of the outer mitochondrial membrane that extended a variable distance in the cytoplasm to become continuous with the membrane of the catecholamine-storage vesicle (figure). The diameter of the tubular extension was about 20 nm and the length varied from a negligible distance when the vesicle appears to be in direct contact with the mitochondrion, to over 1 μm. Due to the tubular extension, the compartment between the inner and outer mitochondrial membranes was in direct continuity with the vesicle contents without an intervening membrane. These continuities involved about 25% of the mitochondria from tissue of control rats (no insulin treatment) as compared to about 40% of the mitochondria seen in tissue from rats perfused 4 h after insulin treatment and 50% of the mitochondria from rats 24 h post-insulin.

Discussion. It has been established that ATP is synthesized principally in the mitochondria and accumulated in the catecholamine-storage vesicles (see above). Although other mechanisms may be involved, our observations suggest that ATP could be transported from mitochondria to catecholamine-storage vesicles directly through membrane-lined channels. This may occur more often during periods of high synthetic activity such as insulin-induced hypoglycemia and the channels may serve only as a supplemental transport process. The channels were seen to occur more frequently in some cells than others in the same gland. This supports the contention that the reaction of the adrenal medulla to stress tends to exhibit an all-or-none response on the cellular level[9].

Although our observations have been restricted to the adrenal medulla, we may postulate the possibility that direct channels exist in other cellular systems where ATP is transported from mitochondria to other organelles. For example, Droz has suggested that direct contacts between mitochondria of neural tissue and axonal smooth endoplasmic reticulum (SER) may provide direct transfer of materials required for the maintenance of mitochondria during their long transit time from the cell body to the nerve terminal[10,11]. He did not speculate that transfer of high-energy nucleotides from the mitochondria to the lumen of the SER might be occurring, but this would be an interesting possibility since he suggested that the transmitter storage vesicles (presumably rich in ATP[2]) 'pinch off' at terminal arborizations of the SER. This possibility would be of additional interest if ATP were shown to have the properties of neurotransmitter as suggested by Burnstock[12]. As discussed by Volk[13], it would be a fortuitous ultrathin section to allow the visualization of an undulating tubular connection such as the one described in this report, and then the observation would be too uncommon to attach any special significance to it. Indeed, other authors argue that extensions from mitochondrial profiles are artifacts of thin sectioning[14-16]. But the HVEM offers the advantage of greater electron penetration of the specimen, thus thicker sections can be examined with reasonable resolution. Examination of thick sections (0.5–1 μm) on a tilt stage allows a 3-dimensional evaluation of the interrelationship of cell organelles through a greater distance in the tissue. Specifically, the tubular connection that we describe can be visualized in 3-dimensions, ruling out the possibility that the images are the result of superimposition of structures.

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Effect of dimethyl sulfate on the secondary structure of DNA

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Summary. Hydroxyapatite chromatography has been used to demonstrate that alkylation of DNA at neutral pH may lead to denaturation under conditions where no significant depurination occurs. Presence of salt has a preventive effect on such denaturation.

Many chemical carcinogens belong to the class of alkylating agents. Both in vivo and in vitro studies have indicated that principle target of these agents are the nucleic acids[2-4]. Lawley and Brooks have shown that the main alkylation sites in double stranded DNA are the N-7 of guanine and N-3 of adenine[5]. In addition, the alkylation of DNA-phosphates may also occur. However, on this aspect there are conflicting reports in the literature[6,7]. Hsiung et al.[8] have postulated that partial denaturation of alkylated DNA at higher pH values may occur through the disruption of hydrogen-bonding due to a base catalysed imidazole ring opening of the quarternized N-7 guanine. Rizvi and Hadi have earlier published a preliminary experiment showing that alkylation may lead to denaturation of DNA under neutral conditions[9]. To our knowledge, these are the only reports available in the literature on the effect of alkylating agents on the secondary structure of DNA. In the present work we have used hydroxyapatite chromatography to demonstrate the denaturation of DNA as a result of alkylation without causing significant depurination. Dimethyl sulfate has been chosen as the alkylating agent, since it is known to cause minimum alkylation of DNA-phosphates[10].

Materials and methods. Calf thymus deoxyribose-nucleic acid (sodium salt, average mol. wt 1 million) was obtained from Sigma Chemical Company, and was used without further purification. Dimethyl sulfate was obtained from May and Baker Ltd, England. Hydroxyapatite was prepared as described by Bernardi[11].

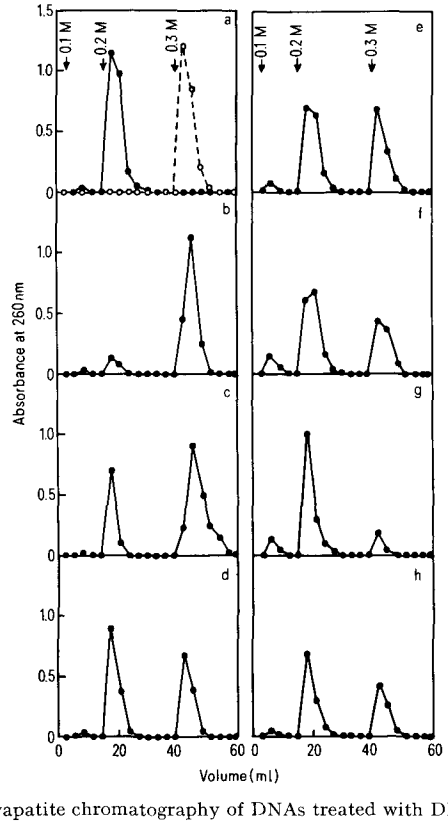
A 2-mg/ml solution of DNA in TNE (0.01 M tris-HCl, pH 7.4, 0.01 M or 0.1 M NaCl and 2×10^{-4} M EDTA), was methylated by adding sufficient dimethyl sulfate (DMS) to obtain the desired DNA nucleotide/DMS molar ratio. The solution was gently shaken at 25 °C for the desired period of time. The acid released by the hydrolysis of the methylating agent was neutralized by the addition of i.m. NaOH and the pH was maintained between 6 and 7. Depurinated DNA was obtained from alkylated DNA by incubating the latter at 50 °C for 4 h[12]. Hydroxyapatite Chromatography was done as described by Bernardi[11]. To determine the alkali labile acid-soluble

Alkaline hydrolysis of dimethyl sulfate treated DNA

DNA/DMS molar ratio	Time of alkylation (h)	NaCl concentration	% DNA denatured (calculated from figure)	Alkaline hydrolysis of alkylated DNA (μmoles acid soluble DNA nucleotide)	Alkaline hydrolysis of depurinated DNA (μmoles acid soluble DNA nucleotide)
No DMS	–	0.01 M	0.0	< 0.005	< 0.005
1:1	1	0.1 M	9.8	< 0.005	0.033
1:4	1	0.1 M	22.5	< 0.005	0.516
1:8	1	0.1 M	46.0	0.110	0.580
1:4	2	0.01 M	50.0	0.013	–
1:4	5	0.01 M	54.0	0.019	–
1:4	10	0.01 M	82.0	0.022	–
1:4	10	0.1 M	51.0	0.010	–

Percent denatured DNA was calculated from the data of the figure. Hyperchromicity of DNA on denaturation was taken into account before calculating the percentage. See text for details of alkaline hydrolysis of alkylated and depurinated DNA.

DNA nucleotides in alkylated and depurinated DNA, the DNAs were first treated with alkali (0.1 M) before precipitating with perchloric acid in the presence of 2 mg/ml of bovine serum albumin (BSA). The acid-soluble material was determined by diphenylamine reaction[13].



