EXPERIENTIA

Vol. 32 – Fasc. 3 Pag. 269 – 400 15. 3. 1976

GENERALIA

HENSEN'S Node – The 'Organizer' of the Amniote Embryo*

by A. LEIKOLA

Laboratory of experimental Embryology, Department of Zoology, University of Helsinki, Arkadiankatu 7, SF-00100 Helsinki 10 (Finland).

'This I will call the node'

The 3rd to 4th issue of the first volume of 'Zeitschrift für Anatomie und Entwicklungsgeschichte', which was published on the 26th of November 1875, contained a lengthy article on the fertilization and development of rabbit and guinea-pig embryos[1]. The article, which was continued in the next issue the following year, was written by the Kieler zoologist, VIKTOR HENSEN (1835–1924), who later earned a high reputation as one of the founders of plankton studies and was actually the creator of the plankton concept. In describing the blastoderm of an early rabbit embryo, Hensen noted that the anterior end of the primitive streak is somewhat enlarged, and he decided to call this formation 'the node'[2]. He found that both layers of the embryos, the epiblast and the hypoblast[3], were, at this spot, tightly united to each other by the growing middle layer, the mesoblast, and he concluded that the node was a most important point where both epiblastic and hypoblastic cells gave rise to the mesoblast cells which then spread in all directions between the two former layers (Figure 1).

HENSEN's description appeared at a time when the origin of different germ layers of both birds and mammals was hotly debated by leading embryologists and histologists, such as HIS, WALDEYER, KÖLLIKER, DURSY and others. Strangely enough, the debate about this central and seemingly simple problem has continued for nearly a century, and only recently the gastrulation movements of a bird embryo – whose pattern the mammals are, with reason, supposed to follow[4] – have been satisfactorily and convincingly described. But it would be bold to say that the node is even now properly understood. It still retains many of its secrets.

The significance of the primitive streak and HENSEN's node or primitive knot, as it has also been called, could not be worked out before the advent of experimental techniques in embryology, which were initiated in the 1880's by W. ROUX and H. DRIESCH. In spite of some brave attempts to work on early chick embryos[5–7], the main focus in vertebrate experimental embryology, headed by SPEMANN during the first decades of the present century, was in Amphibians. A reason for not extending these studies to birds and mammals was the lack of suitable culturing methods for young embryos, and our information on mammals in this respect is still very scanty, mainly owing to similar technical difficulties in experimentation[8].

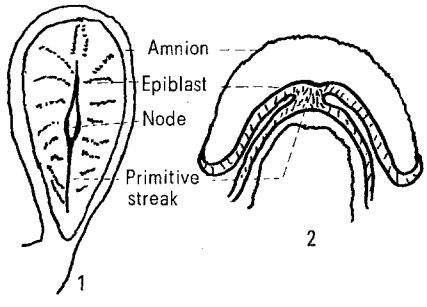

Fig. 1. Human embryo in gastrulation, about 2 weeks after fertilization. 1, dorsal view (the amnion has been cut); 2, section through the primitive streak. After PATTEN and CARLSON[16].

* Dedicated to Professor ETIENNE WOLFF on the occasion of his retirement.

[1] V. HENSEN, Z. Anat. EntwGesch. *1*, 213 (1875); *1*, 353 (1876).

[2] 'Nach vorne bildet sich ein scheibenförmiges Ende an ihm aus, welches ich als Knoten bezeichnen werde', p. 268[1].

[3] The terms 'epiblast' (or 'ectoblast') and 'ectoderm' are in general used synonymously, as well as 'mesoblast' and 'mesoderm' and, respectively, 'hypoblast' (or 'endoblast') and 'endoderm'. I have mainly used the 'blasts' when referring to the positions of the layers and the 'derms' when speaking of the tissues derived from these layers.

[4] J. C. DANIEL JR. and J. D. OLSON, Anat. Rec. *156*, 123 (1966).

[5] F. PEEBLES, Roux' Arch. *7*, 405 (1898).

[6] F. KOPSCH, Int. Monatschr. Anat. Phys. *19* (1902).

[7] R. ASSHETON, Anat. Anz. *27* (1905).

[8] M. BALLS and A. E. WILD, *The Early Development of Mammals* (Cambridge University Press, Cambridge 1975).

Three attacks on the node

In the latter half of the 1920's, the problem of early avian development was attacked simultaneously from three directions. At first, WETZEL[9,10] and GRÄPER[11] published their descriptions of the gastrulation movements, based on VOGT's vital staining method, and showed that an invagination of epiblast occurs anteriorly and laterally through HENSEN's node and laterally through the rest of the streak. The presumptive mesoblast flows in from both sides, and as the node regresses backwards after the streak has reached its maximum length, it leaves gradually condensing notochord behind. Before the regression, there is also a direct mesodermal forward flow through the node, so that the resulting head process – the front end of the notochord – extends well over the most anterior point of the node of the full-grown streak. These studies showed that the node was not a growth centre but an invagination centre of the chordamesoderm, and the rest of the streak was 'no material-bound form, but a state', as WETZEL[10] phrased it. The whole streak was cinematically comparable to the Amphibian blastopore and apparently homologous with it. In the next decade, PASTEELS[12,13] again applied vital staining to chick embryos, and thus a picture of the avian gastrulation emerged that, although now shown to be somewhat erroneous, is presented in most textbooks of embryology[14–16].

At the same time attempts were made, particularly in WILLIER's laboratory in Chicago, to find out the developmental potentialities of different parts of the chick blastoderm, and especially of HENSEN's node in isolation. In these experiments, in which the pieces were grown as explants on the chorioallantoic membrane of older embryos, HUNT[17] found that the node had a great capacity for differentiation and was 'essentially totipotent', which indicated that it was 'a center of differentiation in the normally developing embryo'. The work was carried on especially by RUDNICK and RAWLES in the 1930's[18].

The pioneer of the third attack on the secret of HENSEN's node was the talented young embryologist WADDINGTON[19,20] in Cambridge. He devised a method for cultivating chick and duck blastoderms in vitro on blood plasma clots. Moreover, he could show that the anterior part of the primitive streak, i.e. the part containing the node, was able to induce a new neural plate from the anterior and lateral 'competent' epiblast which had not yet been underlain by the invaginating mesoblast. Thus a parallel could be drawn to the induction of neural plate by the invaginating archenteron roof, the so-called primary induction, that had been discovered by SPEMANN and HILDE MANGOLD[21] some years earlier. The node, or probably rather the notochord rudiment emanating from it, was an organization centre of the embryo, an 'organizer' in the Spemannian sense, whatever that might mean in physicochemical terms. When the news came from SPEMANN's workshop that even a killed organizer could act as an inductor[22], WADDINGTON[23] quickly reported that the case was similar with birds.

