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## Selective Deamination of Nucleosides by 2,4-Dinitrophenyl Hydrazine

NUCLEOPHILIC substitution by semicarbazide in cytidine and its derivatives has been reported[1]. Such alterations of nucleic acid bases is an essential preliminary to the formation of synthetic "genetic messengers" to serve in the chemotherapy of growth abnormalities. Generally, methods for the synthesis of derivatives of nucleosides are of wide applicability to genetic problems. There is particular chemical interest in the reactions of dinitrophenyl hydrazine with nucleosides because the reagent replaces, in a specific manner, amino groups without respect to their position in a pyrimidine or purine ring.

A solution containing 30 mg of 2,4-dinitrophenyl hydrazine in 50 ml. of ethanol with 1 per cent hydrochloric acid and 50 mg of nucleoside was stirred for 24 h at room temperature (22° C). The solvent was removed, *in vacuo*, and the residue washed three times with ether. The residue was then recrystallized twice from a mixture of ethanol and water. Analysis was made using infra-red spectroscopy (Beckman *IR*-8) and ultra-violet spectroscopy (Beckman *DB*). Melting point temperatures were obtained on a micro-Koefler stage (Arthur H. Thomas) and molecular weights were determined by Rast's method using camphor[2].

Derivatives of adenosine, guanosine and cytidine were formed (Table 1). Uridine was unreactive in these experimental conditions.

Table 1. CHARACTERISTICS OF COMPOUNDS INVESTIGATED.

| | Melting point (° C) | $\lambda_{max}$ (mμ) | Molecular weight | Colour |
|---|---|---|---|---|
| Adenosine | 229 | 257 | 267 | white |
| 4 Deamino, 4 dinitro (2,4) phenyl hydrazine | | | | |
| Adenosine | 174 | 262 | 447 | orange-yellow |
| Guanosine | 230–235 | 250 | 283 | white |
| 2 Deamino, 2 dinitro (2,4) phenyl hydrazine | | | | |
| Guanosine | 197 | 260 | 463 | yellow |
| Cytidine | 230–231 | 270 | 243 | white |
| 4 Deamino, 4 dinitro (2,4) phenyl hydrazine | | | | |
| Cytidine | 206 | 279 | 423 | olive green |

Infra-red spectra of the parent compounds showed two double peaks within the range 3,570 to 3,226 $cm^{-1}$. These were interpreted to represent N—H stretching vibrations. N—H bending vibrations appeared at 1,640 $cm^{-1}$ and C—N vibrations at 1,280 $cm^{-1}$. These are all characteristic absorptions for a primary amino group. Spectra of the derivative compounds showed the N—H vibration at 3,448 $cm^{-1}$, N—H bending at 1,610 $cm^{-1}$ and C—N vibrations at 1,315 $cm^{-1}$. These shifts indicate the absence of a primary amino group and the presence of a secondary amino group in the derivatives.

Derivatives also showed absorptions at 1,408 $cm^{-1}$ (nitro group vibrations), 735 $cm^{-1}$ and 704 $cm^{-1}$ (C—H stretching in substituted aromatic rings). Ring vibrations characteristic of substituted pyrimidine and purine ring appeared at 820 $cm^{-1}$ and 980 $cm^{-1}$, respectively, in both parent compounds and the derivatives.

Spectral shifts in the ultra-violet seen when derivatives were compared with the parent compounds were of the same magnitude as that reported by Hayatsu *et al.*[1] for the semicarbazide derivative of cytidine.

The possibility of hydrazone formation or of elimination reactions through *cyclo*purine and *cyclo*pyrimidine[3] as alternative possible reactions has been eliminated by demonstration of the absence of primary amino groups in the derivative compounds. We suggest, therefore, that the reactions reported here are simple nucleophilic substitutions of the first order for which a probable mechanism is partial protonation followed by 2,4-dinitrophenyl hydrazine substitution.

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## Absence of RNA Synthesis in Shed Human Spermatozoa

THE spermatozoon, the carrier of the paternal inheritance, has a brief lifetime *in vitro*, during which it must be viable and motile until it has fulfilled its function of conjugating with the ovum. Mature, though non-motile, sperm can survive for many days in the male reproductive tract before being shed, and they may survive for up to 6 days in the female tract, according to recent observations[1]. On the other hand, *in vitro*, their viability, as judged by motility, is limited to a few hours. The metabolism of the sperm would therefore appear to be different in each of these several circumstances.

Several criteria are used to evaluate the fertility of human sperm: number, morphology and motility[2,3]. Of these factors, motility and the quality of motility appear to be the most significant[7]. When the sperm is not motile, the diagnosis is obvious, but the examining physician not infrequently encounters specimens of apparently adequate quality, including motility, which nevertheless fail to fertilize a normal female. Accordingly, it is necessary to find other criteria to explain these cases of infertility.

The metabolic activity of sperm in the epididymis appears to be very low. The inception of motility on ejaculation ushers in a comparatively high rate of expenditure of energy. The energy of motility of sperm is met by the utilization and conversion of substrates, as in other cells. Human sperm are essentially glycolytic; ATP and Embden–Meyerhof–Parnas enzymes have been found in sperm[4–6].

The energetic outlay for maintaining cellular function involves the conversion of substrates by enzymes. The enzymes, in turn, are normally regenerated on ribonucleic acid templates. In many circumstances, the rate of synthesis of RNA can be used as an indicator of anabolic activity. The rate of synthesis of RNA in viable biological specimens can be measured by autoradiography, after incubation with an appropriate labelled precursor. Cytidine is a precursor of RNA which can be detected by autoradiography when it is labelled with tritium.

Accordingly, a series of autoradiographic measurements of RNA synthesis in specimens of human semen were undertaken in an effort to determine whether or not resynthesis of metabolic enzymes does occur to any significant extent in sperm and, if so, whether or not variations in enzyme resynthesis, as measured by RNA

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