storia forestale documentata dai sedimenti di Monterosi, prendendo in considerazione solo tre specie, cioè *Abies*, *Quercus sessiflora* (specie di quercia favorita da ambiente più fresco), e altre specie di quercia di ambiente più temperato, raggruppate insieme. Come si può osservare il clima va sempre più migliorando dalla situazione tipo «tundra». La punta di *Abies* indica sempre un clima fresco ed umido che va evolvendo verso situazioni più calde con oscillazioni documentate dalle rispettive variazioni dei due tipi di quercia. Più di una interpretazione cronologica è possibile dei diagrammi, ed una definitiva sarà possibile quando in possesso di maggiori dati e di datazioni con il radiocarbonio. La più probabile, anche sulla base di una correlazione con un'altro diagramma pollinico della stessa regione[6], mi sembra quella che attribuisce la zona ad *Artemisia* al pieno «Wurm 3», l'oscillazione di 160 cm (Diagr. B) all'«Allerod»; il massimo di quella che possiamo chiamare «quercia inferiore» di 70–90 cm (Diagr. B) all'«Ipsotermico»[7].

Difficile dare una stima precisa dell'intervallo di tempo racchiuso nel deposito di Monterosi, ma secondo l'interpretazione data sopra dovrebbe essere di poco minore di 20000 anni.

I numerosi dati ricavati dalle analisi polliniche dei sedimenti di Monterosi saranno trattati in dettaglio in una futura nota. Sarà discusso il diagramma completo nel quadro della stratigrafia del Quaternario recente italiano, e molti altri fatti di un certo interesse, quali ad esempio la storia forestale della regione, l'evoluzione delle piante acquatiche del lago (di cui si può avere un'idea dal Diagr. A), etc. Inoltre per quanto riguardo l'archeologia, ritengo sposibile datare gli inizii della pratica dell'agricoltura nella regione. A questo proposito interessante è l'andamento del polline di *Plantago*[8], mostrato nel Diagr. E, e l'aumento percentuale che le specie erbacee mostrano nei 50 cm superiori del sedimento (Diagr. A).

Molto lavoro, sotto la direzione di G. E. HUTCHINSON della Yale University, USA, è in corso sui sedimenti di Monterosi (analisi chimiche, mineralogia della frazione argillosa, microfossili animali, diatomee, etc.). La correlazione dei diversi dati sembra poter fornire interessanti notizie sulla sedimentazione lacustre.

*Summary.* This note is a preliminary report on some research being carried out at present on the sediments of Monterosi, a volcanic lake of Central Italy, under the direction of Prof. G. E. HUTCHINSON, of Yale University, USA. Herein are given some data taken from the pollen analysis of cores of the lake's sediments. The first results seem promising for the stratigraphy of the recent Quaternary Age in Central Italy, and for other related problems.

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## Periodic Acid Oxidation of the Steroid 11β-Hydroxyl Group

The scope of oxidations with periodic acid has been reviewed by BOBBITT[1] but in steroid chemistry the use of this agent has been almost completely limited to the oxidation of glycol or ketol structures. During the course of other work in which cortisol was being oxidized with periodic acid it was noticed that in addition to the expected product, 11β-hydroxyandrost-4-ene-3,17-dione, there was a less polar compound which behaved chromatographically as androst-4-ene-3,11,17-trione. Further investigation has shown that it is possible to achieve with periodic acid a selective oxidation of the 11β-hydroxyl group in the presence of other isolated hydroxyl groups.

The scope of the oxidation was investigated on a micro scale by adding 1 ml aqueous periodic acid solutions (0.18, 0.135, 0.09, 0.045, 0.0225 *M*) to 20 μg steroid in 0.1 ml methanol and allowing the reaction to take place over 24 h in the dark at room temperature. The oxidation products were extracted and examined by paper chromatography. Table I shows the concentration of periodic acid required for 50% conversion of the 11β-hydroxyl group of various steroids and also shows that, as expected, the 11α-hydroxyl group was much more resistant to attack. Table II shows that hydroxyl groups at other positions in the steroid nucleus were only slowly attacked with high concentrations of periodic acid, a detectable change being

[1] J. M. BOBBITT, Adv. Carb. Chem. (New York) *11*, 1 (1956).

Tab. I

| Steroid | Molarity of periodic acid for 50% conversion to the 11 oxo-compound |
|---|---|
| 11β-Hydroxyandrost-4-ene-3,17-dione | 0.045 |
| 11β,17β-Dihydroxyandrost-4-en-3-one | 0.045 |
| 11β-Hydroxypregn-4-ene-3,20-dione | 0.045 |
| 11α-Hydroxyandrost-4-ene-3,17-dione | 0.135 |
| 11β-Hydroxyandrost-1,4-diene-3,17-dione | 0.09 |
| 9α-Fluoro-11β,17β-dihydroxy-17α-methylandrost-4-ene-3-one | > 0.18 |
| 3α,11β-Dihydroxy-5α-androstan-17-one | 0.023 |
| 3α,11β-Dihydroxy-5β-androstan-17-one | 0.023 |

Tab. II

| Steroid | Molarity of periodic acid for any change to be detected |
|---|---|
| 6β-Hydroxyandrost-4-ene-3,17-dione | 0.18 *M* |
| 3β,6β-Dihydroxy-5α-cholestane | no change at 0.18 *M* |
| 3α,7α,12α-Trihydroxy-5β-cholanic acid | no change at 0.18 *M* |
| 11β,17β-Dihydroxyandrost-4-en-3-one (17β-OH → 17-oxo) | 0.09 *M* |
| 3α,11β-Dihydroxy-5α-androstan-17-one (3α-OH → 3C=O) | 0.09 *M* |
| 3α,11β-Dihydroxy-5β-androstan-17-one (3α-OH → 3C=O) | 0.09 *M* |
| 3-Methoxy-16β-hydroxyoestra-1,3,5(10)-triene (16β-OH → 16C=O) | 0.18 *M* |

5% conversion of the starting material. Similar results using chromic acid oxidation were obtained by SCHREIBER and ESCHENMOSER[2]. Oestrogens, unless methylated at position 3, were attacked, presumably in ring A[3] and dehydroepiandrosterone was also oxidized; it has been shown[4] that periodate will attack the $\Delta^5$ bond of cholesterol with the formation of the 5α, 6β-glycol.

