How heavily does the hen sit on her eggs during incubation?

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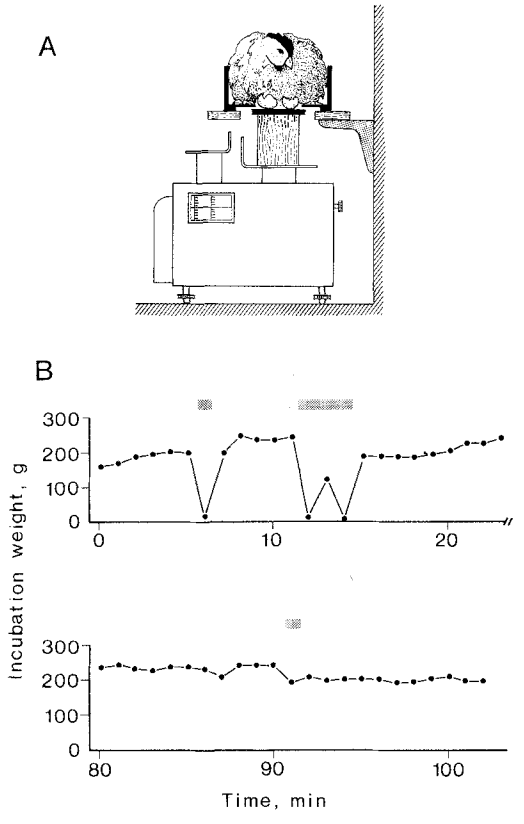
Summary. Incubating hens do not sit with their entire body weight on the eggs. The weight applied to the clutch is carefully regulated and depends on the number of eggs in the nest. Sensory input from the thoracic skin (brood patch) of the incubating hen appears to be important for controlling the tightness of sit.
Key words. Incubation behavior; brood patch; sensory nerves.

The high temperature needed for development of avian embryos is in most species secured by the close contact between the eggs and the naked brood patch of one of the parent birds[1]. Experiments with birds incubating cold eggs indicate that the heat required for incubation is produced by shivering[2] and is delivered to the brood patch by way of the circulating blood[3,4]. In addition to this physiological response, birds regulate the temperature of their eggs behaviorally by varying the time spent on the nest and by adjusting the tightness of sit[5–8]. The incubating bird is apparently faced with the conflicting demands of creating proper contact with the eggs and not pressing so hard that the pressure on the skin interferes with the blood flow in the brood patch. It might therefore be speculated that the incubating bird regulates the pressure applied to the eggs. However, information about how heavily birds sit on their eggs is not available from the literature. In this paper, a simple technique for recording the actual weight that a hen applies to the eggs during incubation is described. The results suggest that the weight is regulated and it appears that the pressure on the brood patch is below that which would be required to compress the blood vessels.

The experiments were performed with two bantam hens *(Gallus g. domesticus)* with body weights of 610 g and 630 g, respectively. The nests were made of plastic buckets placed in cardboard boxes, and the hens were kept in the incubating state by changing their eggs near to the time of hatching. The experimental nest was similar to the hens' own nests, except that it was located on a shelf above an ordinary post-office balance (fig. A). The bottom of the nest was provided with a hole that fitted around the clutch so that the eggs rested on a platform that was mounted directly on the balance. In this way, the hen rested with her feet on either side of the eggs and only the weight that she applied to the eggs was transmitted to the balance. The hens did not appear to be disturbed by the slight vertical movement of the eggs on the balance or the close presence of the observer. The weight was read off with an accuracy of ± 5 g at 1-min intervals and the behavior of the hen was noted. The values given represent means (± SD) of periods of at least 5 min of steady incubation in between resettling movements of the hen. Means have been compared by Student's t-test.