Further on, WADDINGTON[24] demonstrated that the inducing effect was not species-specific, since duck HENSEN's node could induce a neural plate in chick epiblast and vice versa. This, again, was comparable to what had been found in Amphibians[21,25]. Even class barriers were overcome: chick primitive streak could induce neural plate in rabbit blastoderm, and rabbit streak had a similar effect on chick blastoderm[26,27]. All these results, as well as those from the vital staining experiments and the chorioallantoic graftings were reviewed in 1952 by WADDINGTON[28] in his book *The Epigenetics of Birds*, which is already considered as a classic in this field, in many respects the avian counterpart to SPEMANN's[29] *Experimentelle Beiträge*.

A new front in the 1960's

Although there was some progress in the 1940's and the 1950's, such as SPRATT's[30–33] extensive studies on chick gastrulation with carbon grain markings, where also the significance of HENSEN's node was discussed, as well as NEW's[34] invention in 1955 of a new method for culturing blastoderms, a really new phase in the search for the meaning of the node began in the 1960's when several authors, notably VAKAET[35], MODAK[36], NICO-

[9] R. WETZEL, Roux' Arch. *106*, 463 (1925).
[10] R. WETZEL, Roux' Arch. *119*, 188 (1929).
[11] L. GRÄPER, Roux' Arch. *115*, 523 (1929).
[12] J. PASTEELS, Bull. clin. Sci. Acad. Belg. Ser. V, *22*, 737 (1936).
[13] J. PASTEELS, Archs Biol., Liège *48*, 381 (1937).
[14] B. I. BALINSKY, *An Introduction to Embryology*, 3rd edn. (W. B. Saunders Co., Philadelphia 1970).
[15] N. J. BERRILL, *Developmental Biology* (McGraw-Hill Book Co., New York 1971).
[16] B. M. PATTEN and B. M. CARLSON, *Foundations of Embryology*, 3rd edn. (McGraw-Hill Co., New York 1974).
[17] T. E. HUNT, Proc. Soc. exp. Biol. Med. *27*, 84 (1929).
[18] D. RUDNIK, Q. Rev. Biol. *19*, 187 (1944).
[19] C. H. WADDINGTON, Nature, Lond. *125*, 924 (1930).
[20] C. H. WADDINGTON, Phil. Trans. B 221, 179 (1932).
[21] H. SPERMANN and H. MANGOLD, Roux' Arch. *100*, 599 (1924).
[22] H. BAUTZMANN, J. HOLTFRETER, H. SPEMANN and O. MANGOLD, Naturwissenschaften *20*, 971 (1932).
[23] C. H. WADDINGTON, Nature, Lond. *131*, 134 (1933).
[24] C. H. WADDINGTON and G. A. SCHMIDT, Roux' Arch. *128*, 522 (1933).
[25] B. GEINITZ, Roux' Arch. *106*, 357 (1925).
[26] C. H. WADDINGTON, J. exp. Biol. *11*, 224 (1934).
[27] C. H. WADDINGTON, Arch. Biol. *48*, 273 (1937).
[28] C. H. WADDINGTON, *The Epigenetics of Birds* (Cambridge University Press, Cambridge 1952).
[29] H. SPEMANN, *Experimentelle Beiträge zu einer Theorie der Entwicklung* (Springer, Berlin 1936).
[30] N. T. SPRATT, J. exp. Zool. *103*, 259 (1946).
[31] N. T. SPRATT, J. exp. Zool. *128*, 121 (1955).
[32] N. T. SPRATT, J. exp. Zool. *134*, 577 (1957).
[33] N. T. SPRATT, J. exp. Zool. *135*, 319 (1957).
[34] D. A. T. NEW, J. Embryol. exp. Morph. *3*, 326 (1955).
[35] L. VAKAET, J. Embryol. exp. Morph. *10*, 38 (1962).
[36] S. P. MODAK, Experientia *21*, 273 (1965).

LET[37], and ROSENQUIST[38] revised the whole picture of avian gastrulation. They showed, especially with transplantation of radioactively labelled blastoderm pieces, that the endoderm of the embryo actually invaginated through the primitive streak as a 'secondary hypoblast', whereas the 'primary hypoblast', which is formed before the appearance of the streak, only develops to extraembryonic tissue. To quote HARA[39], 'we may think of it as the second milestone in the history of avian organizer studies, the transplantation experiments of WADDINGTON being the first'. On the other front, HARA[40] confirmed experimentally WADDINGTON's old suggestion that the head process, i.e. the prechordal plate and presumptive notochord, which grow out from the node, actually acts as a neural inductor in normal development. Others attacked the problem of primary induction and determination by other means, such as investigating the inducing effects of the node of different ages in different positions, applying different chemicals to the node or using completely foreign tissues as 'heterogenous inductors', which had been widely and successfully applied in Amphibian studies (for reviews on Amphibian work, see[41,42]). The new information on avian gastrulation was reviewed in 1971 by NICOLET[43], and the problem of induction in avian blastoderm at the same time by GALLERA[44]. Even more recently, essential facts and theories on the 'organizer' in the bird embryo have been summarized by HARA[39]. A very detailed and up-to-date account of the various aspects of the early avian and mammalian embryo is also found in BELLAIRS's textbook[45].

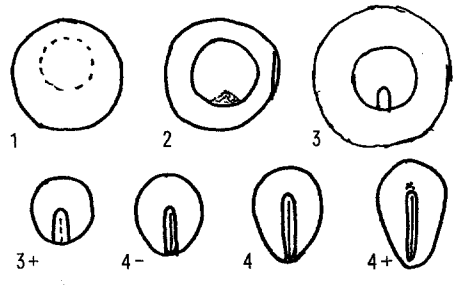

Fig. 2. Stages of the development of the chick embryo, according to HAMBURGER and HAMILTON, modified by HARA[39]. 1. Area opaca and area pellucida indistinctly separated, posterior accumulation of cells. 2. Initial, short and wide streak. 3. Short streak, straight, ungrooved. 3^+. Medium streak, with shallow groove. 4^-. Long streak, with well-developed groove, node still flattish. 4. Definitive, fully developed streak, with rounded node. 4^+. Head process primordium (the prechordal mesoderm is invaginating), not yet rod-shaped. From stage 3^+ in the area opaca is omitted.

