Table II. Micrograms of testosterone, epitestosterone and androstenedione found in 10 g pollen of *P. silvestris* L.

Methods	Testosterone Individual values	Means	Epitestosterone Individual values	Means	Androstenedione Individual values	Means
1	Not detected		Not detected		Not detected	
2	Not detected		0.06		0.03	
3	0.7		0.7		6.6	
	0.6	0.8	1.5	1.1	4.4	5.9
	1.1		1.1		6.7	
3a[a]	0.3		0.3		0.6	
	0.3	0.3	0.3	0.4	0.2	0.8
	0.3		0.6		1.6	
4[b]	Testosterone + Epitestosterone $\frac{2-3}{3}$				Androstenedione $\frac{3-4}{5}$	

[a] The hydrolysis with β-glucuronidase was omitted. [b] The zones of testosterone and epitestosterone overlapped.

identification of the substances determined. In the 3 different solvent systems the Rf-values of free and acetylated reference steroids covered those of testosterone, epitestosterone and androstenedione isolated from pollen. On this base testosterone, epitestosterone and androstenedione are present in the pollen of Scotch pine in amounts from 0.8 to 5.9 μg/10 g pollen.

Zusammenfassung. Im Pollen von *Pinus silvestris* L. wurden die androgenen Steroide Testosteron, epi-Testosteron und 4-Androsten-3,7-dion nach vier verschiedenen Methoden bestimmt.

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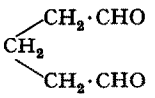
Interaction of Glutaraldehyde with Some Micro-organisms

Glutaraldehyde is a 5-carbon dialdehyde which is active against viruses, fungi and bacterial spores and



vegetative cells. Its antimicrobial activity is enhanced at alkaline pH[1-5], and it is used as a chemical sterilizer[6-8]. The mode of action of glutaraldehyde is unknown, but interactions between the dialdehyde and proteins have been described[9,10], and it has been suggested[5] that glutaraldehyde reacts with amino groups present in the bacterial cell.

Recently, it was found in this laboratory[11] that under alkaline, but not under acid, conditions, glutaraldehyde produced a red colouration with whole cells and penicillin-induced spheroplasts of, and cell envelopes isolated from, *Escherichia coli* NCTC 9001. Since this suggested possible binding sites for glutaraldehyde with components of bacterial cells, the effects of alkaline glutaraldehyde (glutaraldehyde 0.2% + sodium bicarbonate 0.3%) on various bacteria were investigated.

Interaction of glutaraldehyde with some micro-organisms

Organism	Strain of organism	Culture or preparation[a]	Colour after exposure to alkaline glutaraldehyde[b]
E. coli	NCTC 9001	NB 2, 18 h, 37 °C	Red
		Penicillin-spheroplasts	Red
		Cell walls	Red
		Cytoplasmic constituents	Yellow
Klebsiella aerogenes	NCTC 8172	NB 2, 18 h, 37 °C	Red
Serratia marcescens	NCTC 8706	NB 2, 18 h, 30 °C	Red
Proteus vulgaris	Laboratory	NB 2, 18 h, 37 °C	Red
Micrococcus lysodeikticus	NCTC 2605	NB, 18 h, 37 °C	None
Staphylococcus aureus	NCTC 6571	NB, 18 h, 37 °C	Slight yellow
Bacillus subtilis	NCTC 8236	NB, 18 h, 37 °C	Yellow
B. subtilis (fattened)	NCTC 8236	GB, 10 subcultures for 24 h, 37 °C	Light yellow
B. subtilis spores	NCTC 8236	Penicillin assay medium 7 days, 37 °C	Light orange/yellow (24 h only)
B. polymyxa	NCTC 10343	NBY, 18 h, 37 °C	Light orange/yellow
B. megaterium	NCTC 6005	NB, 18 h, 37 °C	Yellow
		Lysozyme-protoplasts	None detectable
		Cytoplasmic constituents	Yellow
Saccharomyces carlsbergensis	NCYC 74	NBG, 36 h, 37 °C	None
Penicillium chrysogenum	Laboratory	S, 7 days, 25 °C	None

[a] NB, nutrient broth (Oxoid); NB 2, nutrient broth no. 2 (Oxoid); GB, nutrient broth + 3% glycerol; NBY, nutrient broth + 1% yeast extract; NBG, nutrient broth + 1% glucose; S, saboraud liquid medium (oxoid). [b] Exposure for 2 h at 37 °C to 0.2% glutaraldehyde + 0.3% sodium bicarbonate.

