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Review

The active migration of germ cells in the embryos of mice and men is a myth

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Much is written about the independent migration of cells during normal embryonic development. However, for any putative migration, few authors give a frame of reference for the movement or a velocity; both are necessary to prove active cell migration. As it is now known that (i) sclerotomal cells do not migrate to form the axial skeleton; (ii) cranial neural crest cells do not migrate to form facial mesectoderm; and (iii) mesodermal cells do not migrate from the primitive streak, it is essential to review the evidence for the active migration of germ cells. The use of a frame of reference in a re-examination of data in the seminal paper postulating amœboid germ-cell migration in humans indicates that there is no active migration at all and that the displacement of germ cells can be explained by the global growth movements of the embryo. Such displacements are unrelated to the artefactual movements of explanted germ cells or the appearances of dead cells. The study of human embryos forces a re-examination of evidence for the active migration of germ cells in other species, such as the mouse, where the impact of growth movements appears to have been too casually dismissed.

The notion that cells in the developing embryo actively migrate like homing animals to attain their final destinations has become almost an axiom, some might say a dogma, of contemporary biology. The migration of mesodermal cells away from the primitive streak, the migration of sclerotomal cells to form the axial skeleton, the migration of neural crest cells to make, for example, the connective tissues of the facial region (mesectoderm), and the migration of germ cells to the gonad are cited frequently as examples of cell migration. Schematic diagrams in textbooks and treatises are replete with arrows indicating the directions of cell migrations and substantial funds are expended annually on the search for the molecular signposts, gateways and stop signs, as well as the cellular mechanisms, associated with the movements. Congenital defects are frequently ascribed to failures of cell migration.

However, it has been shown that mesodermal cells in mouse embryos do not migrate actively between the ectoderm and endoderm (Poelmann, 1981). Further, it has been proven that sclerotomal cells in rat embryos do not migrate to form the axial skeleton (Gasser, 1979). Several authors have argued that differential growth mechanisms are more significant than any assumed independent migration of cranial neural crest cells in the development of facial structures (Vermeij-Keers and Poelmann, 1980; Nichols, 1986). In the embryonic central nervous system, Morest and colleagues have long been providing proof that the translocation of young neurones is not due to amœboid cell migration (Morest, 1970; Book *et al*., 1991).

Bronner-Fraser (1982) reported that inert latex beads implanted in a chick embryo along a so-called 'migration pathway' became displaced in a similar fashion to the neural crest cells that were supposed to be actively migrating. She concluded that neural crest-cell displacement was influenced by 'a large driving force resulting from the environment and independent of active cell migration' and that the nature of this large 'environmentally directed component' was still unknown.

In view of the above claims against active cell migration in normal development and Bronner-Fraser's mysterious driving force, it is essential to review the evidence that germ cells migrate during ontogeny. As early as 1921, it was emphasized that 'migration' was an unfortunate term to describe the displacement of germ cells in fish embryos, as the movement of the sex cells is 'passive, being due to the forces of growth which are altogether external to the cells themselves' (Richards and Thompson, 1921). The more cautious of contemporary views about germ-cell movement is that the actual mechanism for the displacement is unknown for the majority of vertebrates (Zuckerman and Baker, 1977) or that a combination of active and passive phenomena is involved (Nieuwkoop and Sutasurya, 1979, pp. 123–125).

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Table 1. Human embryo data

Embryo Carnegie number	Carnegie stage	Number of somites	Crown–rump length (mm)	Average age (days)	Notochord arc length (mm)	Arc distance (notochord tip – germ cells) (mm)
8005	11	16/17	3	24	2.9	2.3
7852	12	25	3.7	26	6.0	ID
7889	13	32	4.2	28	7.3	3.3
1380	14	38	5.5	32	10–11*	3.4
792	16	NR	8	37	16–19†	ID

Data in this table are compiled from Witschi (1948), Blechschmidt (1960, 1963) and O'Rahilly and Müller (1987). Crown–rump length is the chord distance as conventionally measured with callipers; average age is postovulatory embryonic age in days; notochord arc length is the actual curvilinear length of the notochord, estimated from total reconstructions of the same or similar embryos; arc distance (notochord tip – germ cells) is the curvilinear distance from the cranial tip of the notochord to the location of the most rostral germ cells; *estimate based on reconstructions and lateral photograph of embryo no. 1380 as described in text; †estimate based on lateral photographs of similar Carnegie stage 16 embryos; ID: insufficient data available for estimation; NR: data not recorded.