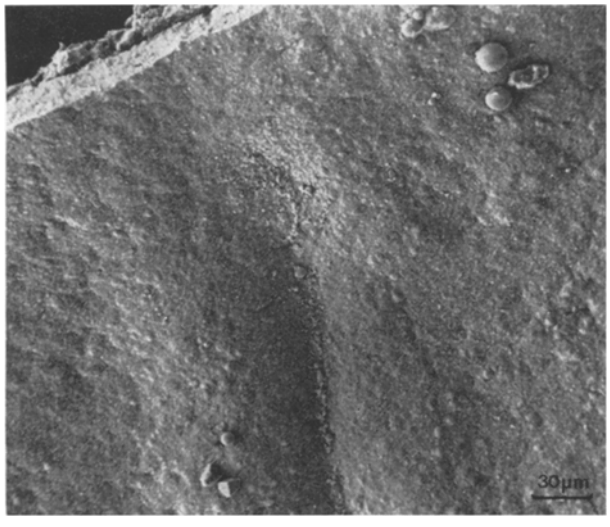

Fig. 3. A scanning electron micrograph of a chick full primitive streak blastoderm showing HENSEN's node and the primitive groove. The anterior border of the blastoderm has been cut to show the thickness of the blastoderm.

A wandering passageway for the cells

There is no doubt by now that the node is a transitory passageway of the notochordal and endodermal cells from the epiblast down to the second and third layers of the blastoderm. Thus it must be borne in mind that the node consists, at any given moment, of different cells, at least until the invagination is over. Some inconsistencies, especially in earlier studies, may be explained by the fact that the developmental stage of the embryo has not been carefully observed, or several different stages have been lumped together.

The situation is still more complicated because of the axial movement of the node (Figure 2). When the streak first appears at the posterior margin of the area pellucida of the blastoderm, its front end is not far from its rear end. Curiously enough, there is no general agreement as to from which stage onwards it is appropriate to use the term HENSEN's node, since it can hardly be applied to the very vague anterior end of the short and broad incipient streak. But either there is a 'node' as soon as the beginnings of the streak become visible, or then there is a true node only from the short-streak stage on, when the streak is more condensed and its anterior end begins to look more node-like.

As the streak elongates, the node moves forward as its spearhead. After some 18 h from the beginning of the incubation at 38 °C the streak is 1.8 mm long and occupies about three-quarters of the length of the area pellucida. All of the streak elongation is, however,

[37] G. NICOLET, Acta Embryol. Morph. exp. *8*, 213 (1965).
[38] G. C. ROSENQUIST, Contr. Embryol. Carnegie Inst. *38*, 71 (1966).
[39] K. HARA, in: *Organizer – A milestone of half a century from Spemann* (Ed. O. NAKAMURA and S. TOIVONEN) in press.
[40] K. HARA, Ph. D. Thesis, Utrecht (1961).
[41] L. SAXÉN and S. TOIVONEN, *Primary Embryonic Induction* (Logos Press, London 1962).
[42] P. D. NIEUWKOOP, Adv. Morphogenes. *10*, 1 (1973).
[43] G. NICOLET, Adv. Morphogenes. *9*, 231 (1971).
[44] J. GALLERA, Adv. Morphogenes. *9*, 149 (1971).
[45] R. BELLAIRS, *Developmental Processes in Higher Vertebrates* (Logos Press, London 1971).
[46] G. C. ROSENQUIST, J. exp. Zool. *180*, 95 (1972).

not due to a stretching forwards, since there seems to be a backward stretching as well in the posterior end of the streak. This is accompanied by an elongation of the whole posterior part of the area pellucida, whose shape changes from roundish to pear-shaped. The causes and mechanisms of these movements are unknown.

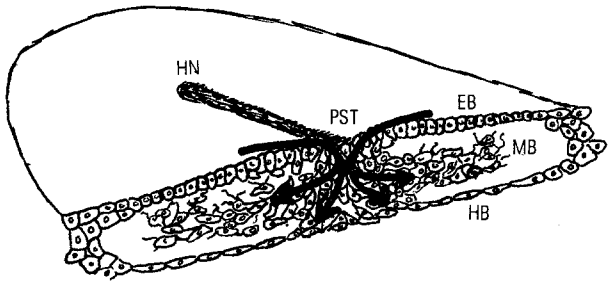

Fig. 4. The invagination of the endoderm and mesoderm through the primitive streak in chick embryo. After BALINSKY, *An Introduction to Embryology*, 3rd edn. (Saunders, Philadelphia 1970).
HN, HENSEN's node; PST, primitive streak; EB, epiblast; MB, mesoblast; HB, hypoblast

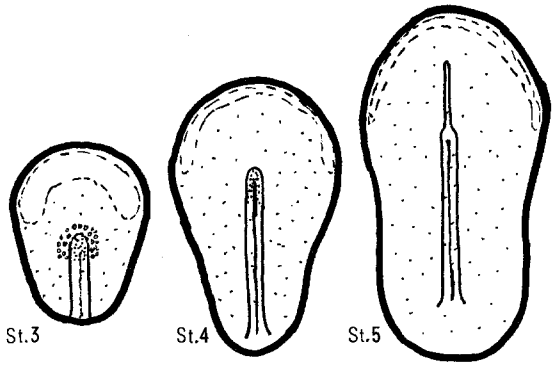

Fig. 5. Invagination of the definitive hypoblast (endoderm) in chick embryo. Future endoderm cells in the epiblast are shown as circles, invaginating endoderm cells in the node as dense dots, already invaginated hypoblast as sparse dots. After NICOLET[43].

