that glucose may play a role independent of its more obvious one of substrate. There is evidence that glucose and other sugars can influence the activity of enzymes.* In addition, glucose might be capable of stabilising an orderly arrangement of water molecules in lattice,[10] and it has been suggested that water molecules play a vital role in the structure and function of the cell membrane.[11] Our own preliminary studies suggest that glucose, (2 mM) under conditions unfavourable for glucose metabolism, reduces the free acid phosphatase activity of cerebral lysosomes subjected to mild osmotic shock. Coldman and Good[12] have observed, using the haemolysis of erythrocytes as a test system, that glucose can increase the energy of activation necessary to convert water molecules to the free (disordered) state. The convulsant metrazol had the opposite effect. Phenobarbitone did not increase the energy of activation and the authors concluded that it must increase the degree of "apolar hydration" in order to account for its narcotic effect. Our work suggests that whatever direct physico-chemical effect phenobarbitone and other anticonvulsants have on the cell membrane may be supplemented by their effect of elevating the cell glucose concentration which, itself, could be involved in the activity of the drugs by influencing water structure at the membrane. This mechanism could be implicated in the biological activity of a variety of drugs.

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Effect of versicolorin C, rosenonolactone and cyclopiazonic acid on bovine pancreas deoxyribonuclease

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VERSICOLORIN C, a metabolite of *Aspergillus versicolor*,[1] rosenonolactone, a metabolite of *Trichothecium roseum* Link[2–4] and cyclopiazonic acid, a metabolite of *Penicillium cyclopium* Westling[5] are toxic compounds* of which at least the first two are potential carcinogens according to their chemical structures.

* I. F. H. Purchase, personal communication.

The activating effect of certain strong carcinogens on pancreatic deoxyribonuclease (deoxyribonucleate oligonucleotidohydrolase EC 3.1.4.5) has recently been reported.[6] The magnitude of activation seemed to depend on the strength of carcinogenicity[6] and may be caused by interaction with DNA or deoxyribonuclease. It was decided to study the effect of these compounds on deoxyribonuclease activity since at least two of them can be regarded as potential carcinogens.

Crystalline bovine pancreas deoxyribonuclease was obtained from Sigma Chemical Company, St. Louis, U.S.A., and highly polymerized calf thymus DNA from the British Drug Houses, Johannesburg. Versicolorin C was a gift from Mr. P. Aucamp and rosenonolactone as well as cyclopiazonic acid were supplied by Dr. C. W. Holzapfel, both from the National Chemical Research Laboratories, C.S.I.R., Pretoria.

The assay method for deoxyribonuclease activity was based on the change in absorption at 260 nm.[7] The concentrations of the different components of the reaction mixtures for the assay of deoxyribonuclease activity were: 85 mM sodium phosphate buffer (pH 6·65); 5 mM $MgCl_2$; 100 μg calf thymus DNA; varying concentrations of versicolorin C, rosenonolactone or cyclopiazonic acid (0–100 μM) in a final methanol concentration of 10% (v/v) and a final concentration of 0·15 mg deoxyribonuclease/ml in a total volume of 2 ml. This methanol concentration had no effect on the activity of the enzyme. The reaction was initiated by the addition of enzyme. The reaction mixtures were incubated at 37° in a temperature regulated waterbath equipped with a shaking device and shaken gently for 60 min. Thereafter 2 ml of ice-cold 1·4 M perchloric acid were added to each incubation mixture and they were then kept in ice for 30 min. The precipitates were removed by centrifugation at 20,000 *g* for 10 min at 0° and the absorption at 260 nm determined in a Beckman model DK-2A spectrophotometer. To correct for the absorption of the different mould metabolites added to the incubation mixtures, the absorption values of blank incubations (also incubated for 60 min and with similar composition as above except for the omission of DNA) at 260 nm were subtracted from those obtained for the reaction mixture to give the corrected 60 min Δ A260 nm values. Corrected zero time absorption values (260 nm) obtained in a similar manner from determinations with and without DNA in the incubation mixtures, were subtracted from the corrected 60-min Δ A260 nm values in order to obtain the final Δ A260 nm values for 60-min incubations. Deoxyribonuclease activity was expressed in terms of the final Δ A260 nm/hr.

TABLE 1. THE ACTIVATING EFFECT OF VERSICOLORIN C, ROSENONOLACTONE AND CYCLOPIAZONIC ACID ON PANCREATIC DEOXYRIBONUCLEASE ACTIVITY*

Metabolite	Final corrected Δ A260 nm/hr	Activation (%)
Versicolorin C	0·540	31·1
Rosenonolactone	0·471	14·3
Cyclopiazonic acid	0·454	10·2
Control	0·412	—

* The assay method for deoxyribonuclease activity is described in the text. The final concentration of each of the different compounds used was 100 μM.

The activation of pancreatic deoxyribonuclease activity by versicolorin C, rosenonolactone and cyclopiazonic acid at a concentration of 100 μM is given in Table 1. Versicolorin C was found to be the strongest activator (31 per cent) while cyclopianzonic acid was a weak activator of this enzyme. The dependence of the activating effect of the different compounds on concentration is illustrated in Fig. 1.

The interaction of these metabolites with DNA or deoxyribonuclease was investigated by means of difference spectroscopy in a Beckman Model DK-2A ratio recording spectrophotometer. The cuvettes used had an optical path length of 1 cm. In the cases of rosenonolactone and cyclopiazonic acid no significant results could be obtained due to the absorption of DNA and deoxyribonuclease in u.v. absorption region of these compounds. In the case of versicolorin C, however, significant binding to DNA and very weak binding to deoxyribonuclease were found (Fig. 2). Difference spectroscopy has been used to demonstrate the interactions of actinomycin D[8,9] nogalamycin,[10] aflatoxins,[11–13] anthracene and anthraquinone derivatives[14] etc., with DNA.