The present review concentrates on the claim for migration of human germ cells, and also analyses recent evidence for germ-cell migration in mice (Molyneaux *et al.,* 2001).

Human

As almost all contemporary claims for human germ-cell migration (for example, Nieuwkoop and Sutasurya, 1979; Baker and Eastwood, 1983; Buehr, 1997) can be sourced to the 1948 Carnegie contribution of Emil Witschi (1948), this review will focus on a re-examination of the evidence presented in that report. Witschi was surprised at how easily he could recognize embryonic germ cells in serial histological sections. By making graphical reconstructions of some early embryos in the Carnegie Collection and marking the positions of individual germ cells, Witschi believed he had provided conclusive proof for active migratory ascent of a 'front' of germ cells at the head of a column.

The youngest embryo graphed by Witschi had 16 somites and a crown–rump length of 3 mm (embryo no. 8005 of the Carnegie Collection). This embryo, which has pronounced cervical lordosis, is now classified as stage 11 by O'Rahilly and Müller (1987); technical details are summarized (Table I). Witschi identified and plotted a total of 109 germ cells, producing his 'famous picture' (Hilscher, 1983) of the topographic distribution of the earliest human germ cells. The essential parts of this picture are redrawn in this review (Fig. 1, bottom left). In this figure, the approximate position of the notochord, indicated by the thick black line, has been superimposed according to the location and extent of the notochord in reconstructions of similar human embryos (Blechschmidt, 1960; O'Rahilly and Müller, 1987). Most germ cells (dots, Fig. 1, bottom left) were found cranial to the allantois (*a*) in the ventral and ventrolateral walls of the hindgut, partly in the endoderm and partly in the mesoderm. Twenty-four germ cells were found in the nearby wall of the yolk sac. From the scales of Witschi's geometrical reconstruction and related photomicrographs, it is possible to determine more accurate positional information as follows. In the transverse plane, the germ cells of embryo 8005 appear to lie in the range 100–600 μm from the axial centre of the notochord. Longitudinally, it is possible to estimate that the most rostral germ cells lie at an arc distance (that is, actual length of the sinuous notochordal arc) of about 2.3 mm from the cranial tip of the notochord, which in these embryos is located dorsal to the buccopharyngeal membrane (*b*, Fig. 1), close to the cephalic flexure.

The choice of the cranial tip of the notochordal process as a reference to locate the 'advancing' germ cells is not arbitrary. In a growing embryo, the notochordal axis has been long recognized as the natural reference for transverse displacements because the notochord manifests relatively little growth in the transverse plane (Blechschmidt, 1960; Blechschmidt and Gasser, 1978). Furthermore, the cranial end of the notochord is a site of zero growth and so provides a null-point for embryonic developmental movements in any direction, for example, transverse and longitudinal (Blechschmidt, 1978). Clearly, the notochordal process increases in length as the embryo grows, but this increase is due to appositional growth at the caudal (primitive steak) end during the so-called unrolling of the embryo (Blechschmidt, 1960, 1978). At the cranial tip and along the length of the notochord, there is hardly any cell division and little interstitial growth. It is as though the notochordal process (and the subsequent solid notochord) elongates like a single queue at a closed ticket window, with ever more people joining the tail of the queue. The cells for notochordal elongation are continually supplied by the surface growth of the embryonic ectoderm cranial to the primitive pit. The growth in length of the notochord that occurs appositionally at its caudal end between Carnegie stages 6 and 9 and the unique reference point that is thereby associated with the cranial tip of the notochord is illustrated (Fig. 2). The cranial tip of the notochord is the most quiescent, fiducial structure in the growing