Recordings of the incubation weight from an experiment where a hen was on the nest for nearly 2 h are shown in figure B. The weight that the hen applied to the eggs appears to be regulated around 200 g, and it oscillated slightly (± 10 g) in phase with the respiratory movements (increase during inspiration). During preening, the hen lost contact with the eggs in most cases, although she hardly rose on the nest. If the hen was approached with a hand the weight similarly dropped to zero, but returned to the normal value when the hand was retracted. The weight that the hens applied to 4 eggs with normal incubation temperatures was 245 ± 45 g (n = 9), which is about 40% of their body weight. When the clutch size was reduced to 2 eggs or 1 egg, the incubation weight decreased significantly ($p < 0.01$) to 180 ± 45 g (n = 10) and 154 ± 69 (n = 9), respectively. With 4 cold eggs (ca 6 °C) in the nest, the hens were sitting with a weight of 251 ± 29 g (n = 6) which is not significantly different from the value found with 4 eggs that have normal incubation temperatures. Unfortunately, it was not possible to increase the number of eggs to more than 4 since the hens' toes tended to slip down the hole if this was further enlarged.

The decrease in the tightness of sit that was observed when the number of eggs was reduced could be considered as an attempt of the hen to keep the pressure per cm^2 brood patch constant. Interestingly, the incubation weight appeared to decrease roughly in proportion to the number of eggs that were removed. Assuming that each egg has a contact surface of 3 cm^2 with the brood patch, the incubation weight recorded with 1, 2, and 4 eggs in the nest corresponds to 51, 30, and 20 g per cm^2. Obviously, the hens did not succeed in keeping the pressure constant, and the low incubation weight recorded with few eggs in the nest could merely reflect difficulties in sitting on a reduced clutch. Indeed, the hens appeared to be more restless and the weight fluctuated more when they were incubating single eggs.



A Experimental conditions for measuring incubation weight. The hen rests with her feet on either side of the clutch and only the pressure from the brood patch on the eggs is transferred to the balance. *B* Recordings of the weight applied to 4 eggs by an incubating bantam hen (body weight: 610 g). The bars indicate periods of feather preening.

Regarding the possibility of compression of the blood vessels in the brood patch, it is seen that, in the case of 4 eggs in the nest, the pressure per cm^2 on the skin equals 14.7 mm Hg, which is far below the ordinary capillary blood pressure of 30 mm Hg. So, during natural incubation in the domestic hen, where the clutch size usually exceeds 4 eggs, the pressure from sitting on the eggs should not interfere with the circulation and the delivery of heat to the brood patch.

In order to assess whether sensory input from the brood patch is involved in controlling the weight that the hen applies to the eggs during incubation, experiments with anesthetized brood patches were made. After intra-cutaneous injection of 1.5–2.0 ml 1% lidocaine along the lateral margins of the brood patch, the incubation weight recorded with 4 eggs in the nest was 293 ± 24 g ($n = 9$), which is significantly higher than the value recorded without local anesthesia ($p < 0.02$). Although cutaneous sensory receptors were blocked, the hens apparently did not lose complete control over the tightness of sit, which suggests that more deeply located receptors in the breast and perhaps also mechanoreceptors in the feet may be involved in the control.

The technique used in the present study was very simple, but the experiments have nevertheless provided some basic knowledge about the weight that the hen applies to her eggs during incubation. By using more elaborate experimental techniques, such as an automatic and continuous recording system, it should be possible to obtain information regarding changes in the tightness of sit during the entire incubation period, or more specifically around the time of hatching. Furthermore, by using artificial eggs, it could also be established whether the shape of the eggs or their surface architecture have any effect on the tightness of sit.

1 Drent, R., in: Avian Biology, vol. 5, pp. 333–420. Eds D.S. Farner and J.R. King. Academic Press, New York 1975.
2 Tøien, Ø., Aulie, A., and Steen, J.B., J. comp. Physiol. *B 156* (1986) 303.
3 Gabrielsen, G., and Steen, J.B., Acta physiol. scand. *107* (1979) 273.
4 Midtgård, U., Sejrsen, P., and Johansen, K., J. comp. Physiol. *B 155* (1985) 703.
5 Davies, S.D., Williams, J.B., Adams, W.J., and Brown, S.L., Auk *101* (1984) 556.
6 Drent, R.H., Postuma, K., and Joustra, T., Behaviour, Suppl. *17* (1970) 237.
7 Haftorn, S., Ornis scand. *10* (1979) 220.
8 White, F.N., and Kinney, J.L., Science *186* (1974) 107.

