromosome aberrations in onion root tip cells[4]. In terms of present concepts, all of these phenomena presumably result from an effect of these agents on cellular DNA. Indeed the alkylation of DNA by DDVP has been reported[5]. In this report the effect of DDVP on DNA is confirmed, moreover, it is shown that TMP and dipterex also alter DNA. This may then provide a chemical basis for the observed biological effects of these substances.

Exposure of DNA to these phosphoric acid esters[6] resulted in noticeable diminutions in the sedimentation coefficient of this biopolymer (Table). Presumably these changes reflect alkylation of DNA followed by some de-

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purination; this in turn causes scission of the phosphodiester backbone[7] leading to lower sedimentation coefficients. Heating of the modified DNA results 1. in further depurination and hence further scission and 2. in separation of the strands and therefore the unmasking of additional 'hidden' (single-chain) breaks that previously were stabilized (i. e. hidden) by the double-stranded structure. These effects cause a further reduction in sedimentation coefficient (Table). The control DNA, on the other hand, does not show this pronounced decrease in sedimentation coefficient upon thermal denaturation.

Additional experiments revealed that exposure of deoxyguanosine to these substances resulted in the formation of a product with chromatographic and spectral properties similar to those of 7-methyldeoxyguanosine. These findings support the above postulated mechanism of action in which alkylation of DNA is the first step in the degradative process, they are also in accord with the known alkylating potential of these substances (see refs.[1] and[5]).

In view of the established relationship between the ability of a substance to react with DNA and its potential to induce detrimental effects (mutations, carcinogenesis and teratogenesis) and because of widespread human exposure to the agents studied, ways to eliminate human contact with these substances should be sought[8].

Effects of phosphoric acid esters on the properties of DNA

Additions	Sedimentation coefficients (s) Before heating	After heating
None	18.4	17.5
Dipterex	11.2	7.4
DDVP	15.7	9.1
Trimethylphosphate	15.7	10.3

To 1 ml of calf thymus DNA (1 mg per ml of 0.015 *M* NaCl in 0.01 *M* phosphate buffer, pH 7.0) either 1 µl of DDVP, 1 µl of trimethylphosphate or 100 µg dipterex were added. The mixtures were incubated at 56°C for 42 h whereupon DNA was precipitated with ethanol, the insoluble fibers washed extensively with ethanol and the DNA redissolved in 0.15 *M* NaCl. Portions of the specimens were placed in a boiling water bath for 10 min. and then immersed into an ice-bath. Sedimentation coefficients were determined in a Spinco Model E analytical ultracentifuge equipped with an ultraviolet optical system.

Résumé. L'addition de phosphate triméthyl, «dichlorvos» (0, 0-dimethyl-2, 2, dichlorovinyl phosphate) et du «diptérex» (0, 0-dimethyl-2, 2, 2-trichloro-1-hydroxyethyl phosphonate) à du DNA provoque la dégradation de cette macromolécule. La réaction semble être due à une alkylation du résidu de guanine du DNA. Ces observations peuvent fournir une explication chimique aux effets biologiques de ces substances.

H. S. Rosenkranz and S. Rosenkranz

Department of Microbiology,
College of Physicians and Surgeons, Columbia University,
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Fluorescence of the Soluble 'Sweet-Sensitive' Protein Complexes with Sugars

The interaction of a 'sweet-sensitive' protein with sugars and saccharin was originally studied by the method of difference spectroscopy[1]. Changes in optical density upon titration with various concentrations of different sugars were recorded at 277 nm, the ultraviolet maximum of the protein using a tandem double compartement technique[2]. The UV-absorption spectra of proteins is mainly due to their content of the aromatic amino acid tyrosine, tryptophan and, to a lesser extent, phenylalanine[3]. The region 278–281 nm is characteristically the maximum peak area[4].

The fluorescence of proteins originates almost entirely from the tyrosyl and tryptophenyl residues. Consequently, conformational modifications can be followed by observing changes in either tyrosine or tryptophan fluorescence intensity. This study of the fluorescence of the 'sweet-sensitive' protein and its sugar complexes was undertaken because of the known sensitivity of the emission of the aromatic chromophores.

Materials and methods. The 'sweet-sensitive' protein was extracted from epithelium of cow and dog tongues by the method previously described[1,5] with the following exceptions: 1. the 75,000 × *g* or 105,000 × *g* supernatant was not subjected to ammonium sulfate but was fractionated by the ultrafiltration in an Amicon cell with a Diaflow membrane of retention of molecular weights 100,000 and up: 2. the retentate from ultrafiltration was subjected to cation exchange on Bio-Rex 63 resin (control No. 7429) and the protein eluted with 1.0 *M* sodium bicarbonate-sodium carbonate buffer pH 10.0 plus 1 *M* NaCl. The protein was dialyzed in the ultrafiltration cell until equilibrium to 0.1 *N* sodium phosphate buffer pH 7.0 was achieved.

Emission spectra were measured with an instrument built in the laboratory[6]. Optical density (OD) recordings were made on a Cary 15 recording spectrophotometer. In

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