not in percent lipid (unchanged) for undisturbed LD 12:12 larvae at 40, 84, and 109 days. Immaculate larvae[8] (fig. 3b) appeared at LD 12:12 when larvae moulted from the 6th to the 7th or the 7th to the 8th stadium. Immaculate sugarcane borer larvae (borers without pinacular pigmentation) always gained weight and were able to moult. In contrast, the southwestern corn borers did not feed or gain weight during diapause or as immaculate larvae (personal communication, G.M. Chippendale).

Burges[14] found that larval Khapra beetles, *Trogoderma granarium* Everts, occasionally fed, gained weight, and molted during delayed pupation, but it was not clear whether this was quiescence (cold or heat stupor) or diapause because in some cases the delay in pupation could be terminated with fresh food[13,14]. A number of other isolated field observations with various insect species were equally as puzzling[5,15,16]. Mansingh proposed an alternative physiological strategy to quiescence and diapause that allowed insects to deal with seasonal changes of a short-term and moderate nature[5]. He referred to this condition as oligopause. Unfortunately, no definitive studies were available as proof. It appears in the sugarcane borer from both field observations[17] and laboratory study (figs 1–3) that feeding and growth is possible during photoperiodically induced delayed metamorphosis. Delayed metamorphosis in the sugarcane borer prevents adult reproduction during a generally unfavorable winter season; but the option of feeding allows the borer to also take advantage of any intermittent periods when favorable conditions for growth may exist. Feeding during delayed metamorphosis is more than simply for the maintenance of a minimum threshold size needed to successfully complete metamorphosis and adult reproduction since borers are able to double their normal weight (fig. 3b). A greater weight increases the chance of surviving periods of unfavorable conditions when feeding is impossible and may affect fecundity in the spring. The example of delayed metamorphosis in the sugarcane borer is an alternative physiological strategy to the well known conditions of quiescence and diapause. This condition in the sugarcane borer is classified by Mansingh[5] as oligopause but is classified by others as weak diapause[3].

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3 Beck, S.D., Insect Photoperiodism. Academic Press, New York 1980.
4 Jungreis, A.M., in: Dormancy and Developmental Arrest, p. 47. Ed. M.E. Clutter. Academic Press, New York 1978.
5 Mansingh, A., Can. Ent. *103* (1971) 983.
6 Andrewartha, H.G., Biol. Rev. *27* (1952) 50.
7 Fuchs, T.W., Harding, J.A., and Smith, J.W. Jr, Ann. ent. Soc. Am. *72* (1979) 271.
8 Katiyar, K.P., and Long, W.H., J. econ. Ent. *54* (1961) 285.
9 Chippendale, G.M., and Reddy, A.S., Ann. ent. Soc. Am. *65* (1972) 882.
10 Chippendale, G.M., Univ. Mo. agr. Exp. Stn Res. Bull. *1031* (1979) 1.
11 Roe, R.M., Hammond, A.M. Jr, and Sparks, T.C., Ann. ent. Soc. Am. *75* (1982) 421.
12 Yin, C.-M., and Chippendale, G.M., J. Insect Physiol. *20* (1974) 1833.
13 Burges, H.D., J. Insect Physiol. *5* (1960) 317.
14 Burges, H.D., Nature *184* (1959) 1741.
15 Silver, G.T., Can. Ent. *90* (1958) 65.
16 Corbet, P.S., J. exp. Biol. *33* (1956) 1.
17 Holloway, T.E., Haley, W.E., and Loftin, U.C., U.S. Dep. Agr. Techn. Bull. *41* (1928) 1.

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Mast cells are present during angiogenesis in the chick extraembryonic vascular system

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Summary. The extraembryonic vascular membranes of 3-day–18-day chick embryos were examined for the presence of mast cells. As early as 3.5 days mast cells were found on the area vasculosa. It is suggested that these cells have a role in angiogenesis of the chick extraembryonic vascular system.

The extraembryonic membranes of the chick embryo have been frequently used to assay potential angiogenic or anti-angiogenic agents. Most often the mature, non-growing vessels of the chorio-allantoic membrane (CAM)[2] have been employed to demonstrate the capacity of various agents such as infarcted myocardial tissue[3], lymphocytes and embryonic tissues[4], to induce new blood vessel growth. In particular, this system has provided a useful assay for determining the angiogenic properties of neoplastic tissues[5].

