tures, the nucleus enlarges considerably, becoming concave. At the same time the cytoplasm is reduced considerably, but it persists in the mature sperm; both the nucleus and the residual cytoplasm form a nucleo-cytoplasmic cup.

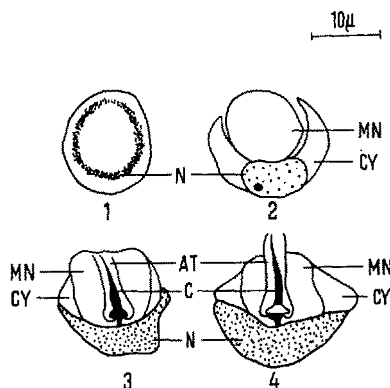
SCHRADER and LEUCHTENBERGER[4] find that the great dilution of DNA in the large cells of *Arvelius albopunctatus* makes the identification of the nucleus impossible in the later stages of spermateleosis after counter-staining with methyl green in the periodic acid-Schiff technique.

The failure of large-sized egg nuclei to stain with Feulgen dye may be due to the dilution of the dye below the concentration at which it is perceptible, as pointed out by MONNÉ and SLAUTERBACK[5] and ALFERT[6]. It may be mentioned that the sperm nucleus of *Clibanarius longitarsis* is inordinately large. ISHIDA[7] attributes the negative Feulgen reaction in histochemical preparations to the presence of some inhibiting substances and an extremely low content of DNA.

SCHRADER and LEUCHTENBERGER[8] conclude that decrease in DNA in salivary gland nuclei is the result of its utilization in the formation of secretory products.

However, the failure of Feulgen nucleal reaction in the sperm nucleus of *Clibanarius longitarsis* seems to be mainly due to the dispersion of DNA in the form of a large number of very fine granules or particles too small to enable recognition of violet stain, as also attributed by BRACHET[9] in the growing oocytes. These particles are easily recognizable with basic dyes and especially in the acid haematein test after pyridine extraction.

The presence of nuclear material in the form of granules in the sperm, as evidenced by the present light microscope investigations, is so far a unique phenomenon. In no organism studied so far has definite nuclear structure been found in mature spermatozoa, the nuclei appearing dense and homogeneous[10]. Other decapods under investigation, including *Diogenes miles*, a hermit crab, possess a homogeneously Feulgen positive nucleus[11].



Figs. 1-4. Stages in Spermatogenesis of *Clibanarius longitarsis*. (1) Primary spermatocyte-nucleus is Feulgen positive. (2) Spermatid-nucleus is Feulgen *negative*. (3) Sperm-nucleus reveals positive granules with crystal violet. (4) Sperm-nucleus reveals positive granules in acid haematein test after pyridine extraction. CY = cytoplasm, N = nucleus, MN = mitochondrial nebenkern, C = tripartite centrosome, AT = axial tube.

Zusammenfassung. Die Desoxyribonucleinsäure (DNS) im Samenkern des Einsiedlerkrebses *Clibanarius longitarsis* lässt sich durch die Feulgen-Reaktion histochemisch nicht nachweisen.

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On the Interaction of 1-Alkyl Pyridinium Couples[1]

Solutions of 1-*n*-propyl-1, 4-dihydronicotinamide (*A*) and 1-*n*-propyl nicotinamide chloride (*B*) interact with each other. Such interaction is shown by the formation of a colored complex reversible upon dilution and an obligatory direct hydrogen exchange with no net oxidation reduction[2,3].

This process seems to be a property of 1-substituted pyridinium couples. Thus the biologically important oxido-reduction pairs, nicotinamide adenine dinucleotide (NAD^+-NADH) and nicotinamide adenine dinucleotide phosphate ($NADP^+$-NADPH), also exchange hydrogen directly and form a reversible colored complex. These complexes show featureless absorption spectra in the visible range 420 mμ to 600 mμ characteristic of charge transfer electronic transitions.

The association constant for the complex was determined from the equation described by FOSTER et al.[4].

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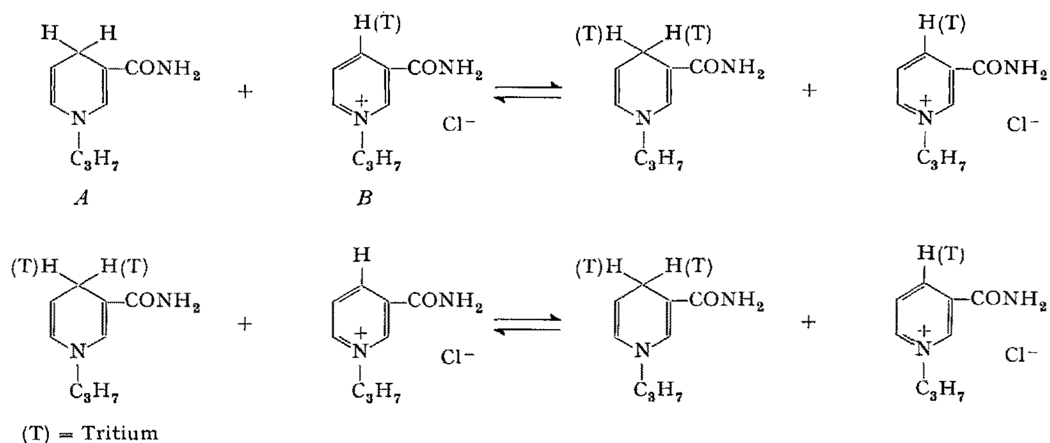

Following their description, the reaction and equilibrium constant are represented by equations (I) and (II), where AB_n is the complex.

(I) $A + {}_nB \rightleftharpoons AB_n$ (II) $K = \frac{(AB_n)}{(A)\,(B)^n}$

Assuming that only one species of complex molecule is formed, K is obtained from (III).

(III) $D/b^n = -KD + Ka_\varepsilon$

In (III), a is the initial concentration of A, b is the initial concentration of B, $b \gg a$, ε is the molar extinction coefficient of the complex and D is its optical density at a wavelength chosen where the optical densities of A and B are negligible. The value of $-K$ was obtained from the plot of D/b^n against D. Other methods for determining K where the concentration of each of the two components is varied were not suitable due to the high absorption intensity of A.

Table I presents data for the interaction of A and B. The concentration of $A(a)$ was kept constant at $0.01\,M$ and the concentration of $B(b)$ was varied from 0.1 to $1.0\,M$. All solutions were in $0.1\,M$ Tris buffer, pH 8.5. The optical densities were measured at 500 mμ immediately (less than 2 min) after mixing the solutions of A and B at room temperature (24°C). This was necessary because an irreversible colored product in addition to the complex appeared in the solution mixtures if they were allowed to stand at room temperature[3]. At 500 mμ the individual absorption intensities of A and B are negligible. The formation of 1:1 complex was demonstrated from the linearity (unique for $n = 1$) of the plot D/b against D yielding $K = 0.80$ and $\varepsilon = 89$.

The effect of temperature on the formation of the complex was also studied. Table II shows that upon increasing the temperature the absorption intensity decreases; this temperature effect was reversible.

Attempts to determine the association constant for the interaction of NAD^+ and NADH did not succeed. The failure to define K for this system was due largely to the uncertainties of the concentrations, purity, and high molecular weight of the components.

Table I

b, mole/l	D_{500}	D/b
0.1	0.067	0.670
0.2	0.124	0.620
0.4	0.215	0.538
0.6	0.291	0.485
0.8	0.352	0.440
1.0	0.410	0.410

Table II

Time (min)	Temperature	D_{500}
1	15°	0.264
3	25°	0.255
5	35°	0.242
7	15°	0.265
10	35°	0.243

Optical density recorded after mixing equal volumes of A ($0.02\,M$) and B ($1.0\,M$). Changes in temperature were applied to the same solution sample.

Résumé. Les solutions de 1-*n*-propyle-1, 4-dihydronicotinamide (A) et de 1-*n*-propyle nicotinamide chloride (B) ont la faculté de présenter des interactions. Cette faculté est demontrée par la formation d'un complexe coloré, réversible suivant dilution et échange obligatoire direct d'hydrogène, sans réduction nette d'oxygène. Le facteur de dissociation du complexe et l'effet de trempage sur la stabilité du complexe sont discutés ici.

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