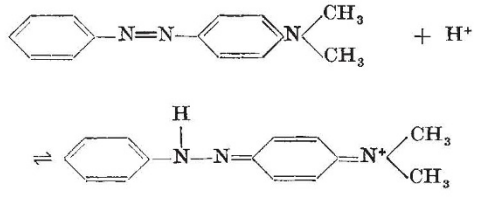
## 'Solubilization' of Carcinogens with Nucleic Acid Solutions. The Interaction of *p*-Dimethylaminoazobenzene with Ribonucleic Acids

Phenomena of 'solubilization' of carcinogens in nucleic acid solutions have been previously described[1,2]. For example, Boyland and Green[2] reported that native DNA solubilizes more benz(*a*)pyrene than heat-denaturated DNA does. A slight bathochromic effect of 10 nm and quenching of the fluorescence were taken, following Steele and Szent-Gyorgyi[3], as evidence of a molecular interaction. However, Giovanella, McKinney and Heidelberger[4] questioned the interpretation of these findings and claimed that they were entirely due to a phenomenon of stabilization of the colloidal suspension of benz(*a*)pyrene by the nucleic acids. They concluded that no non-enzymatic interaction between the carcinogenic hydrocarbons and DNA could be proved.

The general problem of a possible interaction between carcinogens and nucleic acids is of the utmost importance because of obvious genetic implications. It is shown here that there are cases in which such an interaction can be demonstrated clearly although part of the carcinogen is present under a micellar form.

The 'solubilization' of *p*-dimethylaminoazobenzene (butter yellow) in an aqueous solution of nucleic acids is easily achieved at temperatures in the range of 32°–45° C. If the incubation mixture is filtered either on Whatman No. 1 paper or on a fine porosity fritted glass, a liquid, coloured and optically void to the naked eye, is obtained. With most of the samples there is no opalescence and the filtrate has the same appearance as a solution of the dye in an organic solvent, except for its viscosity. However, the ultrafiltration of such a filtrate on a membrane of pore size 0·45 ± 0·02μ ('Millipore' membrane type *HA*) leaves a sediment of very fine yellow particles on the filter. This demonstrates that part of the butter yellow is under a micellar form. If such an experiment is made using a ribonucleic acid with few nucleotides (such as the 'Ribose Nucleic Acid' sold by Nutritional Biochemical Corporation), a red solution is obtained after filtration on Whatman No. 1 paper, but the sediment on the membrane of the ultrafilter is yellow.

It is known[5] that the molecule of butter yellow can exist in two forms, an azoid form which is yellow and a quinoid form which is red; with mineral acids and aliphatic acids of small chain-length, the two forms are part of a reversible equilibrium described by the equation:



It can be assumed, as was implied by Hartley[6] in his examination of long-chain salts on indicators, that the colours observed are indicative of the same electronic configuration as in a non-colloidal environment. Then, the observed colour change demonstrates the existence of an interaction between the carcinogen and the RNA used. All samples of RNA tested gave such a reaction which is practically independent of the ionic strength of the medium. Although the phenomenon is best demonstrated with RNA of low molecular weight, samples of highly polymerized RNA of yeast (Worthington, lot No. 6141) show a definite colour change.

Furthermore, it is possible to show a size-effect in the interaction between RNA and butter yellow. Table 1, which relates the sizes of the particles to the electronic configuration of the dye in the case of the filtrate obtained with Whatman No. 1 paper, leads one to suppose the existence of a correlation between the size of the particles and the electronic configuration of the dye, most, if not all, of the large particles being yellow and most, if not all, of the small ones red. In these conditions, the interaction involves only small micelles and dispersed molecules of butter yellow. This finding suggests the existence of a critical distance of approach between the reacting constituents.

Table 1. Correlation of sizes and colours; the sample used was an aqueous solution of 'Ribose Nucleic Acid' (Nutritional Biochemical Corporation) incubated with butter yellow and filtered on Whatman No. 1 paper

| | Sizes of the particles | |
|---|---|---|
| | 0·45 ± 0·02 | 0·45 ± 0·02 |
| Azoid colour | + | ? |
| Quinoid colour | ? | + |

The mechanism of interaction is at present unknown. Be it an intercalation of dispersed molecules of the dye in the molecule as Boyland and Green suggested for carcinogenic hydrocarbons in the case of DNA, or more related to micelle interaction, it seems that the concept of 'solubilization' must be revised. It is of interest to note, in this context, that Tobolsky and Ludwig[7] came to the same conclusion after their study of a system less complex from a physicochemical point of view.

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## Effect of Synnematin *B* on *Brucella*

In view of the high percentage of relapses in cases of brucellosis treated with the antibiotics now in use, we have examined the *in vitro* effect of synnematin *B* on *Brucella*. This antibiotic is unique among the penicillins in that it possesses remarkable activity against a narrow spectrum of Gram-negative organisms. Synnematin *B* has proved capable of controlling experimental infections in mice caused by *Salmonella* and *Proteus*[1–3] and natural infections in man[4–6]. Cultures of mouse fibroblast cells infected with *Salmonella typhosa* have been freed regularly of infection by treatment with synnematin[7]. Bacteriostasis of *Brucella* by 1–2 units/ml. of the antibiotic and bactericidal action against *Brucella abortus* with 100 units/ml. have been reported[8,9]. No other reports are known.

This communication reports the effect of synnematin *B* on *Brucella* in liquid and solid media and intracellular *Br. abortus* in bovine tissue cells. Additionally the effect of synnematin combined with tetracycline has been probed.

Synnematin *B* (327 units/mg) was supplied by the Michigan Department of Health. Typical, smooth virulent strains of *Brucella* were used. Strains 3076 and 1359