The immature, growing blood vessels of the yolk sac vasculature (area vasculosa) of early chick embryos have recently been used to show that embryonic angiogenesis can be promoted and inhibited by applying specific angiogenic and anti-angiogenic molecules[6]. The experiments demonstrated that locally applied protamine (a basic protein) inhibited the growth and expansion of the blood vessels over the yolk sac, causing the formation of a large avascular zone; and that this inhibitory property of protamine could be overcome by heparin. The discovery of the angiogenic properties of heparin and the inhibitory properties of protamine resulted from a series of experiments in which mast cells, and particularly mast cell heparin, were implicated. For example, mast cell accumulation was found to precede the ingrowth of new capillary sprouts at a tumor site[7]; heparin released by mast cells stimulated migration of capillary endothelial cells in vitro[8]; heparin enhanced tumor angiogenesis on the chick CAM[6], and protamine (an antagonist of heparin) blocked mast cell or heparin stimulation of capillary endothelial cell migration in vitro[8].

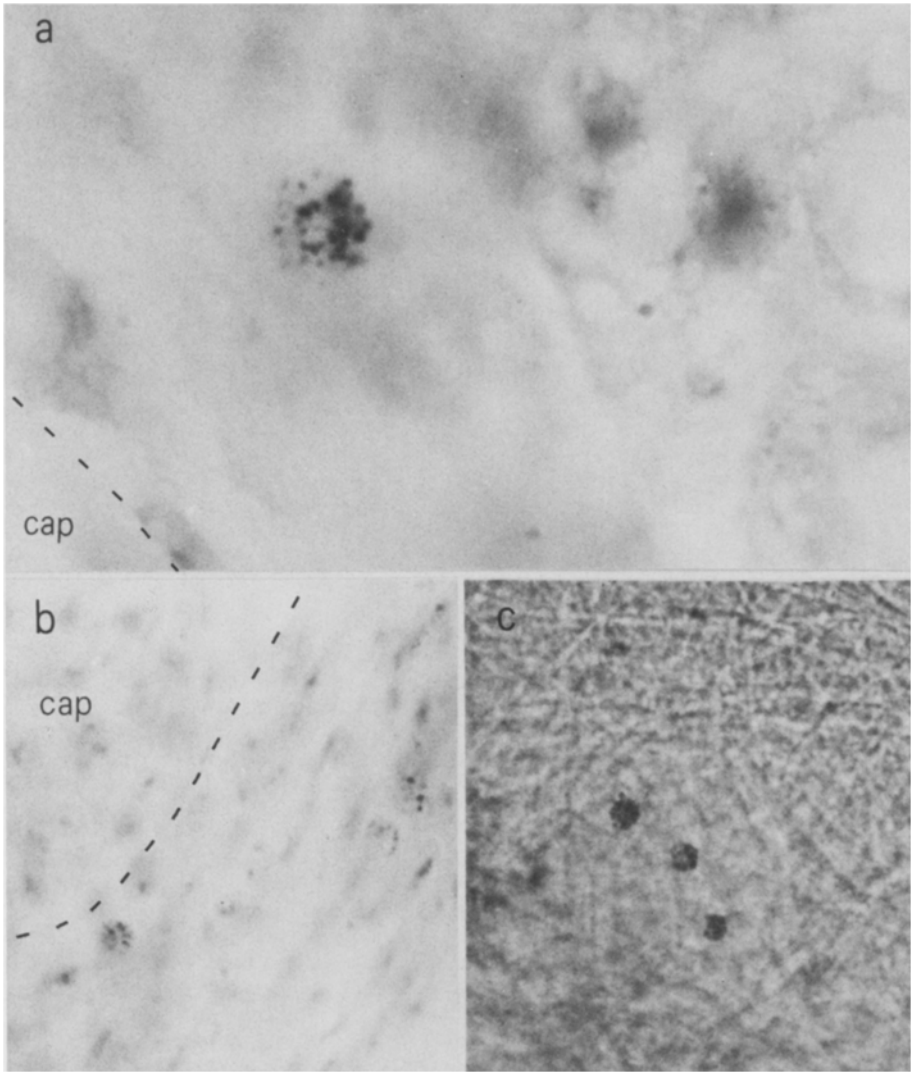
In view of these findings it is possible that mast cells, and the heparin they produce, have a role in normal embryonic angiogenesis. If the angiogenesis accompanying embryonic development is promoted by mast cell heparin, then it is important to demonstrate the presence of mast cells in regions of vascular development. Thus extraembryonic membranes of chick em-

bryos at various stages of development were examined for the presence of mast cells.