Hydroxyapatite chromatography of DNAs treated with DMS under various conditions, 600 μg of DNA in 0.3 ml of TNE was loaded on a 1×3 cm column previously equilibrated with 0.01 M sodium phosphate buffer (pH 7.0) containing 1% formaldehyde. The column was washed with 3 ml of equilibrating buffer and immediately followed by elution with sodium phosphate buffers (pH 7.0) of molarities indicated containing 1% formaldehyde. 3 ml fractions were collected at the rate of 10 ml/h. Recoveries in all experiments were between 85% and 100%. *a* Heat denatured DNA (●—●), native DNA (○----○). DNA nucleotide/DMS ratio, NaCl molarity and time of alkylation respectively for different samples were as follows: *b* 1:1, 0.1 M, 1 h; *c* 1:4, 0.1 M, 1 h; *d* 1:8, 0.1 M, 1 h; *e* 1:4, 0.01 M, 2 h; *f* 1:4, 0.01 M, 5 h; *g* 1:4, 0.01 M, 10 h; *h* 1:4, 0.1 M, 10 h.

Results and discussion. In the figures b, c and d, is shown the hydroxyapatite chromatography of DNAs alkylated with increasing molar ratios of DMS in the presence of 0.1 M NaCl. Increasing number of DNA molecules show strand separation with concomitant decrease in the double-standed material. As to the reason why all molecules are not denatured at the same time, one possibility may be the heterogenous population of molecules in the DNA used in the experiment. The hypochromicity of native DNA as determined by heat denaturation was 24%, whereas for DNA alkylated with DMS molar ratios of 1:1, 1:2 and 1:4 it was 23, 15 and 10.5% respectively. This observation, with the results of figure b, suggests that light alkylation of DNA (e.g. DNA/DMS molar ratio 1:1) does not result in any significant denaturation. Alkaline hydrolysis of DNA alkylated with a DMS molar ratio of 1:1 and 1:4 shows negligible production of acid-soluble nucleotides (table). This suggests that no significant depurination of alkylated sites had occurred[14]. However, when depurinated DNAs obtained from these alkylated DNAs were subjected to alkaline hydrolysis, appreciable production of acid-soluble nucleotides was observed indicating that alkylation of DNA by DMS had earlier taken place. This is further suggested by a control experiment where native DNA was heated at 50 °C for 4 h and then treated with alkali. No production of acid-soluble material was seen. In contrast, when DNA was alkylated with a DMS molar ratio of 1:8 (figure d),

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appreciable alkaline hydrolysis of alkylated as well as depurinated DNA occurred. In order to determine the effect of ionic strength on denaturation, the experiments shown in the figures e, f, g, h were done. DNA was alkylated for different periods in 0.01 M NaCl and subjected to hydroxyapatite chromatography. A DNA nucleotide/DMS molar ratio of 1:4 was chosen since this causes minimum depurination of alkylated DNA. Almost all the DNA molecules are denatured after incubation for 10 h in 0.01 M NaCl (figure g), while only 50% molecules are denatured in 0.1 M NaCl in the same period (figure h). A control DNA sample kept in 0.01 M NaCl at room temperature for 10 h did not show any production of denatured molecules. In all experiments where alkylation was done in the presence of 0.01 M NaCl, some A_{260} absorbing material was eluted with 0.1 M phosphate buffer, which corresponded to mononucleotides.

The major sites of alkylation in DNA with DMS are the N-7 position of guanine and N-3 position of adenine[15]. Hsiung et al.[8] observed a relationship between the decrease in fluorescence of intercalated ethidium and the extent of alkylation of DNA at pH 7.0. This was attributed to the charge repulsion between the quarternized alkylated bases and the positively charged ethidium which resulted in a decrease in the number of ethidium-binding sites. It is possible that the positive charges of the quarternized alkylated bases alone through repulsion may adversely effect the forces stabilizing the secondary structure of DNA. Regardless of the precise interpretation of our results, it is clear that alkylation of DNA makes the secondary structure of DNA relatively unstable compared with that of the native molecule.

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Neuroactifs et tératogenèse vertébrale chez l'embryon d'oiseau

Neuroactive compounds and vertebral teratogenesis in the bird embryo

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Summary. Anticholinesterasic and depolarizing (analogues of acetylcholine) agents administered to quail embryos after 3 days of incubation give rise to vertebral fusions in addition to neck deformities. Antagonists of acetylcholine (gallamine and hexamethonium) produce only vertebral joint fusions. The incidence of all these compounds in the vertebral defects by the way of there neuroactive properties is proposed.