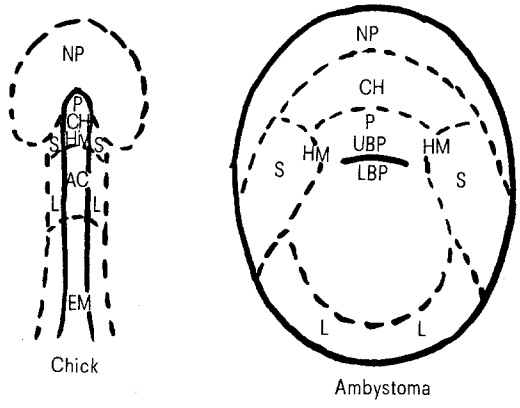

Fig. 6. Fate map of the mesoderm in the gastrulation of chick as compared to *Ambystoma*. The *Ambystoma* gastrula has been distorted about 20°. In the chick blastoderm, only the vicinity of the node is shown. NP, neural plate; CH, notochord; P, prechordal mesoderm; HM, head mesenchyme; S, somites, AC, amniocardiac mesenchyme; L, lateral plate; EM, extraembryonic mesoderm; UBP, upper blastoporal lip; LBP, lower blastoporal lip. After NICOLET[43].

The cell flow through the node begins during the streak elongation and continues until the streak has attained its full length. A groove at first shallow but later sharper appears in the midline of the streak as 'the morphological expression of vigorous invagination of the prospective endo- and mesodermal material'[39]. The end-point of this primitive groove in the middle of HENSEN's node is known as the primitive pit (Figures 3 and 4). On the ventral side of the blastoderm, the new hypoblast spreads out in a semicircular pattern, with the node as its centre[46].

Initially the node consists practically exclusively of future endoblast cells. At the short to medium streak stages, about 80–95% of the cells of the node will go to the endoblast and 5–20% to the mesoblast, whereas at the full streak stage the former percentage is about 60% and the latter about 35%, and the rest, about 5%, will remain in the epiblast[47] (Figures 5 and 6). The distribution of the cells to the left and right side apparently happens at random.

After the full (or definitive) streak stage, the streak begins to shorten again, and the node regresses backwards. There is still some cell flow through the posterior parts of the streak, but no more through the node which leaves notochord and some hypoblast in its former place (Figure 7). There has been some discussion about whether the regression is a real movement of nodal cells or just an apparent shifting of the morphological entity called 'the node'. The general opinion, however, is that the node materially regresses and, simultaneously, there seems to be a regression of the adjacent parts of the blastoderm, which in turn are important in somite formation[48]. Even at a much later stage, when the brain vesicle has closed, the heart is beating and there is a row of somites on each side of the spinal cord and notochord, the node can be seen in the posterior end of the embryo as a rudiment from a time when the foundations of the embryonic axis were laid.

An organization centre

The HENSEN's node is, however, also an organization centre, the first of the four that WADDINGTON[28] distinguishes in the development of the avian embryo, and it possesses some very curious properties. Its physiology as such has been studied in some detail, and it seems to be the focus of some physiological gradients in the blastoderm. It is the point most susceptible to poisons such as hydrogen cyanide[49], and there seems to be a higher need of carbohydrate in the node than in other parts of the blastoderm[50]; but there is apparently no difference in the oxygen consumption between different blastoderm regions[51]. The mitotic

[47] G. NICOLET, J. Embryol. exp. Morph. *23*, 79 (1970).
[48] R. BELLAIRS, J. Embryol. exp. Morph. *11*, 697 (1963).
[49] L. H. HYMAN, Biol. Bull. *52*, 1 (1927).
[50] N. T. SPRATT, Biol. Bull. *99*, 120 (1950).
[51] H. EMANUELSSON, Thesis of the University of Lund (1962).

activity seems to be highest in the node area[52], at least at the definitive streak stage, and the rate of DNA synthesis is high[53], whereas the RNA/DNA ratio is particularly low[54]. As stated by EMANUELSSON[54] 'HENSEN's node and the head region form centres for cell multiplication, while there should be a more decided protein incorporation in the cells of the adjacent parts'. It should be stressed again, however, that the node is not a blastema-like proliferation centre which would produce all notochord material by its own mitoses. The invagination is a real process, and there is no indication that every cell passing through the node will undergo a division. It is not even known whether the mitotic rate of the node has anything to do with its developmental potencies. In general, the relationship of the physiological properties of the node to its functional significance is obscure. There are metabolic values which are focused not in the node but elsewhere, as in the heart-forming areas at both sides of the blastoderm[55]. The node is by no means a centre for everything.

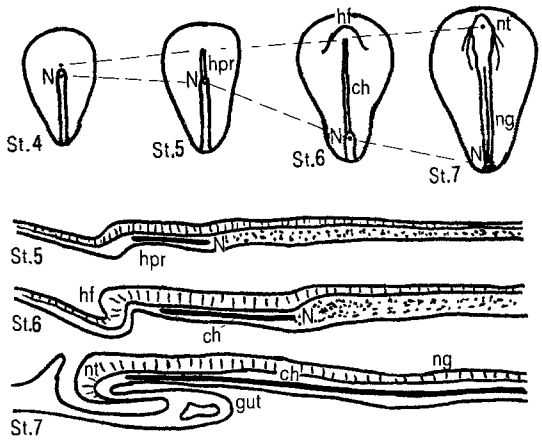

Fig. 7. Regression of the node at different HAMBURGER-HAMILTON stages. Upper row, dorsal view; lower, sagittal sections. After BELLAIRS[45]. N, HENSEN's node; hpr, head process; hf, head fold; ch, notochord; nt, neural tube; ng, neural groove; gut, foregut.

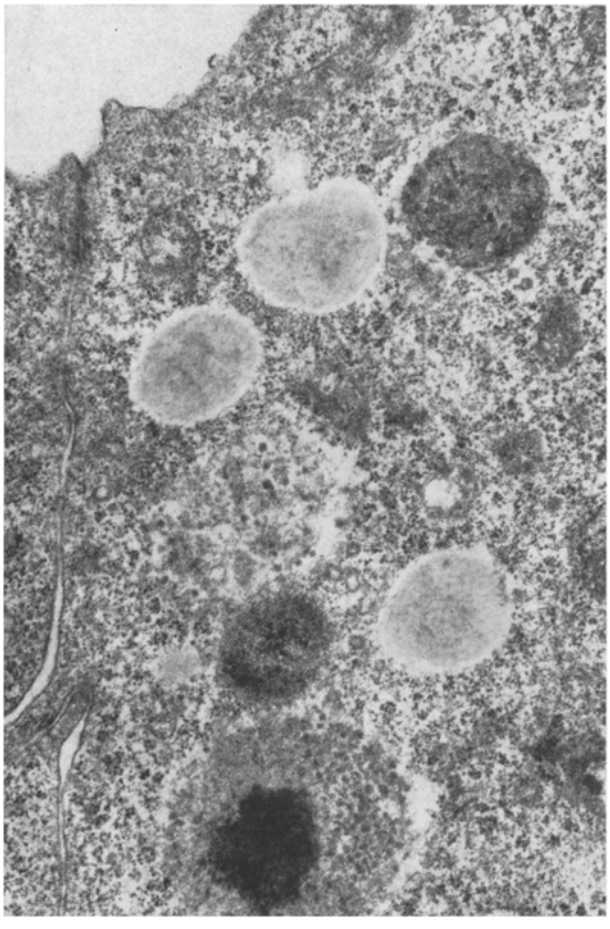

Fig. 8 Electron micrograph of the upper part of HENSEN's node at HAMBURGER-HAMILTON stage 3. Note the tight junction between cells and the large yolk vacuoles. From KÄRNER and LEIKOLA[57].