Materials and methods. Fertile White Leghorn eggs were incubated for 2.5 days, then windowed according to the basic technique described by Summerbell and Hornbruch[10]. Extraembryonic membranes of eggs incubated for 55 h to 10 days were fixed in ovo using absolute methanol. After dissection the membranes were left in absolute methanol for a further 10 min, and then stained for mast cells using a non-aqueous toluidine blue staining procedure[7,11]. After dehydration, the specimens were cleared in xylene and mounted in Clearmount on glass microscope slides. In total, 52 extraembryonic membranes were stained and examined.

Results and discussion. The development of the extraembryonic vasculature over the period in which the membranes were examined can be briefly summarized: capillaries appear in the area surrounding the embryo at about 48 h by the anastomosis of differentiating hemangioblastic masses. Subsequent sprouting of endothelial cells by migration and proliferation produces an expanding network of small vessels over the yolk sac. Rapid expansion of this vascular network continues over the following 6–8 days, eventually anastomosing with the allantoic vessels[2,9].

The stained, whole mounted membranes revealed metachromatically stained mast cells as early as 3 days of development (fig. a). No other metachromasia was observed in the membrane, which stained orthochromatically. The distribution of mast cells on the 3.5-day extraembryonic membranes was non-uniform; the cells aways occurring in clusters and were usually found in close proximity to the capillaries, particularly at branch points in the vasculature (fig. b), when as many as 20 cells/cluster were observed. Mast cells were also identified in the vessel-free mesoderm, again occurring as clusters but of fewer cells (3–7/cluster). The mast cells were found in regions both near the embryo and the advancing edge of the extraembryonic membrane. Up to 7 days mast cells were generally confined to clusters with the number of cells in each cluster varying between 3 and 24 cells. Such clusters of cells were rather rare (at a frequency of $< 2/5$ mm^2 – obtained by examining the 7-day membranes ($n = 5$) at $\times$ 160 using an occular grid in the microscope). However, by 10 days the mast cells were more evenly distributed often occurring singly and at greater distances from blood vessels (fig. c). Sites chosen arbitrarily for examination at 9 days and later always revealed mast cells, at an average frequency of 3.7 ± 1.6 cells/mm^2 (obtained by counting mast cells in 3 1-mm^2 fields on each membrane, $n = 7$). Precise quantitation of the number of mast cells at the earlier times was extremely difficult because a) rather few cells were present and b) the clustering of the cells presented sampling difficulties. The morphology of the mast cells in the early extraembryonic membranes differed slightly from that of mast cells in extraembryonic membranes at older stages; being larger, containing fewer granular inclusions and could thus be



a Mast cell on area vasculosa at 3.5 days, the granular inclusions stained red and the mesenchyme of the membrane stained blue. $\times$ 1777. *b* Cluster of mast cells lying adjacent to a capillary (cap) on 3.5-day membrane. *c* Mast cells on 10-day chorioallantoic membrane. Note that at later stages the mast cells occur more frequently in vessel-free areas of the membrane (both $\times$ 622).

considered to be less differentiated[12]. Few mast cells in the membranes examined had discharged their granular contents. Accounts of when mast cells first develop are conflicting[13]; however this report of the presence of mast cells at 3 days in the chick yolk-sac vasculature is the earliest at which mast cells have been identified. Recently mast cell precursors have been discovered in the early mouse yolk sac at 9.5 days (equivalent to 3 days of chick embryo development), but the method employed was not able to ascertain at what time the precursors gave rise to characteristic mast cells[14].

The presence of mast cells on the yolk-sac vasculature is significant because it shows that heparin-producing cells are present during a phase of extensive capillary growth in a developing embryonic system. Thus it appears, that the mechanism proposed for tumor angiogenesis, that is, heparin produced by mast cells enhances the directed migration of capillary endothelial cells towards the tumor[15], could also apply to embryonic angiogenesis. Such a unifying mechanism would account for the similar effects of protamine on both tumor and embryonic angiogenesis.