Les malformations du rachis provoquées par les insecticides organophosphorés, insensibles à un apport de nicotinamide exogène[1-2], sont au contraire moins accentuées ou abolies si des agents connus pour réactiver les cholinestérases phosphorylées sont administrés conjointement à ces tératogènes[1-3].

D'après ces données il a été suggéré que les processus inhérents aux fonctions cholinergiques pouvaient être impliqués dans les désordres morphogénétiques axiaux induits par les esters phosphoriques. Pour étayer cette idée, il nous a paru important de rechercher si des anomalies vertébrales, semblables à celles induites par les organophosphorés, pouvaient être reproduites par d'autres drogues, également bien connues pour leurs interférences avec la physiologie neuromusculaire. Nous rapportons ici les résultats relevés au niveau de la région cervicale de l'axe vertébral d'embryons de caille, exposés chroniquement à des organophosphorés et à des carbamates anticholinestérasiques, à des analogues structuraux de l'acétylcholine, ainsi qu'à deux antagonistes de ce neurotransmetteur.

Matériel et techniques. Les œufs de *Coturnix coturnix japonica* sont issus de l'élevage du laboratoire et reçoivent à 3 jours d'incubation des injections intra-vitellines de différents produits dont les concentrations et la nature sont précisées ci-après: 75 µg de nitrostigmine (Pestanal), 250 µg de dicrotophos (Shell), 500 µg de sulfate de physostigmine (Prolabo), 500 µg de bromure de néostigmine (Merck), 2,5 mg de bromure de démécarium (Merck Sharp and Dohme), 1,5 mg de bromure de décaméthonium (Fluka) et 1,5 mg de carbachol (Fluka). Pour la gallamine (Rhône-Poulenc) 2 injections de 5 mg chacune sont faites à 3 et 6 jours et pour l'héxaméthonium (Fluka), une première administration de 10 mg à 3 jours est suivie d'une seconde de 5 mg à 6 jours. Les sacrifices sont réalisés aux 10 ou 11èmes jours. Les examens histologiques sont effectués sur des coupes à la paraffine colorées à l'hématoxyline-éosine.

Résultats. Seuls les survivants aux administrations de gallamine et d'héxaméthonium montrent un aspect morphologique externe voisin de la normale. Les sujets ayant survécu à tous les autres traitements sont dotés de malformations cervicales (figures 1 à 4) dont la gravité décroit conformément à la sériation suivante: nitrostigmine $\geqslant$ néostigmine $>$ carbachol $\geqslant$ décaméthonium $>$ dicrotophos $\geqslant$ physostigmine $>$ démécarium. Avec le dicrotophos et la physostigmine, s'ajoutent aux déformations du cou (court et tordu), des anomalies du bec et des pattes (figure 3).

Les examens histologiques montrent que les anomalies cervicales sont dues, d'une part, à un retard de développement des pièces squelettiques (comparer les figures 6 et 7 avec la figure 5) et d'autre part, à des torsions de l'axe vertébral (cyphoses et/ou lordoses). Les torsions les plus accentuées sont situées à un niveau postérieur à la 8e vertèbre cervicale (figure 6); la partie antétieure du rachis étant généralement dépourvue de violents plissements (figures 6 et 7).

Tous les agents éprouvés ici provoquent la fusion de certaines pièces vertébrales. Les soudures sont particulièrement évidentes au niveau des premiers neurarcuaux (figures 6, 7 et 8) et dans la région du joint crânio-vertébral.

L'étendue des zones ankylosées est toutefois sujette à des variations; elle est d'autant plus développée que les déformations sont plus accentuées. Une autre caractéristique, évidente avec les agents les plus actifs, réside dans une atrophie de la musculature vertébrale.

Discussion. Parmi les tératogènes envisagés ici, seuls la physostigmine et le dicrotophos entrainent la micromélie et les anomalies du bec; ceci est un argument supplémentaire pour considérer que les défectuosités de la morphogenèse du bec et des membres obéissent à un déterminisme différent de celui impliqué dans les malformations axiales[1-4]. Par contre, tous les toxiques que nous avons étudiés sont capables d'induire des anomalies axiales ma-