It seems that the secret of the node is not to be found in simple physiological gradients. Nor has it been found in the morphological and ultrastructural properties of its cells. At both sides of the primitive groove, the epiblastic cells form essentially a columnar epithelium. In the groove, they become flask-shaped, with long necks extending to the upper surface, as if the cell bodies were stretching themselves downwards in order to reach the inner depths of the node[43,56,57]. In the necks, microtubules are seen which can be destroyed by colchicine and vinblastine and seem to be essential for a proper invagination[58,59]. Further down, the cells assume a more irregular mesenchyme-like shape, having been released from the tight pressure of the invagination process. There is acid phosphatase activity between the cells, which may loosen their initially tight junctions[57] (Figures 8 and 9). Some cells seem to become necrotic and are phagocytozed by their neighbours, especially in the deeper layers[57]. Inside the cells, there is the usual cell complement with mitochondria, Golgi apparatus and ribosomes. There are also numerous yolk drops of various sizes which seem to be gradually digested during the streak elongation, so that a definitive streak node contains less yolk drops but more vacuoles of digested yolk than the middle streak node[57] (Figures 8 and 9). But there is no evidence as to whether the intensive yolk utilization or the phagocytic processes have anything to do with the movements and differentiation of the node cells.

[52] H. EMANUELSSON, Acta physiol. scand. *52*, 211 (1961).
[53] H. EMANUELSSON, Expl. Cell Res. *42*, 537 (1966).
[54] H. EMANUELSSON, Acta physiol. scand. *44*, 336 (1958).
[55] M. REPORTER and G. C. ROSENQUIST, Science *178*, 628 (1972).
[56] B. I. BALINSKY and H. WALTHER, Acta embryol. morph. exp. *4*, 261 (1961).
[57] J. KÄRNER and A. LEIKOLA, Differentation (in press).
[58] N. H. GRANHOLM, Am. Zool. *9*, 615 (1969).
[59] N. H. GRANHOLM, Am. Zool. *10*, (1970).

Extensive differentiation and regulation potentialities

Thus, nothing morphologically or physiologically distinct has been found to account for the self-differentiation and induction capacities, these most prominent features of the node. The former is far greater in the node than in any other part of the blastoderm, although there is no sharp limit, especially backwards, between the very potent node area and the less capable posterior parts of the streak. If the node is cultured on a chorioallantoic membrane, or better still, in the coelomic cavity of another embryo, it will differentiate into almost every possible type of tissue. The change in this capacity has recently been investigated by VEINI and HARA[60]. They found, using the coelomic grafting method, that the node gave rise to thyroid, parathyroid, pancreatic and thymic tissue, different parts of the digestive tract, notochord, muscle cartilage, mesonephros and adrenal gland, and nervous tissue (rhombencephalon and/or spinal cord). The endodermal differentiation tendencies decreased from the medium-streak to the pre-head-process stage, and were then completely lost, whereas the frequency of

[60] M. VEINI and K. HARA, Roux' Arch. *177*, 89 (1975).

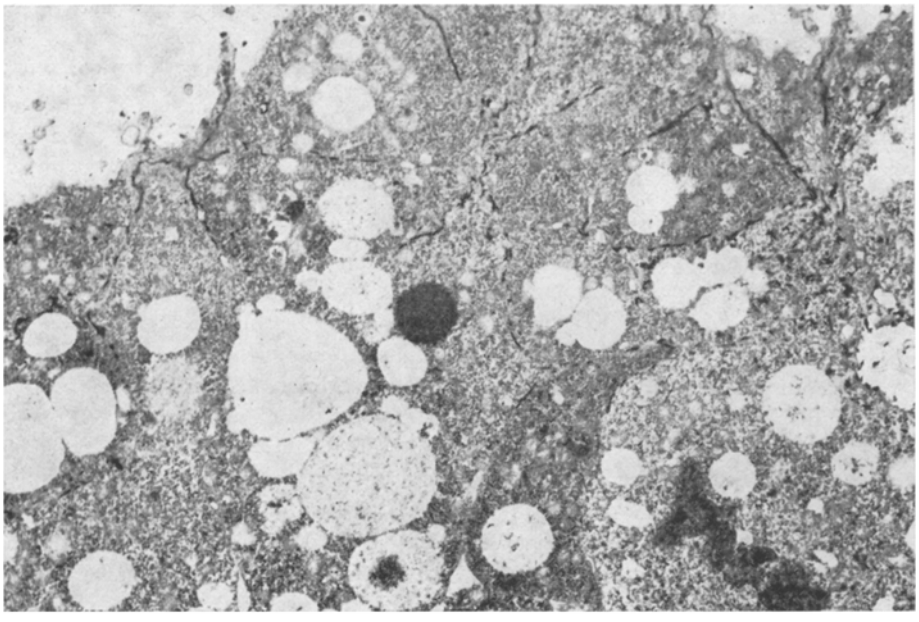

Fig. 9. Electron micrograph of the upper part of HENSEN's node at stage 4. Note the distribution of acid phosphatase (shown dark) between the cells and large yolk vacuoles from which the yolk has been utilized. From KÄRNER and LEIKOLA[57].