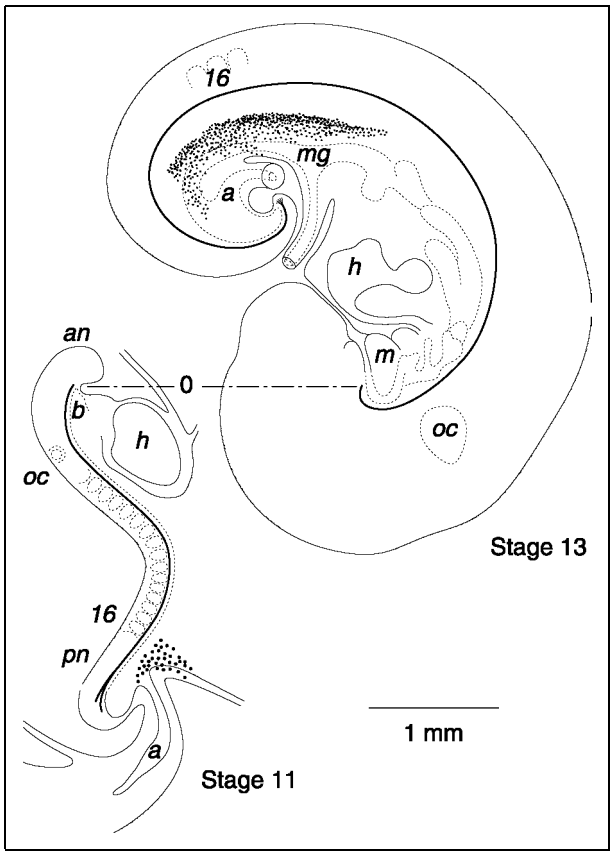

Fig. 1. Outline drawings of sagittal views of two human embryos (bottom left: 3 mm crown–rump length, 16 somites, stage 11; top right: 4.2 mm crown–rump length, stage 13) with the positions of germ cells indicated by black dots. The outlines of the embryos, which are drawn to the same scale, are based on reconstructions and diagrams by Witschi (1948, 1956). Sufficient germ cells have been marked in each embryo to indicate the spatial extent of the population. In each embryo, the notochord has been superimposed as a solid black line ventral to the somites, its position being based on the location and cranial extent of the notochord in similar embryos (Blechschmidt, 1960, 1963; O'Rahilly and Müller, 1987). The two embryos have been mutually oriented with the tip of the notochord at the same level (reference level: dot–dash line marked 0) and with similar curvature in the most cranial section of the notochord; the oldest part of the notochord therefore serves as a reference for the subsequent growth movements of the whole embryo. Note that as the embryo grows, the germ cells are carried further from the cranial tip of the notochord, but remain within about 500 μm of the notochordal axis at all times. In both embryos, the endoderm is indicated by the dashed line(s) ventral to the notochord; it can be seen that between stages 11 and 13, the endoderm grows ventrally away from the notochord. In the stage 11 embryo, the caudal end of the notochord cannot be defined precisely as it arises from an undifferentiated population of cells, which includes the primitive streak, the incipient neural folds, and the most caudal endoderm of the hindgut. *an*: anterior neuropore; *pn*: posterior neuropore; *oc*: developing otocyst; *a*: allantois; *b*: buccopharyngeal membrane; *h*: heart; *m*: mandibular arch; *mg*: midgut; *16*: somite 16. Scale bar represents 1 mm.

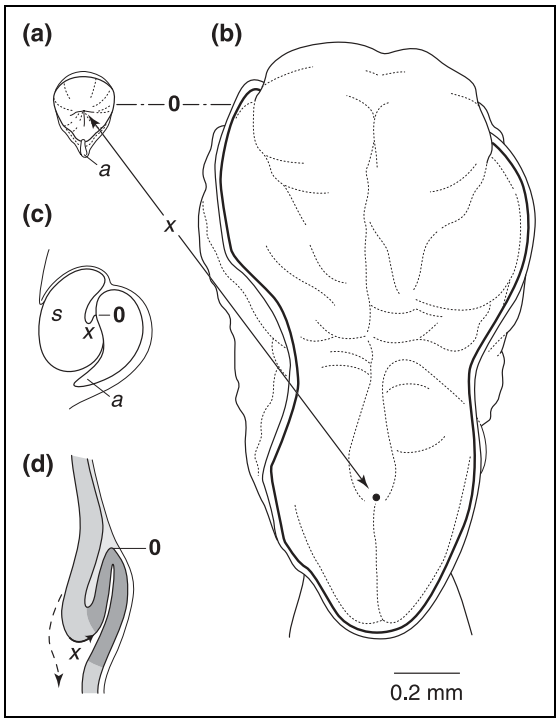

Fig. 2. (a) and (b) Drawings based on three-dimensional total reconstructions of two human embryos of different ages, reproduced at the same scale. The ectoderm is viewed from dorsal after removal of the amnion. The cranial end of each embryo is at the top and the embryos have been aligned so that the cranial tip of the notochordal process of each is in register (dot–dash line marked 0). The caudal end of the embryo is indicated by the allantois (*a*) in the younger embryo (a), and the connecting stalk in the older embryo (b) (above the scale bar). The primitive pit region, marking the entrance to the notochordal process at its caudal end, is indicated by *x* in each embryo. Embryo (a) is about 0.23 mm long (Carnegie no. 10318; stage 6; about 13–14 days; