lized coconut milk gave similar results with proembryos in the 150- to 250-μ size range, but autoclaved coconut milk inhibited differentiation of proembryos in the 45- to 75-μ size range (Fig. 2*B*, plate *b*), causing them to grow as peculiar flattened masses on the agar. An enlarged view of embryos on the glutamine plate (Fig. 2*B*, plate *d*) is shown in Fig. 2*C*.

Microtome sections of preglobular proembryos reveal considerable histological disorganization and often no evidence of polarity. Small meristems apparently overcome this disorganization to develop normal axial polarity. In large irregularly shaped meristems, or in tissue masses where small meristems are partially fused, the polarity which is initially established seems to be radial rather than axial. Thus embryos developing from large meristems, or from dense clusters of small meristems, do so by bud-like outgrowth in such a manner that most of the tissue from the original meristem or cluster is left as a suspensor-like remnant. Embryos from small single meristems may lack this suspensor-like component.

The techniques described here allow experimental control only of the postglobular stages of embryogenesis. Modifying these techniques may permit some degree of experimental control on defined media over the ontogenetic sequence starting with single cells.

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6 July 1964

Placenta of the Indian Elephant, Elephas indicus

Abstract. *The placenta of the Indian elephant is incompletely annular and zonary macroscopically and occupies the equator of an ovoid chorioallantoic sac. The amnion is fused with the chorion over the zone. Microscopically, the placenta is labyrinthine and endotheliochorial with a rudimentary marginal hematoma. Both macroscopically and microscopically it resembles the placentas of the carnivores, particularly the racoon, the cat, and the dog.*

Classification of the Indian elephant placenta (*1*) is based on scattered reports, almost all published prior to 1908 (*2–5*). Some are incomplete as regards microscopic anatomy (*2*); one is based on less than a full-term gestation (*3*), and others on only portions of the placenta (*4*). In the report of an extensive survey of Eutherian mammalian placentation by Mossman in 1937, the Indian elephant placenta is described as "zonary with villous patches at each end of the chorionic sac. Zone apparently hemochorial and villous patches syndesmochorial, but this is uncertain" (*1*). Recently, two full-term intact Indian elephant placentas were obtained, examination of which indicates that placentation in the Indian elephant is not hemochorial.

On 15 September 1963 and 24 September 1963, respectively, viviparous full-term male and female elephant calves (*Elephas indicus*) were born at the Portland Zoological Gardens. Both calves were sired in captivity by the same bull, a Siamese Indian elephant (Bangkok, Thailand). The mothers were, respectively, a Siamese Indian elephant (Bangkok, Thailand) and an Asiatic Siamese elephant (Saigon, Vietnam). The gestational period for both pregnancies was 634 days plus or minus several days. Intact placentas were delivered and obtained at periods of 2 hours and 30 minutes and 3 hours and 28 minutes after calving, respectively. Weights and dimensions are given in Table 1.

For both calves, the chorionic sac was ovoid (Fig. 1*A*) and was encircled on the maternal surface by an annular equatorial placental zone (Fig. 1*B*). On cross section, the zone was dome-shaped. The lateral convexities of the maternal surface of the zone were smooth and green-brown, and bordered a central elevated spongy area which, like the bulk of the sectioned surface, was a deep red-purple (Fig. 1*C*). In the placenta of the male calf, the annulus was bilobed, consisting of one small and one large lobe separated by distances of 5 and 12 cm, while in that of the female calf the zone was incomplete over a distance of 15 cm. Small (2 to 3 cm in diameter) flattened areas of brown friable tissue were present at the poles of the chorionic sac. The remainder of the maternal surface was smooth, opaque, and pink-tan. The fetal surface was opaque with a faint purple hue. It was characterized by an intricate network of fetal vessels with complex vascular crossings. Numerous plaques averaging 2 cm in diameter were distributed along the course of these vessels (Fig. 1*D*). The amnion was filmy and delicate, and was attached to the chorion over that portion bordered by the placental ring. Over the remainder of the chorionic surface it seemed to be separated from the chorion by a well-defined space (see *1*, plate 22, Fig. D). The umbilical cords, at the fetal extremity, consisted of two arteries and one vein, each measuring 1 cm in diameter. As a result of two venous branchings, but only one arterial branching, the cord entered the chorionic surface of the placenta as four separate arteriovenous pairs, two of which supplied the margins of the villous ring and two entered the center of the ring. In the placenta of the male calf, a pedunculated purse-like yolk sac, measuring 7 by 7 by 1.5 cm, was in proximity to one of the four umbilical vessels. This structure was not identified in the female placenta.

