of sclereids develops near the wound. The sections of such damaged portions reveal the organization of a wound periderm in the subsurface layers of the damaged portions. The cells at the cut surface assume a wavy outline with broken cell walls and the plastids in them disappear; cell walls are suberized. The 3rd or 4th layer of epidermal cells situated inwards from the damaged layer of cells enlarges and divides periclinally, organizing a wound meristem (Figure 3). Some of the cells above the wound meristem and closest to the damaged surface develop into brachysclereids with extremely thick (10–16 μ) lignified walls. The brachysclereids are very much smaller in size when compared with the other epidermal or mesophyll cells. Hence it is obvious that certain cells, derived as a result of wound meristem activity develop into brachysclereids. Normally brachysclereids are not found in the lamina portion of this species, but are abundant in the petiole region. It appears that, under the influence of wound hormones, certain epidermal cells can redifferentiate into brachysclereids. Another interesting change is in the thickness of cuticle on the damaged surface. In a normal uninjured leaf, the cuticle is 6.5 μ thick, and the same on the damaged portion is 84 μ thick, and thus the newly formed cuticle as a result of wound response is very much thicker (Figure 3). Other studies on wound healing in leaves and the associated histological changes are summarized by BLOCH[6].

In *Camellia japonica* most of the sclereids are densely arranged near the leaf margin, and in the rest of the lamina they are very sparsely present[1]. When incisions are made parallel or perpendicular to the long axis of the lamina in very young leaves, the parenchyma cells near the new margins which normally would have developed into mesophyll cells differentiate into sclereids, showing a dense arrangement. In *F. fragrans*, some of the mesophyll cells which would have normally developed into sclereids remain as such without undergoing any change when they are present near the wound region. The response of mesophyll cells to wounds, when natural or artificial in these 2 cases, are fundamentally different. The wound or the wound hormone either promotes or retards the development of mesophyll cells into sclereids as in *C. japonica* and *F. fragrans* respectively. It is thus evident that the formation and developmental pattern of sclereids are under the control of certain other factors surrounding the sclereid initials, perhaps including the wound itself, activity of the wound meristem or the wound hormone as is obvious from the present studies. Other experiments conducted concomitantly with this investigation reveal the probability of hormonal control in the morphogenesis of foliar sclereids in *F. fragrans*[7,8].

Résumé. L'effet d'une blessure naturelle sur le développement du tissu sclérifié et la distribution des feuilles est étudié sur la *Fagraea fragrans*. Un méristème blessé est préparé et son histologie décrite. L'influence inhibitrice de la blessure et de l'hormone de blessure sur le développement du tissu sclérifié est très prononcée. Certains points importants de l'étude présente sont discutés, et comparés à des observations antérieures.

A. N. RAO

Department of Botany, University of Singapore, Singapore, 10 April 1969.

[6] R. BLOCH, Bot. Rev. *7*, 110 (1941).
[7] A. N. RAO and M. SINGARAYAR, Experientia *24*, 298 (1968).
[8] I am thankful to Dr. C. R. METCALFE, Royal Botanic Gardens, Kew, England, for some helpful suggestions.

Histological Changes in the Skeletal System of the Developing Quail Embryo Treated with Sodium Salicylate

Skeletal abnormalities are frequently seen in teratological studies but only on rare occasions have histological studies on their early manifestation been carried out with a view to elucidating the mechanisms of their induction. Administration of high doses of sodium salicylate[1–10], acetyl salicylate[2,4,11–15] and phenyl salicylate[15] to pregnant rats, mice, hamsters, rabbits and guinea-pigs has been shown to induce skeletal abnormalities but no effects have been demonstrated in avian embryos. However, acetyl salicylate is known to enhance egg production in the domestic fowl[16]. In this study, the effect of sodium salicylate on the developing quail embryo was investigated both by injecting the laying hen and by treating eggs. The abnormal embryos were examined histologically and a possible mechanism of teratogenesis suggested.

20 adult hen quail were injected i.p. with a single dose of 2 ml of 0.5% aqueous sodium salicylate (buffered to pH 7.0 with dilute acetic acid), and 20 control birds were injected with 2 ml of dilute acetate buffer (pH 7.0). Approximately 100 eggs were collected from each group of birds from 1–20 days after treatment, and incubated

