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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Department of Histology, Free University, Amsterdam (The Netherlands), February 10, 1961.

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

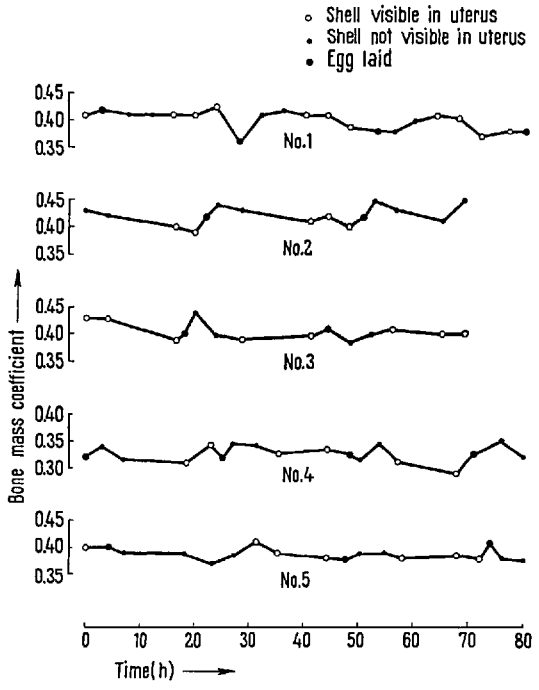
The medullary bone of birds has been observed to undergo marked changes in mineral content and cellular elements in association with the egg laying cycle[1]. Bloom et al.[2] who studied these changes in laying chickens with histological methods noted that there was no correlation between the amount of 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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varies during a laying cycle. The pattern of these changes is not always consistent for the same hen and varies between animals. For hen number 1 the bone mass values decreased as the egg shell calcified. Ovulation of the next egg which usually occurs about $^1/_2$ h after an egg is laid[4], was generally followed by an increase in bone mass suggesting that a stimulus for medullary bone formation occurred possibly associated with estrogen release. The pattern for this hen appeared to be regular. The patterns exhibited by hens 2 and 3 also show a rise in bone mass near the time of ovulation with a leveling off or decline in value as shell calcification proceeds. The pattern demonstrated by hen number 4 is not so clear although shell calcification appears to occur when the bone mass values are low; this hen also had the lowest bone mass values. Hen number 5 did not lay on the day following the first egg. The bone mass decreased apparently until ovulation occurred for the next egg when rising values were noted preceeding shell formation. The accumulated bone mineral then seemed to decrease as shell formation proceeded. The second egg was laid at a low point followed by slightly increasing values coincident with the time of ovulation of the next egg. The third egg was laid at a peak. Either bone formation took place at this time for reasons that are not presently obvious or an error in technique occurred since ovulation evident from egg laying records did not occur for at least 15 h more; this hen also showed the smallest variability in bone mass values during the period of observation (see standard deviation ranges above).

While the number of hens used in this study are insufficient for statistical treatment they seem to confirm the results of BLOOM et al.[2] who found no correlation between the extent of medullary bone calcification and the position of the egg in the oviduct.

The technique of quantitative roentgenography is particularly useful for studying those physiological and nutritional factors associated with skeletal metabolism. In applying this technique to avain studies in the future, certain facts relating to calcium metabolism in birds appear important. For example, it has been observed that certain chickens lay eggs with thick shells while others lay thin shelled eggs. This may be indicative of individual differences in the mobilization of bone calcium for shell production[5]. Species which lay clutches of eggs spaced so that the events of the laying cycle are clearly distinguishable might produce more regular bone mass patterns[6].

Zusammenfassung. Der Mineralstoffgehalt der Tibia-Fibula von Legehennen, mit quantitativer Röntgenographie *in vivo* bestimmt, scheint während des Legezyklus mehr zu variieren als auf Grund des Fehlers der Methode erwartet werden kann. Eine Korrelation zwischen diesen Änderungen in der Knochenmasse und der Position des Eies im Eileiter konnte bisher nicht gefunden werden.

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(+) Lupanine in *Lupinus hilarianus* Benth.

DEULOFEU and GATTI[1] isolated (+) hydroxylupanine from the seeds of *L. hilarianus* Benth. This alkaloid seemed to be the only base present in this part of the plant. A re-investigation of the alkaloids present in seeds harvested in December 1959 near the city of Santa Fé (Argentina) permitted again the isolation of a fairly large amount of (+) hydroxylupanine.

When the ether mother liquors remaining after the separation of this base were essayed on ascending paper chromatography on Whatman 1, employing butanol:acetic acid:water (80:3:17) as mobile phase, two spots were detected employing Dragendorff reagent. One, with Rf 0.29, corresponds to hydroxy-lupanine; and the other, with Rf 0.41, to another base, present in a very small amount.

Evaporation of the solution gave a residue that was chromatographed on a column of alumina (Woelm, grade III). Elution with a mixture of benzene-cyclohexane (50:50) gave the base with Rf 0.41. Elution of the hydroxylupanine was effected by using a larger amount of benzene in the mixture (70:30).

The base giving the spot with Rf 0.41 was identified as (+) lupanine. On evaporation of the solution, a syrup was obtained from which the following derivatives could be prepared: perchlorate, m.p. 211–213° $(\alpha)_D^{25°}$ + 40.0° (*c*, 0.3 ethanol); hydriodide m.p. 188–189°; methiodide m.p. 256–257°; all values in agreement with those given in the literature[2]. The m.p. of the perchlorate was not depressed when mixed with an authentic sample of (+) lupanine perchlorate. The Rf 0.41 was also given by the authentic base.

It is interesting that all species of *Lupinus*, seven in total[2], in which (+) hydroxylupanine has been found, contain also (+) lupanine, both bases having the same stereochemical configuration in carbons 6 and 11. *L. albus* is the only species from which, in addition, the stereoisomeric (–) lupanine has been isolated as such[3]; the latter isomer is present in form of the racemate also in the seeds of *L. angustifolius*[4].

Zusammenfassung. (+)-Lupanin wurde aus den Samen der *L. hilarianus* Benth. isoliert.

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