0014-4754/87/11/121232-02$1.50 + 0.20/0

Isolation of a hexaprenylhydroquinone sulfate from the marine sponge *Dysidea* sp. as an H,K-ATPase inhibitor[1]

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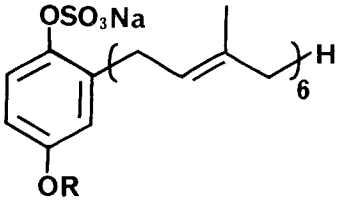
Summary. A hexaprenylhydroquinone sulfate has been isolated as an H,K-ATPase inhibitor from a marine sponge *Dysidea* sp. It also inhibited phospholipase A_2 as well as secretion of gastric acid in rats.
Key words. Marine sponge; *Dysidea*; hexaprenylhydroquinone sulfate; H,K-ATPase inhibitor; gastric secretion.

Little is known of pharmacologically active principles of marine origin, though a high incidence of activity has been reported in the extracts of marine organisms[2–4]. In the course of our search for bioactive substances from Japanese marine invertebrates, we found that the lipophilic extract of a sponge of the genus *Dysidea*, which was collected in Hachijo-jima Island of the Izu Archipelago, inhibited H,K-ATPase in an in vitro assay[5]. From the sponge we have isolated the active principle, which was identified as a hexaprenylhydroquinone sulfate.

The ethanol extract of the frozen sponge (1 kg) was partitioned between water and diethyl ether. The ether soluble materials (9.81 g) were chromatographed on a silica gel column with chloroform-methanol-water (88:12:1, followed by 90:20:1). The active fractions were collected and subjected to HPLC on a YMC-ODS column (Yamamura Chem. Co.) with 30% aq. CH_3CN to yield 144 mg of pure active compound (**1**) as a colorless gum.

Compound **1** had a molecular formula of $C_{36}H_{53}O_5SNa$ which was established by FABMS[$(m/z$ 643$(M + Na)^+$, 659$(M + K)^+$] as well as combustion analysis (C, 67.66; H, 8.32; S, 5.74%). The UV spectrum[λ_{max} 281nm(ε 2200)] was reminiscent of a hydroquinone chromophore[6], while the IR absorption at 1240 and 840 cm^{-1} indicated that it contained a sulfate moiety[7]. The ^{1}H-NMR and ^{13}C-NMR spectra implied the presence of a monosubstituted hydroquinone[δ 7.19(1H,d,J = 8.0Hz), 6.65 (1H,d,J = 3.0Hz) 6.55(1H,dd, J = 8.0,3.0Hz); 153.7s(C–4), 121.0s(C–2), 113.6d(C–5), 142.8s(C–1), 116.8d(C–3), 131.1d(C–6)][8], and an hexaprenyl moiety [δ 138.0s, 136.6s, 135.4s, 135.1s, 134.9s(2C), 124.3d(3C), 124.0d(2C), 123.1d, 39.8t(6C), 26.9t(5C), 25.7t, 17.7q, 16.0q(5C)][9]. These structural features were further established by EI mass fragment ions at m/z 518, 449, 381, 313, 245, 177 and 109. Detailed NMR studies including NOE and LSPD[10] experiments led to unambiguous assignment of



1 R = H
2 R = Me

the hexaprenylhydroquinone sulfate structure. To determine the position of the sulfate, **1** was treated with CH_3I and anhydrous potassium carbonate in acetone[8] to give rise to the methyl ether (**2**) which was studied by nuclear Overhauser enhancement difference spectroscopy. Irradiation of the O-methyl signal at δ 3.75(s, 3H) enhanced the *ortho* protons at C–3 (δ 6.70) and C–5 (δ 6.65), indicating that the sulfate must