[1] K. S. LARSSON, H. BÖSTROM and B. ERICSON, Acta paediat., Stockh. *52*, 36 (1963).
[2] K. S. LARSSON and H. BÖSTROM, Acta paediat., Stockh. *54*, 43 (1965).
[3] K. S. LARSSON, B. ERICSON and H. BÖSTROM, Acta morph. neerl. scand. *6*, 35 (1963).
[4] J. WARKANY and E. TAKACS, Am. J. Path. *35*, 315 (1959).
[5] E. TAKACS and J. WARKANY, Teratology *1*, 109 (1968).
[6] A. S. GOLDMAN and W. C. YAKOVAC, Proc. Soc. exp. Biol. Med. *118*, 857 (1965).
[7] A. S. GOLDMAN and W. C. YAKOVAC, Archs envir. Hlth *8*, 648 (1964).
[8] K. S. LARSSON, Acta paediat., Stockh. *55*, 569 (1966).
[9] K. S. LARSSON, Acta path. microbiol. scand. *66*, 560 (1966).
[10] K. S. LARSSON, Br. med. J. *105*, 352 (1963).
[11] H. J. K. OBBINK and L. M. DALDERUP, Lancet *565* (1964).
[12] D. G. TRANSLER, Lancet *606* (1965).
[13] A. V. JACKSON, J. path. Bact. *60*, 587 (1948).
[14] J. D. MCCOLL, M. GLOBUS and S. ROBINSON, Toxic. appl. Pharmac. *7*, 409 (1965).
[15] T. BABA, Osaka Cy med. J. *12*, 23 (1966).
[16] B. L. REID, A. A. KURNICK, J. M. THOMAS and B. J. HULETT, Poult. Sci. *43*, 880 (1963).

freshly in a forced air incubator at 99.7 °F and 60% relative humidity. In a second experiment, freshly laid eggs were incubated in a similar manner but were inoculated with a single dose of 0.5 ml of 0.5% salicylate (or with acetate buffer) after 1, 3, 7 or 11 days incubation. 10–15 embryos from each group were examined macroscopically and histologically on days 11, 12, 13 or 14; none being allowed to hatch naturally at 16–17 days.

The incidence of abnormal embryos was high in both test groups, particularly in the embryos derived from the treated hens (Tables I and II). The range of abnormalities in both groups was similar and they invariably involved the skeletal system.

In the skeletal tissues from deformed embryos examined at 11 days incubation, the preosseous cartilage was grossly abnormal, the intercellular areas were greatly reduced and the matrix was considerably less metachromatic with toluidine blue dye[17] than usual. In most severely affected embryos the matrix was reduced to a thin intercellular strand and the lacunae appeared to be dilated. In abnormal embryos of 14 days incubation, poorly developed perichondria were seen investing the diaphyses of the primary long-bones and irregularly distributed sites of calcification occurred in the defective cartilage both in long-bones (Figures 3a and b) and vertebrae (Figure 4). In these areas, the trabeculae of the newly formed bone were less intensely calcified and were narrower than normal.

The reduced metachromasia of the cartilage matrix suggests a reduced deposition of acid mucopolysaccha-

Table I. Percentage incidence of abnormalities in quail embryos following inoculation of eggs with a single dose of 2.5 mg sodium salicylate

	% incidence[a] of abnormalities following inoculation on day			
	1	3	7	11
Abnormal embryos	72.4	59.4	45.9	35.0
Dead embryos	20.7	12.5	30.1	27.5
Abnormality				
Micromelia	27.6	25.0	33.3	27.5
Deformed beak	6.9	21.9	23.8	2.0
Deformed skull	6.9	21.9	23.8	2.0
Unfused sterna	6.9	–	2.4	–
Retarded growth	13.8	6.3	4.8	7.5
Abnormal vertebral torsions	10.3	21.8	7.1	–

[a] All percentages are based on the total number of embryos examined. Eggs were incubated for a total period of 11–15 days. All embryos developing in eggs treated with 0.5 ml of 0.001 *M* acetate buffer were normal when examined at 11, 13, 14 or 15 days incubation.

Table II. Percentage incidence of abnormalities in quail embryos in eggs collected up to 20 days after a single i.p. injection of 10 mg sodium salicylate to the laying hen

	% incidence[a] of abnormalities
Abnormal embryos	88.9
Dead embryos	11.1
Abnormality	
Micromelia	88.9
Skeletal deletion	3.7
Abnormal vertebral torsion	7.4
Beak deformity	14.8
Skull deformity	14.8
Hemorrhage	3.7

[a] All percentages are based on the total number of embryos examined. Eggs were incubated for a total of 11–15 days. All embryos developing in eggs obtained from hens dosed with 2 ml of acetate buffer were normal when examined.

