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 Δ^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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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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The relationship of the two types of ribonucleoprotein in the nucleolus to the nucleolini[6–8] and the nucleonemata[8] could also be solved with the data obtained for the intestinal tumour of *Triturus*. Also in the tumour cells of *Triturus*, hollow and solid structures resembling the true nucleolini could be demonstrated after staining with iron haematoxylin. The physiochemical differences in ribonucleoprotein, determining differential staining by the toluidine blue-molybdate method, may therefore also be responsible for some differences in the affinity for the haematoxylin. As these results are in good agreement with those obtained in the Ehrlich ascites tumour cells[4] and the squamous-cell carcinoma of *Lacerta*, it would therefore seem that they are certainly of general importance.

Zusammenfassung. Durch cytologische Untersuchungen von Tumorzellen des experimentell beim Molch *Triturus alpestris* erzeugten Darmcarcinoms kann gezeigt werden, das der Nucleolus dieser Tumorzellen zwei Typen von Ribonucleoprotein enthält.

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Bone Mass Changes in Hens Observed *in vivo* During the Egg Laying Cycle

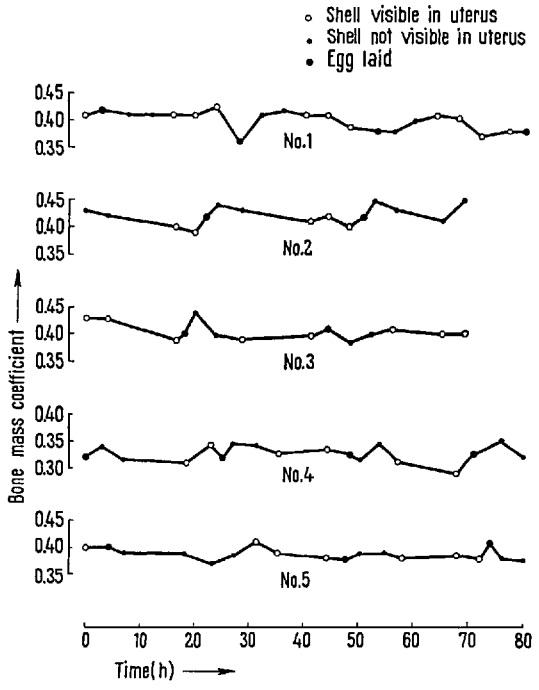
The medullary bone of birds has been observed to undergo marked changes in mineral content and cellular elements in association with the egg laying cycle[1]. Bloom et al.[2] who studied these changes in laying chickens with histological methods noted that there was no correlation between the amount of medullary bone and the position of the egg in the reproductive tract; the observations were terminal ones made on each of a series of birds. The histology of the medullary bone was highly variable among the hens at each stage of the laying cycle, and therefore the magnitude of the mineral changes in a given bird could not be assessed.

Serial quantitative roentgenography is a method for studying the bone changes in a given hen during an egg-laying cycle. Individual patterns of bone formation and destruction can be studied in individual birds over extended periods of time. The technique of quantitative roentgenography has been shown to give a reliable estimate of the bone mineral content or bone density in live rats[3]. In the present study, cyclic changes in the bone mineral content of laying hens are described by employing this technique.

Five white leghorn hens about 18 months of age were kept in individual cages and fed a standard breeder mash. Each hen was roentgenographed several times a day for at least three days. Whenever possible, x-ray films were also taken at the time of egg laying. The films were taken in the following manner. An unanesthetized hen was placed on its left side on a cardboard cassette containing an $8 \times 10''$ no-screen film. The left leg was extended and constrained with a clamp. An aluminum-zinc alloy calibration wedge was placed next to the leg and an exposure was made at 55 KVP, 20 MAS and a focal point to film distance of 48 inches. After development, a line across the midpoint of the tibia-tarsus image was selected for the determination of the mineral content at that site by densitometric analysis of the film. The value obtained as a result of this analysis is termed the bone mass coefficient which represents the mass of the aluminum-zinc alloy that would absorb the same amount of x-radiation as the bone site traced. The bone mass coefficient was found to be proportional to the weight of the bone slice scanned[3]. The bones were evaluated twice for each film and the average bone mass coefficient was used as the final value.

To determine the error in the procedure, each of the three control hens was x-rayed ten times in close succession at the end of the experiment. The mean bone mass coefficient, the standard deviation and the range for each hen was 0.27 ± 0.009 (0.26–0.29); 0.40 ± 0.008 (0.39–0.41); and 0.49 ± 0.012 (0.47–0.52). These standard deviations and ranges are almost 2 times smaller than those of the experimental birds whose values are as follows: (1) 0.40 ± 0.018 (0.34–0.43); (2) 0.42 ± 0.017 (0.39–0.45); (3) 0.41 ± 0.016 (0.39–0.44); (4) 0.33 ± 0.016 (0.29–0.35); (5) 0.39 ± 0.011 (0.37–0.41). The magnitude of the standard deviations in the experimental birds probably reflects the rise and fall in medullary bone mineral associated with the egg laying cycle.

Examination of the record of bone mass coefficient versus time for the five hens (Figure) indicates that the bone mineral content (indicated as bone mass coefficient)



The bone mass coefficient records of hens observed for two or more egg laying cycles.

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