Strong carcinogens such as the butter yellow derivatives increased the activity of pancreatic deoxyribonuclease.[15,16] It is known that they bind to DNA.[17] Melzer[6] concluded from his results that certain strong carcinogens like β-butyrolactone and diepoxybutane had an activating effect on deoxyribonuclease whereas certain much weaker carcinogens like ascaridole had no effect, and other weak

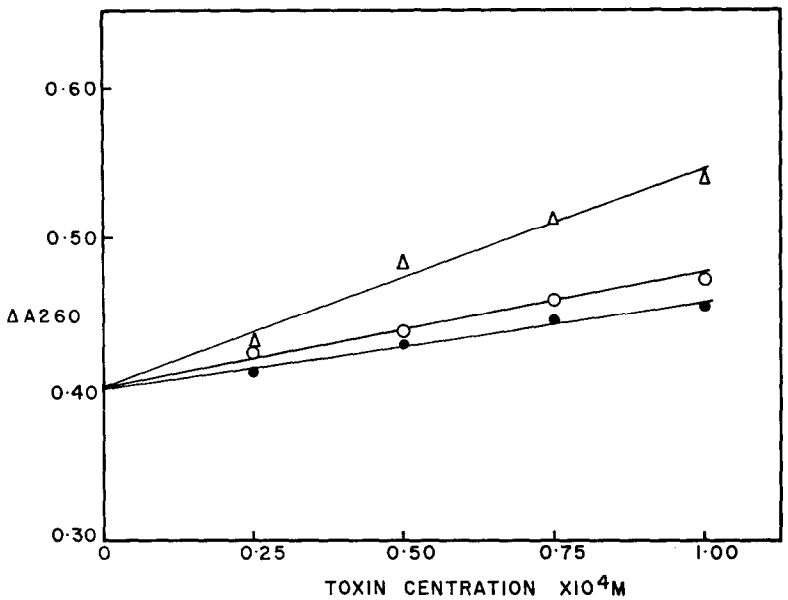

FIG. 1. The effect of concentration of versicolorin C (△) rosenonolactone (○) and cyclopiazonic acid (●) on their activation of pancreatic deoxyribonuclease activity. The assay method is given in the text.

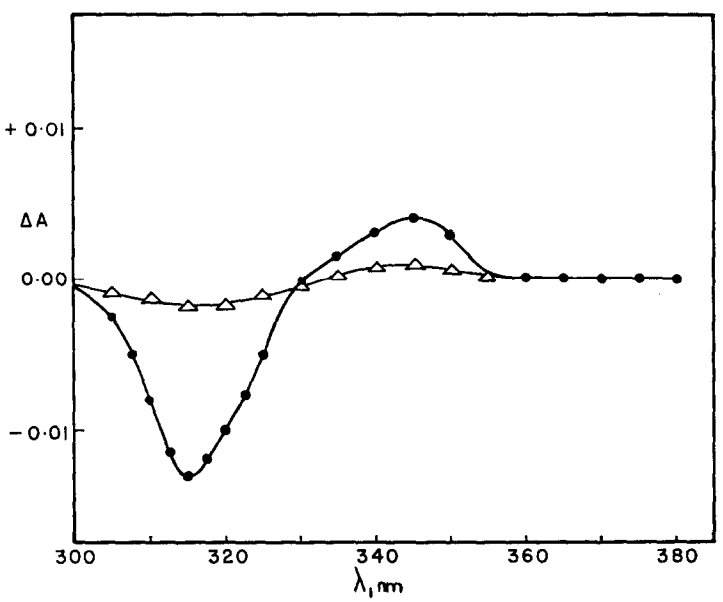

FIG. 2. Difference spectra of versicolorin C (16·7 μM) in the presence of 33 μg calf thymus DNA/ml (●) and 67 μg/ml pancreatic deoxyribonuclease (△). The very sensitive 95–105 per cent transmission range of a Beckman model DK-2A ratio recording spectrophotometer was used for these determinations. The cuvettes used had a 1 cm optical path length. The sample cell contained mixtures of versicolorin C and highly polymerized DNA or pancreatic deoxyribonuclease dissolved in 0·1 M sodium phosphate buffer (pH 6·65). The reference cell contained only versicolorin C in the above mentioned buffer.

carcinogens had an inhibitory effect on the activity of this enzyme. An inhibitory effect on pancreatic deoxyribonuclease activity by actinomycin D and nogalamycin was claimed to be due to their interaction with DNA.[18,19]. Their mode of interaction with DNA, however, differ very much from that of the aflatoxins and they also bind much more strongly to DNA than the aflatoxins do.[12] Aflatoxins B_1, B_2 and M_2 were found to activate pancreatic deoxyribonuclease activity most probably due to its binding to DNA.* Ts'o[20] stated that carcinogens entering into a living cell are more likely to interact with DNA than anything else. The activating effect of versicolorin C, rosenonolactone and cyclopiazonic acid on pancreatic deoxyribonuclease may possibly also be related to interaction with DNA.

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Tolerance to the hypnotic effect of *l*-methylphenobarbital induced by its nonhypnotic stereoisomer*

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RACEMIC methylphenobarbital N. F. (Mebaral, Winthrop; Prominal, Bayer) has been used for many years as an anticonvulsive and hypnotic. Knabe and Philipson[1] succeeded in resolving the compound in 1966. The effects of the two isomers in rats were then studied by Büch *et al.*[2] Only the levorotatory

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