The organisms used and their method of growth are listed in the Table. Suspensions were centrifuged, the deposit resuspended in water and an aliquot treated with alkaline glutaraldehyde for 2 h at 37 °C. The treated suspensions were then centrifuged, and the colour of the cell deposit examined visually. The results (Table) show that a red colour develops only with Gram-negative bacteria, although a yellow or light orange colour is also produced with vegetative and spore forms of various bacilli and with fattened cells of *Bacillus subtilis*.

In *E. coli*, the red colour is the result of combination of alkaline glutaraldehyde with the non-mucopeptide layers of the cell wall, probably the protein components, and is not caused by interaction with cytoplasmic constituents. The cell wall of *Proteus vulgaris* is similar in structure to that of *E. coli*[12], and if it is assumed that interaction of glutaraldehyde with cell wall protein is responsible for the red colour in all cases (this is also borne out by the finding that fattened cells of the Gram-positive organism, *B. subtilis*, give no increase in visual colour over unfattened cells) then this can be accounted for by the similarities in the structure and chemical composition of the walls of Gram-negative bacteria. In contrast to the large number of amino acids in these walls, the cell walls of Gram-positive cocci contain mucopeptide and teichoic acid[13], the mucopeptide consisting of some 5 main amino acids. It is, however, unlikely that the red colour observed when Gram-negative bacteria are treated with alkaline glutaraldehyde is contributing to their death, as a) cell death is apparent some time before the colour is visible, b) Gram-positive cocci, which do not show a red colour, are killed just as rapidly. The development of the red colour is thus concerned with differences in cell wall chemistry.

Résumé. La glutaraldéhyde produit une couleur rouge chez quelques genres de bactéries. Cette couleur est prise par la paroi cellulaire des organismes.

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7 July 1970.

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PRO EXPERIMENTIS

Vital Staining of Neurosecretory Material with Acridine Orange in the Insect, *Periplaneta americana*

Neurosecretory materials vary in their chemical composition between different neurosecretory cells and no one staining technique has been found to stain all types of neurosecretory material[1]. In this paper it is shown that acridine orange can be used as a vital stain for all neurosecretory materials.

Vital staining of cells with acridine orange produces an orthochromatic green fluorescence in nuclei and sometimes a metachromatic red fluorescence in cytoplasmic granules. It is now generally accepted that green nuclear fluorescence is due to nucleic acids and that the red cytoplasmic granules are lysosomes[2-8], however, see AUSTIN and BISHOP[9] for an alternative interpretation. As well as staining with acridine orange, lysosomes show acid phosphatase activity amongst other hydrolytic enzymes[2-5].

Materials and methods. Adult and nymphal forms of the cockroach, *Periplaneta americana* from laboratory colonies were used in this study. The following stains were obtained from Chroma-Gesellschaft; acridine orange, acridine yellow, coriphosphine, acriflavine, phosphin 3R, euchrysin 3RX, and euchrysin 2G. All are acridine derivatives. They were dissolved in 0.9% NaCl at a concentration of 0.1 mg/ml.

Pieces of nervous tissue were dissected from the insect under saline and placed in a drop of stain solution on a microscope slide. The tissue was stained for 1 min and then moved to a drop of saline further along the slide. A coverslip was added and excess saline removed. The whole mount was then viewed with blue light from a Wild microscope fluorescence system.

For acid phosphatase localization, tissue was fixed in formol-calcium over-night. The Gömöri lead method and the simultaneous coupling azo dye method using naphthol AS-TR and hexazotized pararosaniline were used[10]. Incubations without substrate were used as controls.

The stains were analyzed by thin-layer chromatography using Kieselgel and *n*-butanol:ammonia:ethanol:water (16:0.15:5:5) as developing solvent[11]. The chromatograms were viewed with long-wave UV-light.

Results. Differentiation of neurosecretory cells and 'ordinary' nerve cells was obtained with acridine orange, euchrysine 3RX, and coriphosphine, however the latter showed only weak differentiating ability. Negative results were obtained with the other stains. Neurosecretory material appeared as red fluorescing granules while the cytoplasm of 'ordinary' nerve cells and axons was a uniform weakly fluorescent green. Nuclei of glial and nervous tissue showed a strong green fluorescence with distinct nucleoli. The cytoplasm of glial cells was not stained. The red fluorescence was not stable under continuous irradiation and faded after 5 min. The nuclear