The rate of oxidation of the 11β-hydroxyl group was increased by increasing the concentration of methanol in the reaction mixture but decreased by ethanol. It has been shown that methanol decreases the pH of periodic acid solutions[5]. The rate of reaction at pH 4.5 was less than one tenth of that at pH 1.6, the pH of 0.09 *M* periodic acid.

To obtain conclusive evidence of the structure of the oxidation product of 11β-hydroxyandrostenedione, 8.6 mg of this steroid in 43 ml methanol were oxidized with 430 ml 0.09 *M* periodic acid solution in the dark at room temperature for 24 h. The oxidation product was separated from a small amount of unreacted steroid by chromatography on Celite using the solvent system toluene, light petroleum, methanol, water (6.6:3.3:8:2) and recrystallised from ethanol-light petroleum. The crystalline product had m.p. 220–223° (evac. tube) which was not depressed by admixture with authentic androst-4-ene-3, 11, 17-trione, and had similar ultra violet and sulphuric acid adsorption spectra to androst-4-ene-3, 11, 17-trione.

It is interesting to note (Table I) that the introduction of certain substituents into 11β-hydroxyandrost-4-ene-3, 17-dione stabilised the 11β-hydroxyl group towards oxidation with periodic acid. The introduction into the cortisol molecule of these substituents increases the biological activity[6] and plasma half life of the compound[7,8].

*Zusammenfassung.* Die 11β-Hydroxylgruppe im Steroidmolekül kann selektiv mit Perjodsäure oxydiert werden. Ihre Oxydationsgeschwindigkeit wurde von der Gegenwart substituierender Gruppen im Molekül beeinflusst.

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*Clinical Endocrinology Research Unit (Medical Research Council), University of Edinburgh (Scotland), February 8, 1961.*

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## Two Types of Ribonucleic Protein in the Nucleolus of Intestinal Carcinoma in the Newt *Triturus alpestris* Following Injection of Herring Sperm Deoxyribonucleic Acid

Recently, we demonstrated two types of ribonucleoprotein in the nucleolus of tumour cells in the squamous-cell carcinoma of the lizard *Lacerta agilis*[1]. To establish whether a more general significance should be attached to this result, we performed a corresponding investigation with the experimental carcinoma of the intestine in the newt *Triturus alpestris*, following injection of herring sperm deoxyribonucleic acid (injection for eight days with 0.1 ml of 5% solution)[2].

After sacrificing the animals on the 40th day of the experiment, the nucleolar differentiation of the tumour cells was studied by the toluidine blue-molybdate method[3,4]. As in *Lacerta*[1], in this way the ribonucleoprotein of the nuclear sap or parachromatin was coloured deeply purple, the nucleolus partly assuming a green colour. Also in *Triturus*, the existence of two types of ribonucleoprotein, if not actually demonstrated, had thus become highly probable. In the nucleoli, a variable quantity of metachromatic ribonucleoprotein as well as a number of very small vacuoles appeared to be present, remaining unstained by the method referred to. In the interphase cells the metachromatic ribonucleoprotein was most distinct in the form of small granules, hollow spheres or clusters of hollow spheres. Phase-microscopically, the nucleoli of the living tumour cells contained numerous very small vacuoles.

Comparing the nucleolus in the toluidine blue-molybdate stained sections with that in the living tumour cell, it could be ascertained that the metachromatic ribonucleoprotein was situated around or in some very small vacuoles. After a pretreatment with ribonuclease or hot trichloroacetic acid[5], this nucleolar ribonucleoprotein was not coloured. In all phases of staining by the toluidine blue-molybdate method, staining could be obtained in the same way as the nuclear parachromatin. Following LOVE and BHARADNAJ[3,4], in this intestinal tumour of *Triturus* therefore it can also be called the nucleolar parachromatin. Just as in the squamous-cell carcinoma of the lizard and the Ehrlich ascites tumour[4], the tumour cells in *Triturus* often persisted throughout the mitosis, without being incorporated in the daughter nucleus. During mitosis the nucleolar parachromatin exhibited a different localization: in the early prophase it was frequently situated at the rim of the nucleolus, disappearing from view during the latter prophase to become clearly visible again in the metaphase, the anaphase and the telophase as a vacuolated nucleolus green-coloured in the toluidine blue-molybdate sections.

LOVE[3] suggested that the nucleolar parachromatin is probably extruded from the nucleolus during the prophase to contribute to the accumulation of the granular parachromatin, which takes place in this period. The data obtained for the *Triturus* tumour may certainly be an argument for this reasoning. The earliest symptom of reformation of the nucleolus in the daughter nucleus was a metachromatic inclusion, afterwards acquiring an outer coating of ribonucleoprotein, staining green in the toluidine blue-molybdate sections, which could be vacuolated as well as amorphous. This nucleolar differentiation of the tumour cells in *Triturus* is undoubtedly related to the special structures known for a long time already in the nucleolus.

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