Microscopically, the placenta is labyrinthine and consists of delicate

Table 1. The weights and dimensions of the Indian elephant placentas.

Placental weight (kg)	Diameters of placental zone (cm)	Dimensions of sectioned surface of zone (cm)	Length of umbilical cord (cm)
Male calf, weighing 68.1 kg			
11.3	50 × 42	13 × 5.5	110
Female calf, weighing 109 kg			
11.8	58 × 48	16 × 6.0	114

terminal ramifications arising from major chorioallantoic vascular and connective tissue stalks (Fig. 1*E*). In these terminal branches, capillary and venous vessels run in a fine central core of connective tissue covered by a layer of individual trophoblastic cells which, in cross section, appear as linear columns of single uninuclear polygonal cells. The trophoblast impinges upon maternal capillary vessels from whose lumens they are separated by endothelial cells and a prominent basement membrane which reacts positively with periodic acid–Schiff reagent (Fig. 1*F*). No residua of glands or endometrial stroma are present. Larger, non-capillary maternal vessels are invested with a dense eosinophilic hyaline material at the borders of which are embedded numerous trophoblastic cells. The elevated central portion of the convex sectioned surface is similarly labyrinthine when examined microscopically, and is covered at its maternal border by a dense zone of amorphous eosinophilic hyaline material. The lateral pigmented convexities, which have a depth of approximately 0.5 cm, differ significantly in structure and consist of distinct club-like papillary projections embedded in a zone of thrombus devoid of maternal vasculature (Fig. 1*G*). The trophoblastic cells in these regions maintain their individuality. Histochemically, the trophoblastic cells in the labyrinth react positively for alkaline phosphatase, adenosine triphosphatase, and succinic dehydrogenase, while those in the marginal areas are positive for acid phosphatase, neutral fat (Oil red O) and iron (Prussian blue) (Fig. 1*H*).

The histology of the chorioallantoic-maternal vascular relationships in the Indian elephant placenta is typically labyrinthine and endotheliochorial rather than hemochorial as previously reported (*1*, *4*). Only in the marginal pigmented zone and in the areas at the ends of the chorionic sac are there villi resembling the hemochorial villous pattern seen in man. These apparently defunctionalized areas probably arise by extravasation of maternal blood with degeneration of maternal structures (*1*).

The elephant placenta is strikingly similar to those of some of the carnivores, especially the dog (*Canis familiaris*) (*1*, *6*), the racoon (*Procyon lotor*) (*7*), and the cat (*Felis domestica*) (*6*). Macroscopically, all have zonary and annular placentas. The dog differs in that there is a large marginal hematoma, while in the other species the hematoma is rudimentary. In the elephant, dog, and racoon the placenta is microscopically labyrinthine and endotheliochorial. In the cat the pattern is similar but is modified by the persistence of decidual giant cells in the labyrinth. The distribution of acid and alkaline phosphatase in the elephant placenta is identical to that in the cat and the dog (*6*). The similarity between