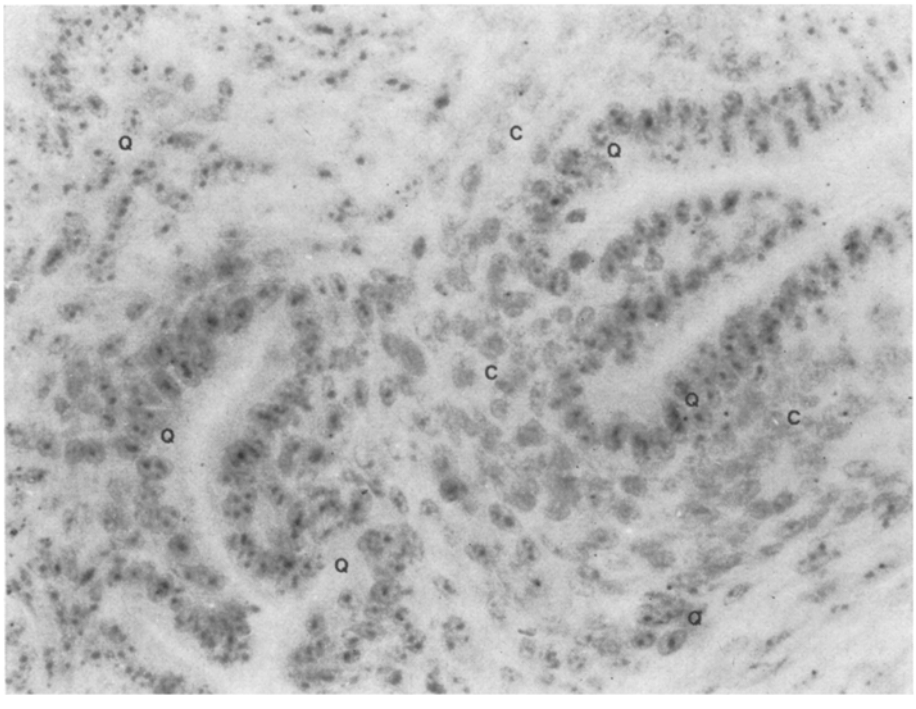

Fig. 10. Digestive epithelium and connective tissue derived from quail HENSEN's node grown in chick coelomic cavity. The quail cells (Q) are readily distinguished from the chick cells (C) by the presence of a DNA-containing nucleolus which appears as a dark dot in Feuglen-stained nuclei.

notochord, muscle and cartilage was all the time nearly 100%, and the mesonephric differentiation tendency was even increased. Also the neural differentiations increased from 75% at the middle-streak stage to 100% at the definitive streak stage. The decrease in endodermal differentiation tendency apparently results from the decrease in the percentage of prospective hypoblast cells. A multipotentiality of the nodal cells was also shown by grafting quail node into chick coelomic cavity, and since the quail cells can be distinguished from chick cells by a nuclear peculiarity (a DNA-containing nucleolus[61,62]), it could be seen that the differentiating node or its derivatives can combine intimately with host tissue and even induce cartilage and muscle from the host mesoderm[63] (Figure 10).

The multipotentiality of the node indicates that the determination of its cells is still labile. Normally, medium-streak node does not give rise to any nervous tissue, but, when isolated, it readily does so. Do some of the node cells act on their neighbours as neuralizing inductors? Or is there an inherent tendency in the nodal cells to begin neural differentiation, a tendency which normally is suppressed? If the hypoblastic and mesoblastic parts of the node are, as far as possible, removed and the epiblastic part alone is grafted into a coelomic cavity, it still forms both neural, mesodermal and endodermal tissues[64]. On the other hand, when the definitive streak node is implanted into the posterior end of another, a younger blastoderm, it is completely incorporated into the host hypoblast[65]. Thus even those nodal cells which normally would have formed notochord were converted into endodermal ones. But if a similar node is grafted into the same place in an older blastoderm, the 'dissolution' into the hypoblast no longer occurs[65].

Actually, we have no definite idea about how and when the cells passing through the node get their final determination. Do the 'endodermal' and 'mesodermal' cells differ from each other somehow even before they enter the node? At least no such differences have been detected. Or are the cells initially similar and become determined only in the node according to the position in which they happen to land, the lowest ones becoming endodermal and those remaining above them becoming mesodermal? Although the latter possibility seems to be the more plausible, it must be admitted that we are utterly ignorant about the whole process of cellular determination. In any case, there must be a great amount of reorganization and regulation in the node when it is isolated and subjected to a foreign environment.

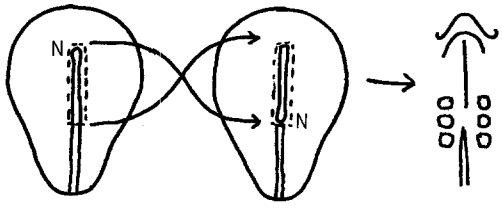

Fig. 11. A reversal of a great part of the primitive streak can result in a normal embryo. N = node. After BELLAIRS[45], based on ABERCROMBIE[84].

Another example of the regulation capacities of the node is its orientation. If a definitive streak node is grafted into another blastoderm of the same age at the level of the host node, it will form another embryonic axis. If the graft has been implanted far from the host node, it will retain its original axial orientation, but if it happens to be close to the host axis, it will accommodate its orientation according to that of the host[24,66]. If, on the other hand, the orientation of the node or even of the whole anterior third of the streak is reversed, a normally oriented embryo may result, indicating that the adjacent tissue of the area pellucida can have a considerable influence on the streak, and that even posterior parts of the streak can assume nodal functions, if so required[67] (Figure 11). Actually, even the posterior third of the streak can be 'nodalized' by various chemicals so that it acquires differentiation and induction capacities which are normally lacking[68–79].

