The bound copper was not removed upon lyophilization, heating, or treatment with 8 M urea. However, the copper could be removed, and the expected yield of DNP-aspartic acid obtained, by precipitation of the Cu-albumin with trichloroacetic acid.

On the basis of these results, it is suggested that the site of binding of the first mole of copper by bovine serum albumin is, at least in part, the free α-amino residue of the terminal aspartic acid. The binding is strong enough to resist attack by fluorodinitrobenzene at pH 9. Since free aspartic acid does not bind copper with the avidity shown by albumin\(^1\), the copper probably interacts also with other groups which are present in the proper spatial relationship.

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Spectrophotometric studies on the binding of acridine orange to ribonucleic acid and deoxyribonucleic acid

BEERS, Hendley and Steiner\(^1\) have reported the formation of two types of complexes between acridine orange and polyadenylic acid in solution. The dye–acid complex I was formed when the relative concentration of the acid to the dye was low and it showed an absorption spectrum with a peak at 465 m\(\mu\). The complex II observed at relatively high concentration of the polymer acid (ratio of its bases to dye 100:1) was associated with a different absorption spectrum with a peak at 502 m\(\mu\) and strong fluorescence in the green. Working with ribonucleic acid and deoxyribonucleic acid obtained from Micrococcus lysodeikticus, these authors observed that

Abbreviations: AO, acridine orange; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.
acridine orange formed a mixture of complexes I and II with RNA and only complex II with DNA. On the basis of these observations they have suggested that the failure of DNA to form complex I would provide a highly specific method for determination of DNA/RNA ratio in the mixture of these two nucleic acids. We have been carrying out similar experiments on the binding of AO to RNA and DNA and some of our results are reported here.

The acridine orange used in these experiments was "Grubler" stain manufactured by Chroma-Gesellschaft Schmid and Co., Stuttgart. The RNA was supplied by Nutritional Biochemical Corporation, Cleveland, U.S.A. The DNA from calf thymus was obtained from Dr. J. A. V. BUTLER's Laboratory, Chester Beatty Research Institute, London. The spectral absorption results were measured on a Beckman DU Spectrophotometer at room temperature (22°--25°). The binding studies were conducted at two different concentrations of AO viz. 0.001 % and 0.005 % at pH 7.4 for DNA and at pHs 6.3, 7.4 and 10.3 for RNA.

The changes observed in the spectral absorption of the dye (0.001 %) with varying amounts of RNA and DNA in solution at pH 7.4 are shown in Fig. 1. No qualitative differences in the formation of complexes I and II are seen between RNA and DNA. At relatively low concentrations of nucleic acid to the dye, the effect in both cases is to suppress the intensity of the AO band at 490 mμ. When the relative concentration of the acid goes above a certain critical range, the formation of complex II shown by a strong band at 502 mμ is evident in the mixture of AO and RNA as well as in that of AO and DNA. Both the solutions exhibited strong green fluorescence, whenever the formation of complex II was indicated from the spectral absorption data. The observations at higher concentration of the dye (0.005 %) are shown in Fig. 2. Allowing for some quantitative variation the results are essentially of the same nature.

PEACOCKE AND SKERRETT found two types of effects in their spectrophotometric study of the interaction of acridine derivatives with nucleic acids. They observed that the peak of the characteristic band of acridine derivatives was displaced to a higher wavelength in the mixtures of proflavine with DNA, acid-treated DNA, RNA and purine deoxyribonucleotides and in mixtures of 5-amino-acridine with DNA. On the other hand, only lowering of over-all intensity of the same band, with no shift in the peak wavelength was noted by them for the mixtures of proflavine with apurinic acid and with 5-amino-1,2,3,4-tetrahydroacridine with DNA. In the present work with AO, it appears that while the principal effect of the formation of complex I is to suppress the AO band at 490 mμ without much affecting its neighbouring band at 460 mμ, the effect of formation of complex II is to shift the 490 mμ band to 502 mμ. In such a case it would not be necessary to expect a clear peak at 460 mμ in order to indicate the presence of complex I. The appearance of a distinct peak at 460 mμ would depend upon (a) the relative intensities of the 460 mμ and 490 mμ bands of AO, a factor which is sensitive to pH and concentrations of AO as shown by ZANKER, and (b) the relative rates of formation of complexes I and II which determine the maximum depression at 490 mμ through formation of complex I, before the formation of complex II increases the spectral absorption in the same region. In our results there is a clear indication of suppression of the 490 mμ peak of AO at relatively low concentrations of DNA and of a displacement of wavelength when the critical range of the relative concentration of the acid has been exceeded. The same is also true for RNA. These observations are in conformity with those of

Fig. 1. Absorption spectra of acridine orange (concentration 0.001%) in veronal buffer, pH 7.4, with the admixture of nucleic acids.

Fig. 2. Absorption spectra of acridine orange (concentration 0.005%) in veronal buffer, pH 7.4, with the admixture of nucleic acids.

Peacocke and Skerret which showed no basic differences between the interactions of DNA and RNA with aminoacridines.

It appears to us that the differential behaviour between DNA and RNA observed by Beers et al. was chiefly due to the limited ranges of concentrations of the two nucleic acids used by these authors. We have shown in Table I the combined observations of Beers et al. pertaining to DNA and RNA and those from the present work tabulated in order of the NA/AO concentration ratios. A closer study of the data in this table suggests that if Beers et al. had used sufficiently low concentrations of DNA the formation of complex I with DNA would also have been observed by them.

<table>
<thead>
<tr>
<th>Complexes of acridine orange with DNA</th>
<th>Complexes of acridine orange with RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AO$ concentration (mg/ml)</td>
<td>Concentration ratio DNA/AO (w/w)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>5.0 $\times 10^{-2}$</td>
<td>0.10</td>
</tr>
<tr>
<td>1.0 $\times 10^{-2}$</td>
<td>0.25</td>
</tr>
<tr>
<td>5.0 $\times 10^{-4}$</td>
<td>0.40</td>
</tr>
<tr>
<td>1.0 $\times 10^{-2}$</td>
<td>0.50</td>
</tr>
<tr>
<td>5.0 $\times 10^{-2}$</td>
<td>1.0</td>
</tr>
<tr>
<td>1.0 $\times 2$</td>
<td>1.0</td>
</tr>
<tr>
<td>1.35 $\times 10^{-3}$</td>
<td>2.26</td>
</tr>
<tr>
<td>1.35 $\times 10^{-3}$</td>
<td>4.53</td>
</tr>
<tr>
<td>5.0 $\times 10^{-2}$</td>
<td>8.0</td>
</tr>
<tr>
<td>1.0 $\times 10^{-2}$</td>
<td>10.0</td>
</tr>
<tr>
<td>1.35 $\times 10^{-3}$</td>
<td>340</td>
</tr>
<tr>
<td>2.65 $\times 10^{-3}$</td>
<td>517</td>
</tr>
</tbody>
</table>

A, Beers, Hendley and Steiner; B, this work.

The fluorescence shift from green towards red shown by AO-stained chromatin matter of the cells exposed to an X-ray dose above about 2000–3000 R suggests that in the chromatin there exists some weakly bound structural lattice which keeps the local DNA concentrations so high that acridine orange can form complex II with it and at the critical dose of X-radiation this lattice is dissolved or so damaged that the local DNA concentrations are reduced and in consequence the equilibrium for AO-DNA binding is shifted from complex II to complex I.

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