

diphenyl-3,3'-diindolylmethane. Dahlbom and Misiorny were quick to reply³ to our unworthy suggestion, and in the light of further work to be described here, we offer our sincere apologies to these authors for suggesting that they had failed to perform a mixed melting point between 2-phenylindole and the product, m.p. 184–185°.

In the present work 2-phenylindole was refluxed in ethanol, containing a trace of hydrochloric acid, with ethyl orthoformate for 24 h. The product was 2,2'-diphenyl-3,3'-diindolylmethane which was isolated as its perchlorate (II) according to the procedure of Harley-Mason and Bu'Lock⁴. Crystallization from acetic acid afforded orange-red prisms, m.p. 289–290° (decomp.) (Found: C 69.83; H 4.34; N 5.58. Calc. for $C_{29}H_{21}O_4N_2Cl$: C 70.09; H 4.26; N 5.64.) This methene salt was hydrogenated in ethanol in the presence of platinum oxide. Evaporation of the filtered solution yielded I which was obtained as colorless needles from ethanol, m.p. 188–189° (Found: C 87.50; H 5.77; N 6.78. Calc. for $C_{29}H_{22}N_2$: C 87.40; H 5.57; N 7.03). The di-1,3,5-trinitrobenzene complex of 2,2'-diphenyl-3,3'-diindolylmethane was obtained as reddish brown needles from methanol, m.p. 161–162° (Found: C 59.86; H 3.62; N 13.82. Calc. for $C_{29}H_{22}N_2 \cdot 2C_6H_3O_6N_3$: C 59.71; H 3.42; N 13.59). In order to confirm that we had obtained authentic I, the compound m.p. 188–189° was N-methylated according to the procedure of Potts and Saxton⁵ to yield 1,1'-dimethyl-2,2'-diphenyl-3,3'-diindolylmethane, m.p. 185–186°, identical (mixed m.p., I.R. spectrum) with material previously obtained¹ by the self-condensation of 3-hydroxymethyl-1-methyl-2-phenylindole.

It is thus evident that Dahlbom and Misiorny obtained authentic I (the slight difference in our observed melting points for this compound is probably not significant) and it is remarkable that 2-phenylindole, 2,2'-diphenyl-3,3'-diindolylmethane and its 1,1'-dimethyl derivative should melt so close together. We have also reinvestigated the self-condensation of 3-hydroxymethyl-2-phenylindole. In agreement with our previous results we obtained 2-phenylindole when this compound was refluxed with 10 % sodium hydroxide. However, when exactly neutral or slightly acidic conditions were used the product was in fact the diindolylmethane I. We suspect that traces of alkali caused the formation of 2-phenylindole when 3-hydroxy-

methyl-2-phenylindole was refluxed with water in our previous experiments.

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Acid Soluble Nucleotide Linked Peptides in Extracts of Ascites Tumor Cells

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In a recent paper¹ it was reported that trichloroacetic acid (TCA) extracts of rabbit livers contained at least four ultra violet (UV) absorbing peaks with bound ninhydrin positive material. These four fractions were eluted immediately after adenosine diphosphate (ADP) and guanosine diphosphate (GDP) with a formic acid-ammonium formate system² from a Dowex 1 column. The positions on the elution curve of the UV-linked ninhydrin positive material were in the same region where we previously had found the uridine nucleotide bound peptides in *L. casei*³. Using a modification of the method of Strominger and Threnn⁴ it was possible to isolate from the four fractions ten UV-absorbing spots. Seven of these contained from three up to seven amino acids, some of them at least bound to uridylic acid⁵.

Since these data seemed to prove that animal tissues also contained acid soluble nucleotide bound peptides of a type previously only observed in extracts of different

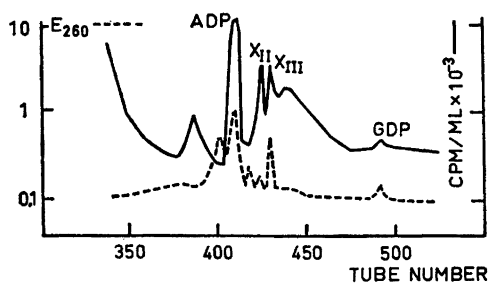


Fig. 1. Acid soluble nucleotides from 3.8×10^6 tetraploid tumor ascites cells. The nucleotides were separated on a 3×50 cm Dowex 1 formate column by gradient elution with reservoir content changed at the tubes numbered as follows: 72, 1 N formic acid; 164, 4 N formic acid; 390, 0.2 M ammonium formate + 4 N formic acid; 510, 0.4 M ammonium formate + 4 N formic acid. The continuous line represents radioactivity and the broken line represents optical density values at $260 \text{ m}\mu$ (E_{260}).

microorganisms it was of interest to look for them in other animal cells. In connection with other experiments the TCA-extracts of ascites tumor cells were chromatographed in the usual way². In Fig. 1 a section of an elution diagram is presented consisting of the peaks immediately before and after ADP. The tumor cells had been separated by counter-streaming centrifugation⁶ (by prof. Lindahl) into mainly tetraploid (3.8×10^6) and octaploid cells (10.9×10^6) and incubated for 30 min. with ³²P in relation to cell numbers. The TCA extract in Fig. 1 was from the tetraploid cells. The TCA extract from the octaploid cells qualitatively gave a similar elution diagram. In view of the low UV-absorbance values the XII and XIII peaks from the tetraploid cells were taken together with the corresponding peaks from the octaploid cells. After charcoal-absorption and elution less than 0.5 mg material was obtained from each of the combined XII

and XIII peaks. No attempt was therefore made to separate the material on an electrophero-chromatogram. After acid hydrolysis all material was put on each of two papers for two dimensional paper chromatography. Both papers showed stronger spots in positions corresponding to glycine, serine, glutamic acid and aspartic acid. Weaker spots were obtained in the positions of leucine or isoleucine, lysine, arginine and threonine. At least four more unidentified spots were also observed on both papers.

Preliminary estimations of the specific activity calculated as counts per minute per μmole of nucleotide compound gave the following values:

	XII	XIII
Tetraploid	40×10^5	8.9×10^5
Octaploid	11×10^5	3.0×10^5

The figures are of interest since the tetraploid cells are the more rapidly dividing of the two cell types⁷ and nucleotide linked peptides in microorganisms have been claimed as precursors in the formation of cell wall proteins⁸.

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