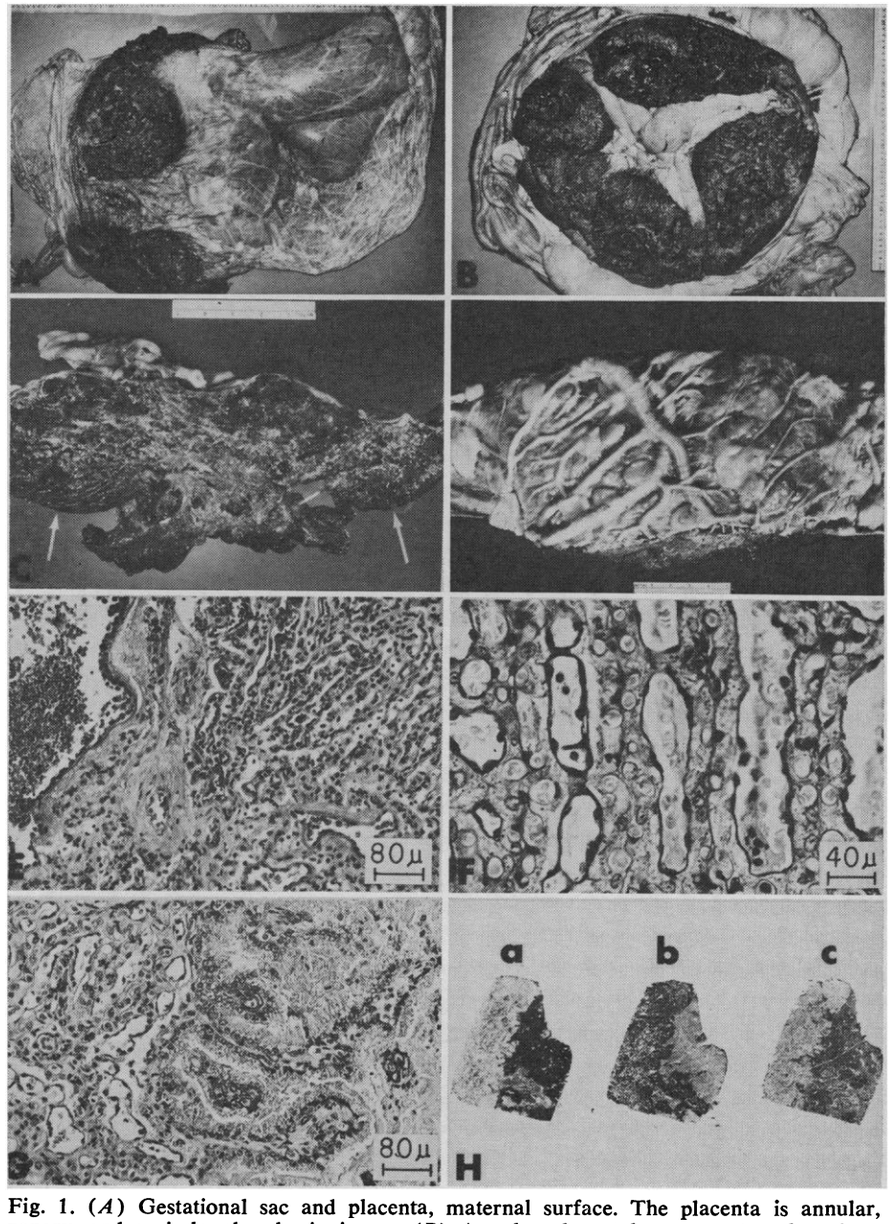

Fig. 1. (*A*) Gestational sac and placenta, maternal surface. The placenta is annular, zonary, and encircles the chorionic sac. (*B*) Annular placental zone, maternal surface. Defects subdivide the zone into two unequal lobules. (*C*) Sectioned surface of zone. The elevated central area is bordered bilaterally by superficial marginal hematomas having a more homogeneous appearance (arrows). (*D*) The fetal surface over the placental zone. The amnion and chorion are fused over the zone and overlay an intricate network of allantoic vessels. The oval, subchorionic, perivascular hyaline nodules can be seen. (*E*) The placental labyrinth. The maternal vessel at the far left is bordered by a zone of amorphous eosinophilic material. Fetal vascular stalks give rise to fine ramifications (hematoxylin and eosin). (*F*) Maternal capillary vessels separated by double columns of trophoblastic cells. Note distinct maternal endothelium and prominent capillary basement membrane. Delicate capillary-venous vessels and a fine connective tissue core are present in the center of trophoblastic columns (hematoxylin periodic acid-Schiff). (*G*) Marginal hematoma. Villi lie in a coagulum of blood and detritus. Note origin of villi from underlying labyrinth (hematoxylin and eosin). (*H*) Serial sections incorporating both marginal hematoma and underlying labyrinth. *a*, Positive reaction for alkaline phosphatase, labyrinth; *b*, positive reaction for acid phosphatase, marginal hematoma; *c*, positive reaction for adenosine triphosphatase, labyrinth.

the placenta of the elephant and those of the carnivores probably represents convergent rather than divergent evolution (*1*).

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Thyrocalcitonin: Hypocalcemic Hypophosphatemic Principle of the Thyroid Gland

Abstract. *A factor that lowers serum calcium and inorganic phosphate in rats has been purified 500-fold from 0.1*N *HCl extracts of hog thyroid glands. It is distinct from thyroxine and triiodothyronine and appears to be a polypeptide.*

Thyrocalcitonin (*1*) is the name given to a hypocalcemic principle readily extracted from thyroid tissue of numerous mammalian species. It was discovered (*2*) as an outgrowth of an investigation of the difference in the acute effect on serum calcium in the rat between parathyroidectomy by cautery and parathyroidectomy by surgical excision (*3*). Apparently, cautery of the thyroid gland during the operation of parathyroidectomy stimulates release of thyrocalcitonin, provoking a greater fall in serum calcium than that which occurs after simple removal of the parathyroid glands.

Since extracts of numerous other tissues, liver, kidney, and salivary gland of the rat, and thymus, pituitary gland, and parathyroid gland of the ox were found not to be hypocalcemic in the rat, thyrocalcitonin-like activity appears to be present in high concentration only in the thyroid gland. Furthermore, the effects of thyrocalcitonin are not duplicated by thyroxine or triiodothyronine.