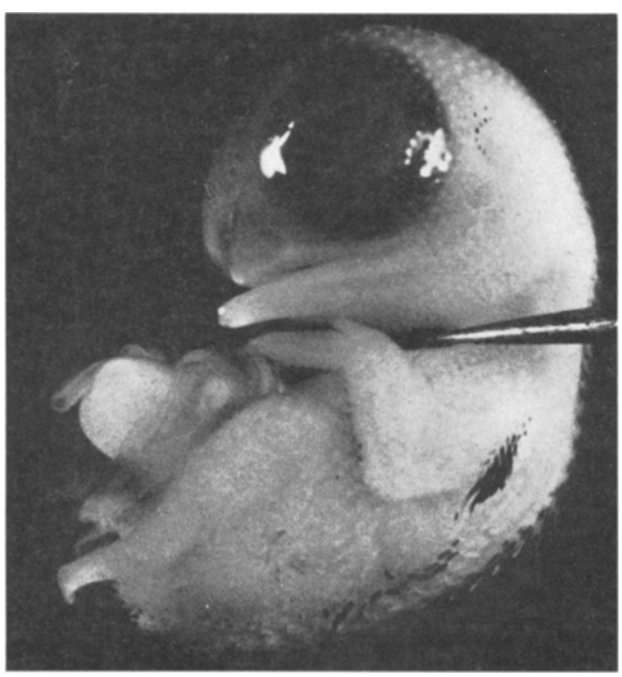

Fig. 1. Embryo at 12 days incubation showing micromelia, unfused sternum, beak deformity and ectopic viscera. × 4.

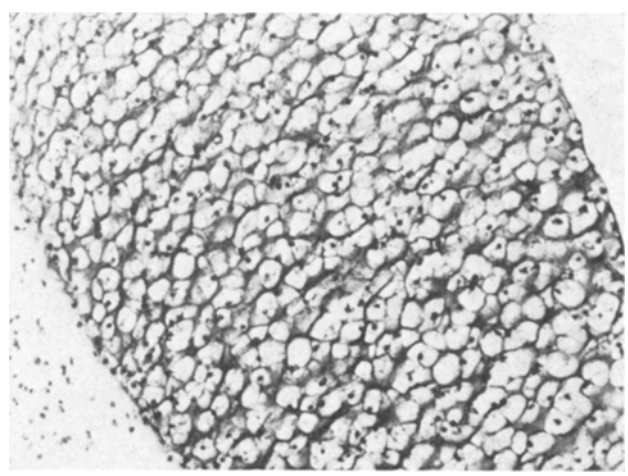

Fig. 2. Diaphyseal cartilage from the femur of an 11-day-old embryo showing dilated lacunae and loss of staining intensity in the cartilage matrix indicative of a reduction in chondroitin sulphate (Toluidine blue). × 100.

[17] A. B. G. Lansdown, Histochemie *13*, 192 (1968).

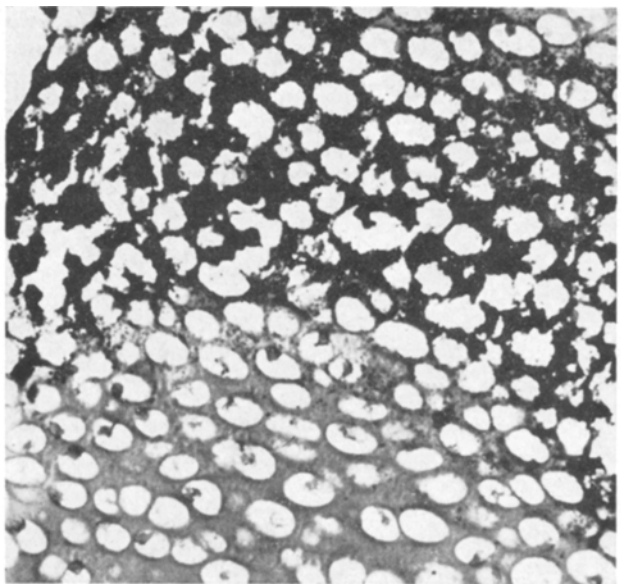

Fig. 3a. Diaphsis of the femur of 11-day-old embryo showing irregular deposits of calcium and defective cartilage matrix (Toluidine blue and silver impregnation). × 100.

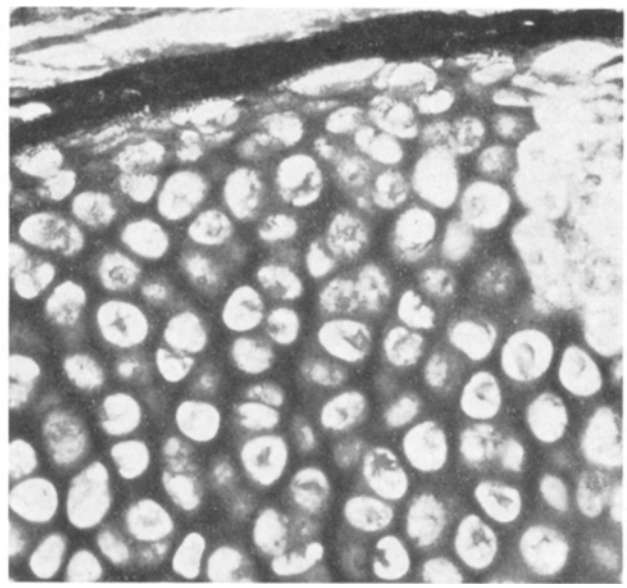

Fig. 3b. Diaphysis of the femur of a normal 10-day-old embryo showing well developed cartilage matrix and strict perichondral deposition of calcium (Toluidine blue and silver impregnation). × 150.

