

The vertical component of the coil's field in two-thirds of the coil's length adjacent to its middle cross-section is negligible and so is the transverse component.

Results. The behavior and building activity of hornets subjected to these magnetic fields was studied in 4 experiments. In the first three experiments the current inside the coil was 1.36 A which corresponds to 23.3 Oe, whereas in the fourth experiment the current in the coil was reduced to 0.1 A which corresponds to $H_c = 1.7$ Oe. Every experiment lasted 16 days, which was long enough to enable hornet workers to build a proper comb¹². Breeding boxes with 15–25 hornets in each were placed both inside the coil where the magnetic field is uniform and outside the coil where the magnetic field rapidly decreases with the distance from the coil's winding.

The hornets were fed sugar solution, pieces of codfish and hornet or honeybee pupae, and were provided with a clump of soil as building material. 3 ABBs with hornets were kept outside the magnetic field as control. In all 4 experiments, the adult hornets died within 4–5 days without performing any building. The juvenile hornets built at least one comb in each ABB, but in all the ABBs, they were almost motionless during the first 4–5 days, assembled mostly on the wall (or floor) closest to the coil's winding where the additional magnetic field was stronger. Building activity of the juveniles began 5–7 days after that of the control hornets. The results are summarized in the table.

In the ABB provided with an inverted comb, the hornets built upwards, constructing a stalk on the surface of the original comb with a knob-like comb on top of it (figure). The openings of the cells were oriented in all directions of

the horizontal plane. The queen oviposited in the inverted cells, and the workers attended the larvae eclosing in these cells. As the distance of the new cells from the coil's winding reached 7–8 cm, their openings gradually began to face downwards.

After-effect: Upon switching off the coil's current we placed inside the coil 3 incandescent lamps which dissipated about 70 W and restored the previous temperature. The hornets now continued to build cells whose openings were gradually oriented downwards.

Discussion. These findings suggest that the hornets are affected by an additional magnetic field. In nature, the hornets most probably build under the combined influence of both the vertical component of the earth's magnetic field and the gravitational force, whose directions coincide. An additional horizontal magnetic field is, for some unknown reason, lethal for the adult hornet and larvae. The juvenile hornets, although they are less active and start building later than usual, are capable of adapting to the additional magnetic field, whether uniform or non-uniform, and build combs with irregular cells, commencing in regions of high intensity but proceeding subsequently in the direction of decreasing field intensity. It should be remembered that by the time the hornets start building they are no longer juveniles, and their building is clearly not influenced by gravity force alone. It is not clear as yet why these hornets do not build while in their first days of life, as they usually do in an ordinary environment.

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Binding of indole-acetic acid to cytosol proteins of embryonic chicken liver

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Summary. ³H-Indole acetic acid bound to cytosol proteins of embryonic chicken liver in vitro to an average capacity of at least 20 pmoles/mg binding protein. The auxin-binding protein complexes could be resolved into 4 major zones by anion exchange chromatography; they sedimented at 3–7 S in sucrose density gradients, and were also heterogeneous in agarose gel electrophoresis.

Indole acetic acid (IAA), one of the regulators of plant growth, also occurs in higher animals^{1,2} which are able to synthesize it³. It has also been shown that the amount of IAA synthesized by chicken embryo increases during embryogenesis⁴. On the other hand, it has been found that auxins promote incorporation of uridine into ribonucleic acids of human leucocytes⁵, and Ihl⁶ reported the presence in soybean cotyledons of IAA-binding proteins probably acting as translocators of the hormone to the cell nucleus. In this paper we report evidence that IAA-binding proteins exist in the cytosol of embryonic chicken liver; we also present data concerning some properties of IAA-binding protein complexes formed in vitro.

Material and methods. Liver cytosol prepared from decapitated 18-day-old chicken embryos⁷ was incubated with 10 μ Ci/ml indole acetic-5-³H acid (29 mCi/mM; Schwartz/Mann, Orangeburg, New York, USA) for 30 min at 25°C and sieved through a column of Sephadex G-25. Macromolecular fractions containing bound ³H-IAA were pooled, dialyzed overnight, concentrated in Sartorius membrane

filter apparatus, and then subjected to analysis by chromatography⁸, centrifugation⁹ and electrophoresis¹⁰. Protein was determined by the method of Lowry et al.¹¹

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Radioactivities were measured in Beckman scintillation spectrometer CPM-100, using Bray's¹² or toluene-based scintillation mediums.

Results. DEAE-Sephadex chromatography of the void volume zone material from Sephadex G-25 gel filtration of ³H-IAA-labelled cytosol resolved the protein-bound radioactivity in at least 4 major zones eluting at progressively higher molarities of NaCl (figure 1); as seen, all zones were distinctively heterogeneous and obviously contained a number of subfractions.

The density gradient centrifugation of the same material resulted in a single broad radioactivity zone with a mean sedimentation rate of about 5 S as shown in figure 2.

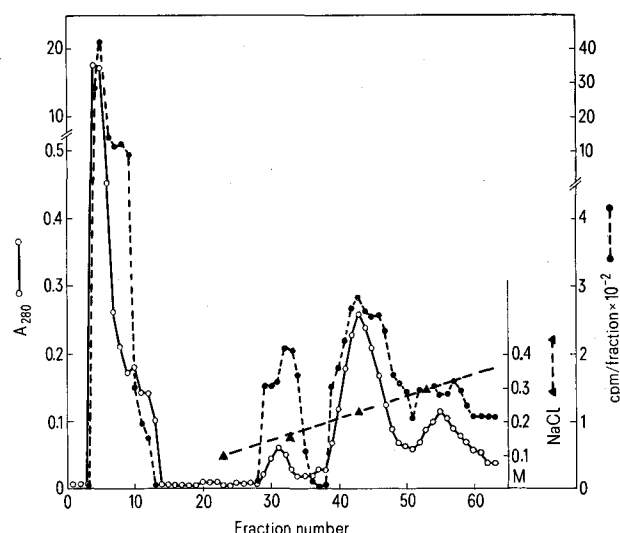


Fig. 1. Elution pattern of the IAA-binding protein complexes on a DEAE-Sephadex column. After molecular sieving chromatography, macromolecular fractions were pooled, dialyzed and concentrated in a Sartorius membrane filter apparatus and then subjected to ion-exchange chromatography on DEAE-Sephadex A-50 column (20 × 2.8 cm). After washing with the homogenization medium, the column was eluted with a linear NaCl gradient (0–0.4 M). Protein content = solid line, radioactivity = dashed line.

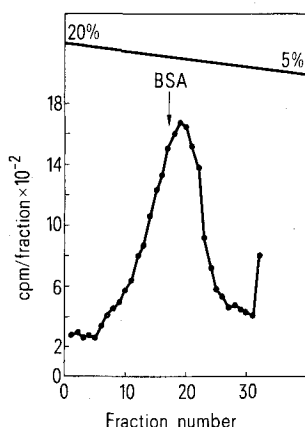


Fig. 2. Sedimentation profile of ³H-IAA-binding protein complexes. 0.3 ml of sample or bovine serum albumin were applied to a 4.6 ml of linear 5–20% sucrose gradient and centrifuged for 20 h at 39,000 rpm in the SW 39 rotor of a Spinco-Beckman model L₃₋₅₀ ultracentrifuge. Fractions were taken from the bottom of the centrifuge tubes directly into Bray's scintillation medium.

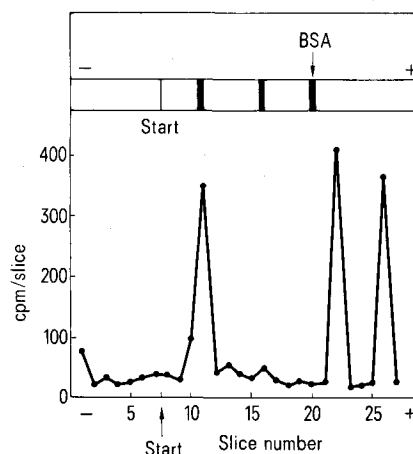


Fig. 3. Agarose gel electrophoresis of IAA-binding protein complexes. 10 µl aliquots of the concentrated sample were filled into the sample slots on an 0.8% agarose gel in 0.075 M barbital buffer plate, pH-8.6. The electrophoretic run was for 60 min at 160 mA and 220–240 V.

The gel electrophoresis resolved the radioactivity bound to protein in 3 fractions at the anodic part of electrophoretogram, and it also gave 2 further peaks of monomeric radioactivity very probably corresponding to free ³H-IAA and products of its degradation (figure 3).

Discussion. Hardin et al.¹³ in 1972 found that an auxin, 2,4-dichlorophenoxyacetic acid, released a protein factor from soybean plasma membranes which, when translocated to the cell nucleus, stimulated RNA-polymerase activity. Farrow et al.⁵ have shown that auxins could increase labelling of human leucocyte RNA with ³H-uridine. On the other hand, Ihl⁶ recently demonstrated IAA-binding proteins in soybean cotyledons. These and other results¹⁴ suggested that IAA might act on genome activity of eucaryotes, including the higher animals, in a way similar to steroid hormones, involving binding of hormone to more or less specific 'soluble' cytoplasmic proteins and translocation of such complexes to the cell nucleus.

From the results presented in this paper, it is evident that a number of species of IAA-binding proteins exist in the cytoplasm of embryonic chicken liver. Analysis of IAA-binding to cytosol proteins suggests that a variety of cytoplasmic proteins could bind the hormone to form relatively stable complexes. The molecular and functional identity of different proteins which bind IAA in this system is not known. If IAA proves to be capable of modifying DNA transcription in higher animal cells, it is conceivable that certain of the proteins binding will be shown to function in ways analogous to specific classes of steroid-binding polypeptides, including transport, protection and processing of the hormone, as well as the derepression proper.

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