We have demonstrated thyrocalcitonin activity in extracts of thyroid tissue of the rat, rabbit, dog, hog, ox, and monkey. In addition, Foster *et al.* have prepared an extract of goat thyroid gland that is hypocalcemic in the goat (*4*), and Foster and Hirsch have reported hypocalcemic effects of thyroid extracts in the dog (*5*). Marked hypocalcemic activity in the rat was found in the extract of one sample of human thyroid tissue obtained at surgery, but six other human samples were inactive.

Subcutaneous injection of the extract of as little as one-third of a single rat thyroid gland into an intact rat results in a fall of 20 to 30 percent in serum calcium below the normal level within 1 hour. Hog thyroid extract, selected for purification because of easy availability in quantity, is also very active; the extract of one hog thyroid gland is sufficient to produce a significant fall in serum calcium in more than 1000 rats. Contamination with parathyroid tissue was not a problem since the parathyroid glands of the hog are embedded in the thymus gland at sites remote from the thyroid gland (*6*).

Chemical and physical properties, including behavior during purification, indicate that thyrocalcitonin is a polypeptide. Ashing destroys the activity, an ether extract is inactive, and the activity is lost during treatment with pepsin or trypsin.

A three-step procedure consisting of ultracentrifugation, fractionation on Sephadex G-50, and fractionation on carboxymethyl-Sephadex G-25 has resulted in a 500-fold purification of thyrocalcitonin from the starting extract, which is prepared by homogenizing fresh hog thyroid tissue with 10 ml of 0.1*N* HCl per gram of tissue for 30 seconds in a Waring blender in the cold. The supernatant obtained by centrifuging the homogenate at 600*g* for 30 minutes in an International refrigerated centrifuge can be stored frozen for at least 6 months without loss of activity. A nine- to tenfold purification of this starting extract is achieved by centrifuging it at 100,000*g* for 24 hours in the No. 40 rotor of the Spinco preparative ultracentrifuge. Presumably, the inactive sedimented material is largely denatured thyroglobulin.

A biological assay method was developed to guide purification. The test animals are intact male rats (150 to 180 g) (Holtzman Co.) maintained on a purified diet low in calcium for 4 days before the assay. (Rats on a stock diet react in a similar manner, but their responses are somewhat more variable.) Standard and unknown solutions are injected subcutaneously into parallel groups of test rats. One hour later, blood samples are drawn by cardiac puncture under ether anesthesia, and the serum calcium is analyzed (*7*). A dose-response curve for thyrocalcitonin is shown in Fig. 1. The relative potencies of the unknowns in arbitrary units per milligram of nitrogen are calculated from the results of the bioassay by standard statistical procedures (*8*) and from analysis of nitrogen content by

Table 1. Effect of thyrocalcitonin on serum calcium and inorganic phosphate in rats. There were five test rats in each group, treated as described for thyrocalcitonin assay. Thyrocalcitonin or vehicle was injected subcutaneously 3 hours after parathyroidectomy by surgical excision. Blood was drawn for analysis 1 hour after the injection. The standard errors were: for calcium, 0.2 mg/100 ml; for phosphate, 0.4 mg/100 ml.

Thyrocalcitonin (units/rat)	Serum values (mg/100 ml)	
	Ca	Inorganic P
	Intact	
0	9.3	11.1
10	7.2	8.5
	Parathyroidectomized	
0	8.1	12.4
10	6.0	10.8

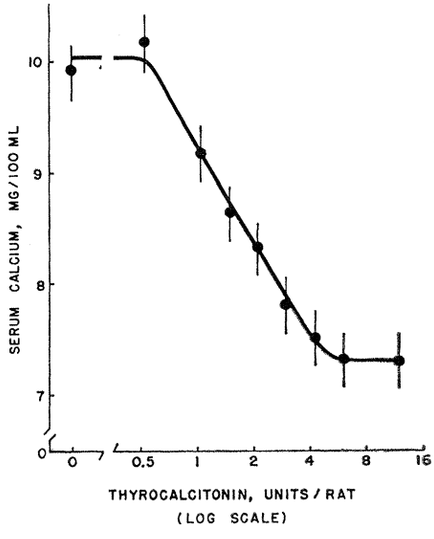

Fig. 1. Log dose-response curve for hog thyrocalcitonin in young male rats. Blood was drawn for calcium analysis 1 hour after subcutaneous injection of the extract. Each point represents the mean value for seven rats, and the vertical lines represent the standard errors.