rides (chondroitin sulphate) and this is in accordance with results in which salicylates are shown to inhibit mucopolysaccharide synthesis in vitro[18,21]. Two possible explanations for this inhibition have been postulated: either that there is a failure in the incorporation of the sugar moiety into the mucopolysaccharide molecule[22], or that there is an inhibition of the sulphation of the polysaccharide[23,24]. For this sulphation, oxidative phosphorylation must occur in order to create an 'active sulphate' group. Since salicylates have been shown to be 'uncoupling agents' in oxidative phosphorylation[25–27], it seems likely that this defect underlies to formation of the abnormal cartilage. In normal osteogenesis, a reduction in the metachromasia of the cartilage matrix normally precedes calcification, and where a poor metachromasia exists, e.g. in the formation of the clavicle[28], the ensuing ossification is accelerated and irregular. The reduced metachromasia seen in these abnormal quail embryonic skeletons therefore, seems to suggest that a defective cartilage matrix and the onset of precocious and irregular ossification, underlies the production of teratological effects by salicylates.

It is suggested that when similar deformities occur after injection of the hen and the egg, the conclusion on the teratogenic properties of a compound has greater validity than when the egg alone is treated[29].

Zusammenfassung. Injektionen von Natriumsalicylat direkt in Quail-Eier oder direkt in Eileiter von Quail-Hühnern ergab kurzgliedrige, embryonale Missbildungen. Die histologische Untersuchung zeigte Hemmung des Knorpelbaues sowie frühzeitige und unregelmässige Knochenbildung.

A. B. G. LANSDOWN and P. GRASSO

The British Industrial Biological Research Association, Carshalton (Surrey, England), 14 April 1969.

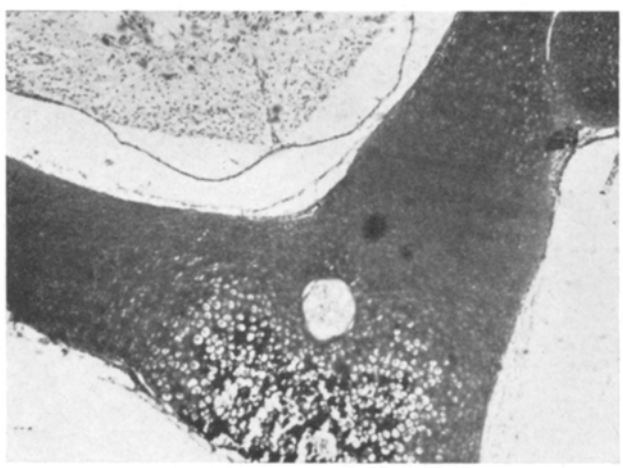

Fig. 4. Transverse section of a thoracic vertebra of a 14-day-old embryo to show irregular deposits of calcium in the pericentral area (Toluidine blue and silver impregnation). × 80.

[18] W. O. FOYE, J. Pharmac. Sci. *54*, 1365 (1965).
[19] R. J. BLATTNER, J. Paediat. *66*, 1102 (1965).
[20] M. W. WHITEHOUSE and H. BÖSTROM, Biochem. Pharmac. *7*, 135 (1961).
[21] M. W. WHITEHOUSE and M. BÖSTROM, Biochem. Pharmac. *11*, 1175 (1962).
[22] D. A. KOHLBHAN, Med. pharmac. Exp. *16*, 185 (1967).
[23] H. W. BÖSTROM and B. MANSSON, J. Pharm., Lond. *1*, 185 (1955).
[24] J. W. LASH and M. W. WHITEHOUSE, Lab. Invest. *10*, 388 (1961).
[25] J. E. LEADER, Biochem. Pharmac. *15*, 1379 (1966).
[26] J. T. MIYAHARA, Biochem. J. *97*, 194 (1965).
[27] M. W. WHITEHOUSE, Biochem. Pharmac. *13*, 319 (1964).
[28] A. B. G. LANSDOWN, J. Zool. *156*, 307 (1968).
[29] We are indebted to Dr. R. F. CRAMPTON, Director of B.I.B.R.A. for his interest in this work and for his permission to publish the results. We are also grateful to Mrs. J. DAVIES for her assistance in the photography and to the Beecham Research Laboratories, Tadworth, Surrey, for providing the birds used in this study.