But regulation will also happen in the rest of the blastoderm. Important as the node may be, it is not indispensable. Since the pioneering experiments of WADDINGTON[20], it has been known that if the node is extirpated and the wound heals properly, another node will form in its place, and a normal embryo will develop. This will only happen, however, if the blastoderm is sufficiently young. After the definitive streak stage, the adjacent epiblast is no longer able to form a node, and the embryo remains notochordless. Even at the

[61] N. LE DOUARIN and G. BARQ, C. r. Soc. Biol., Paris *169*, 949 (1969).
[62] N. LE DOUARIN, Bull. biol. Fr. Belg. *103*, 436 (1969).
[63] A. LEIKOLA, Experientia *31*, 1087 (1975).
[64] A. LEIKOLA, unpublished.
[65] J. GALLERA, Experientia *28*, 1217 (1972).
[66] M. ABERCROMBIE and C. H. WADDINGTON, J. exp. Biol. *14*, 302 (1937).
[67] M. ABERCROMBIE, Phil. Trans. B *234*, 317 (1950).
[68] J. M. BUTROS, J. exp. Zool. *143*, 259 (1960).
[69] G. V. SHERBET and L. MULHERKAR, Roux' Arch. *154*, 506 (1963).
[70] G. V. SHERBERT and L. MULHERKAR, Roux' Arch. *155*, 701 (1965).
[71] S. SANYAL and M. C. NIU, Proc. natn. Acad. Sci., USA *55*, 743 (1966).
[72] M. A. WAHEED and L. MULHERKAR, J. Embryol. exp. Morph. *17*, 161, (1967).
[73] M. C. NIU and A. LEIKOLA, Biol. Bull. *135*, 200 (1968).
[74] M. A. WAHEED and D. J. MCCALLION, Ann. Zool. fenn. *6*, 448 (1969).
[75] S. P. S. CHAUHAN and K. V. RAO, J. Embryol. exp. Morph. *23*, 71 (1970).
[76] M. Z. KHAN and L. MULHERKAR, Proc. 60th Indian Sci. Congr. *3*, 456 (1973).
[77] M. Z. KHAN and L. MULHERKAR, Rev. Can. Biol. *33*, 9 (1974).
[78] M. C. NIU and A. K. DESPHANDE, J. Embryol. exp. Morph. *29*, 485 (1973).
[79] M. C. NIU, A. K. DESPHANDE and L. C. NIU, Proc. Soc. exp. Biol. Med. *147*, 318 (1974).

definitive streak stage, there should be no more prospective notochordal material outside the node, but apparently such material will be formed from the adjacent epiblast, maybe under the influence of hypoblast. It should be noted that, even at the earliest stages, the primary hypoblast plays an important role in determining the orientation of the future streak and actually functions as a 'streak inductor'[20,80–82]. Even the secondary hypoblast, i. e. the definitive hypoblast which has been formed by invagination through the node, may be capable of inducing a notochord from an isolated piece of competent epiblast in vitro[83].

A powerful inductor

For more than 40 years it has been known that, when HENSEN's node is grafted under a competent epiblast, a neural plate will arise above the node and a whole new embryonic axis is formed[20,84–92] (Figure 12). The details of this primary induction are, however, still obscure. HARA[40] showed that, in normal development, the inductor is most probably the mesoderm derived from the node. He implanted isolated pieces of epiblast, together with pieces of prechordal or chordal mesoderm, into coelomic cavities and found that the prechordal plate of a pre-head-process stage embryo induced forebrain, whereas hindbrain was induced by a more posterior notochord piece taken from an older blastoderm. Thus the regionality of the induction result was determined by the inductor. Essentially similar results have been obtained with HENSEN's node: a node from the definitive streak stage induces all brain parts, but an older, retreating node induces only hindbrain and spinal cord[93,94] In the parts of blastoderm close to the axis, there is also a 'position effect': the induction result depends on the level of the reacting epiblast, so that grafts located anteriorly tend to induce more anterior structures than those located posteriorly[24,94].

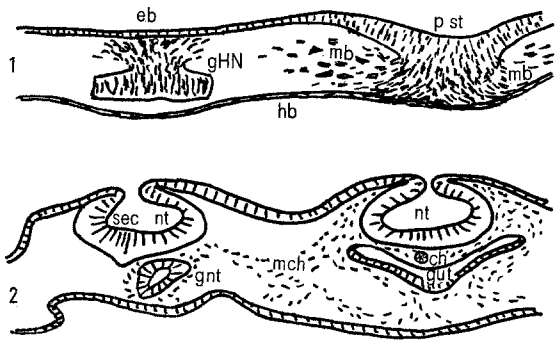

Fig. 12. 1. An additional HENSEN's node has been inserted into a blastoderm between the competent epiblast and hypoblast. 2. As a result of an induction by the graft, a secondary neural plate develops from the host epiblast. After BELLAIRS[45]. eb, epiblast; pst, primitive streak; gHN, grafted HENSEN's node; mb, mesoblast; hb, hypoblast, nt; neural tube; sec nt, secondary neural tube; gnt, graft neural tube; mch, mesenchyme.

The node, however, does not induce only neural structures but it can also cause the formation of a new streak[24,95–98]. This 'streak induction', as HARA[39] proposes to call it, depends on the stage of the inducing node, but also on the stage and area of the reacting epiblast. As shown by GALLERA[97], the neural competence is high in both area opaca and area pellucida, whereas the 'streak competence' is spatially more limited and does not extend much outside the pellucida. On the other hand, the neural competence is higher in the anterior end of the area pellucida, but the streak competence is more evenly distributed.

There is, at present, no evidence for a direct mesodermal induction – a mesodermalization of epiblast cells without formation of a streak – in birds, although it is a well-known phenomenon in Amphibians. It is, however, known that endodermal tissues, such as gut structures, can be induced by some heterogenous inductors[99]; but there is no information about such an induction by the node or its derivatives. It looks as if the epiblast facing an inductor – at least a natural inductor – had two possibilities: to form a streak with a node or to become neuralized. The former would happen under the influence of the hypoblast, or, in experimental conditions, of the hypoblastic element of a grafted node. If mesoblastic elements are present, as they are in the definitive streak node, the epiblast is neuralized under their influence[96]. It must be noted, however, that the neuralizing influence is not exclusively a nodal property. A killed node or its derivative will neuralize the epiblast which is in contact with it[23,100,101], and also several foreign tissues or chemicals

80 L. VAKAET, Mem. Acad. R. Med. Biol. *5*, 235 (1967).
81 H. EYAL-GILADI, J. Embryol. exp. Morph. *23*, 739 (1970).
82 H. EYAL-GILADI and M. WOLK, Roux' Arch. *165*, 226 (1970).
83 I. ROSTEDT, unpublished.
84 M. ABERCROMBIE, Nature, Lond. *144*, 1091 (1939).
85 G. L. WOODSIDE, J. exp. Zool. *75*, 259 (1937).
86 J. GALLERA and J. CASTRO-CORREIA, C. r. Soc. Biol. *154*, 1728 (1960).
87 L. PASTERNAK and D. J. MCCALLION, Can. J. Zool. *40*, 585 (1962).
88 S. P. SHIEH, I. L. NING and S. D. TSUNG, Acta Biol. exp. sin. *8*, 441 (1963).
89 J. GALLERA and I. IVANOV, J. Embryol. exp. Morph. *12*, 693 (1964).
90 J. GALLERA, Experientia *21*, 218 (1965).
91 L. VAKAET, C. r. Soc. Biol., Paris *159*, 232 (1965).
92 D. J. MCCALLION and V. A. SHINDE, Experientia *29*, 321 (1973).
93 C. T. GRABOWSKI, Am. J. Anat. *101*, 101 (1957).
94 S. D. TSUNG, I. L. NING and S. P. SHIEH, Acta Biol. exp. sin. *10*, 69 (1965).
95 L. VAKAET, C. r. Soc. Biol., Paris *158*, 1964 (1964).
96 J. GALLERA and G. NICOLET, J. Embryol. exp. Morph. *21*, 105 (1969).
97 J. GALLERA, Archs Biol., Liège *82*, 85 (1971).
98 J. GALLERA, Experientia *28*, 1217 (1972).
99 I. ROSTEDT, Ann. Med. exp. Biol. fenn. *49*, 186 (1971).
100 A. LEIKOLA and D. J. MCCALLION, Can. J. Zool. *46*, 205 (1968).
101 J. R. VISWANATH, A. LEIKOLA and I. ROSTEDT, Ann. Zool. fenn. *5*, 384 (1968).
102 D. J. MCCALLION and A. LEIKOLA, Ann. Zool. fenn. *4*, 588 (1967).
103 G. V. SHERBET, Naturwissenschaften *20*, 471 (1962).
104 G. V. SHERBET, J. Embryol. exp. Morph. *11*, 227 (1963).

will elicit a neural response in the epiblast[87,102–106]. Under certain conditions, the epiblast seems to be neuralized even without any specific inductor[107,108]. In all these respects, the avian epiblast resembles Amphibian ectoderm; evidently the neural tendencies are inherent in the cells, and maybe only some metabolic block has to be removed at a proper time in order to start the neuralization.

The biochemical nature of this neuralization is practically unknown. A treatment of the node with pyridine[109] or colchicine[110] will destroy the inductive power of the node, whereas actinomycin D, which blocks the synthesis of messenger RNA, does not affect it[111]. But we do not even know whether these facts are relevant to the actual biochemistry of the induction and determination.

Conclusion

If the hundred years of study on the HENSEN's node – i.e. on gastrulation and early determination of the embryos of amniote vertebrates – teach anything, they teach in the first place how limited and fragmentary our knowledge is about one of the most central problems of the whole developmental biology. We know that the events in early amniote development – or early avian development, on which our data and ideas are nearly all based – in many ways resemble those in early Amphibian development, which is only slightly better understood, but we also know that direct extrapolations from anamniotes to amniotes cannot be made without proper reservations and without studying the amniote embryos themselves. And we have practically no idea of what is really going on in the cells of the blastoderm when they move, invaginate, induce or are induced, interact, become determined and begin their differentiation. We know that at the stages of gastrulation, the node, and indeed the whole blastoderm, is in a very labile state and can be regulated in many ways to produce a harmonious whole – or a monster – although we only understand very poorly the modes of this regulation. The progress made during the decades, and particularly in recent years, shows, however, that useful information is accumulating to produce a coherent picture, and there is no reason to be pessimistic[112].

[105] B. BJERRE and L. NORD, Roux' Arch. *171*, 38 (1972).
[106] B. BJERRE, Experientia *30*, 534 (1974).
[107] B. BJERRE and L. NORD, Experientia *29*, 1018 (1973).
[108] I. ROSTEDT, demonstration, 11th Int. Embryol. Conf., Sorrento (1974).
[109] M. S. LAKSHMI and G. V. SHERBET, Naturwissenschaften *49*, 501 (1962).
[110] B. A. DIWAN, J. Embryol. exp. Morph. *16*, 245 (1966).
[111] J. GALLERA, J. Embryol. exp. Morph. *23*, 473 (1970).
[112] The work done on avian and Amphibian embryology in our laboratory is supported by the Academy of Finland (National Research Council for Natural Sciences, project No. 413-2 551-3 01084670-8). I wish to thank Dr. IRMA ROSTEDT for helpful criticism and Mrs. KIRSTI HORSTIA and Mrs. SINIKKA TÄHKÄ for technical assistance in preparing the manuscript. Thanks are also due to Dr. KOKI HARA, Hubrecht Laboratory, Utrecht, The Netherlands, for reading the manuscript and giving permission to quote his unpublished work. Figure 3 was taken at the Laboratory of Electron Microscopy, Faculty of Agriculture and Forestry, University of Helsinki, with a JEOL-JSM-S 1 scanning electron microscope. I am grateful to that Laboratory for permission to use the microscope and to Mrs. MARJA-LIISA LINDELL for technical assistance.

SPECIALIA

Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. – El responsable de los informes reducidos, está el autor.

Novel Reactions of Rotenone

G. R. BROWN and B. WRIGHT

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderlevy Park, Macclesfield (Cheshire SK10 4TG, England), 15 September 1975.

Summary. Novel reactions of rotenone are described. Demethylation of rotenone under mild conditions was observed. A compound with oxidation state between rotenone and rotenonone was isolated.

A programme of work aimed at blocking in vivo hydroxylation sites of rotenone and examining reactions of the carbonyl group has led to the synthesis of some novel rotenoids.

Material and methods. Action of cupric nitrate on rotenone. Cupric nitrate (400 mg) was added in portions to rotenone (1.3 g) in acetic anhydride (25 ml). After 18 h the mixture was poured on to ice. The gum which separated was extracted with chloroform and chromatographed on alumina in chloroform. Evaporation of chloroform eluates gave yellow solid (160 mg, 12%) **4** mp. p 243–245° (petrol/ethyl acetate) $C_{22}H_{18}O_7$ ($CDCl_3$/$DMSOd_6$): 3.86 (3H, s, 2–OCH_3); 6.07 (1H, d, J6.0, 6–CH); 6.61 (1H, s, 4-ArH); 7.62 (1H, d, J6.0, 6–OH); 8.42 (1H, s, 1-ArH) m/e 394 (M^+ 394).

Action of hydrogen bromide on rotenone. Rotenone (2.0 g) in acetic acid (100 ml) was saturated at 0° over 2 h. After 72 h, the mixture was poured into water, the solid col-