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2 Ausprunk, D.H., Knighton, D.R., and Folkman, J., Devl Biol. *38* (1974) 237.
3 Kumar, S., West, D., Shahabuddin, S., Arnold, F., Haboubi, N., Reid, H., and Carr, T., Lancet *2* (1983) 364.
4 Auerbach, R., Kubai, L., and Sidky, Y., Cancer Res. *36* (1976) 3435.
5 Phillips, P.J., Steward, J.K., and Kumar, S., Int. J. Cancer *17* (1976) 549.
6 Taylor, S., and Folkman, J., Nature *297* (1982) 307.
7 Kessler, D.A., Langer, R.S., Pless, N.A., and Folkman, J., Int. J. Cancer *18* (1976) 703.
8 Azizkhan, R.G., Azizkhan, J.C., Zetter, B.R., and Folkman, J., J. exp. Med. *152* (1980) 931.
9 Romanoff, A.L., The Avian Embryo. Macmillan, New York 1960.
10 Summerbell, D., and Hornbruch, A., in: Culture Techniques-Applicability for Studies on Prenatal Differentiation and Toxicity. Eds D. Neubert and H.J. Merker. Walter de Gruyter, Berlin 1981.
11 Padawar, J., J. Histochem. Cytochem. *7* (1959) 352.
12 Combs, J.W., J. Cell Biol. *31* (1966) 563.
13 Seyle, H., The Mast Cells. Butterworth, London 1965.
14 Sonada, T., Gayashi, C., and Hitamura, Y., Devl Biol. *97* (1983) 89.
15 Folkman, J., Taylor, S., and Spillberg, C., in: Development of the Vascular System, p. 132. Ciba Foundation Symposium No. 100, Pitman, London 1983.

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Evidence for reopening of the cranial neural tube in mouse embryos treated with cadmium chloride

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Summary. Use of the whole-embryo culture technique resulted in experimental evidence that the pathogenesis of exencephaly in mouse embryos after cadmium chloride treatment results from reopening of the cranial neural tube.
Key words. Mouse embryos; neural tube closure; cadmium chloride; embryo cultures; exencephaly; teratogenicity.

Neurulation is one of the earliest and most important processes that developing embryos undergo during the organ formation phase. This process is a very complex one, during which various morphogenetic events take place simultaneously at an extremely rapid rate. In the mouse, for example, the central nervous system (CNS) primordium is established within about a two-day period.

It is known that chemicals can cause CNS abnormalities during embryonic and fetal development. The most common CNS defects are anencephaly in humans[4,5] and exencephaly in experimental animals (for review, see Lemire et al.[6]). The pathogenesis of these anomalies is as yet unknown, as is whether they are due to non-closure or reopening of the neural tube. This study reports the effects of cadmium chloride ($CdCl_2$) on the neural tube closure of mouse embryos. $CdCl_2$ was selected for this study, as this chemical was shown to produce high incidences of exencephaly in term mouse fetuses when administered to pregnant mice during organogenesis[8], as well as to cause changes in the neuroepithelium during the process of neural tube closure[9]. $CdCl_2$ administration to pregnant rats at various intervals after copulation has shown the greatest percentage of accumulation in embryos which were at the early phase of organogenesis[10].

Investigations were carried out with the aid of the postimplantation embryo culture technique[7]. This method not only provides access to embryos at the early stages of organogenesis but also enables direct extracorporeal observation and manipulation of the embryos.

Material and methods. Medium preparation. The medium consisted of 100% male rat serum. Blood for the serum was taken from the dorsal aortas of CD-rats (Charles River Laboratories, Wilmington, MA), and centrifuged immediately. The serum was then decanted and stored at −20 °C. All sera were carefully thawed and heat-inactivated at 56 °C for 40 min prior to use. *Culture conditions.* Early organogenesis CD-1 mouse conceptuses (Charles River Laboratories, Wilmington, MA) were explanted on day 8 (plug day = 0) and the maternal decidua and Reichert's membrane removed. The embryos (3–5 somites) within their yolk-sacs and amnions were cultured at 37.5 °C for

Effects of cadmium chloride on mouse neural tube tube development in vitro observed after a) 24 h and b) 48 h exposure periods

	Cadmium chloride ($\times 10^{-6}$ M)	No. of embryos treated	Embryos showing open neural tubes	Mean morphological score
a)	0.0	25	0 (0)	28.2 ± 0.1
	1.1	14	0 (0)	26.1 ± 0.1
	1.3	14	0 (0)	26.3 ± 0.2
	1.6	6	0 (0)	26.2 ± 0.2
b)	0.0	82	0 (0)	43.3 ± 0.2
	1.1	13	4 (30.8)	39.5 ± 1.3
	1.3	10	2 (20.0)	42.6 ± 0.4
	1.6	14	7 (50.0)	37.1 ± 1.9

Numbers in parentheses: percentages.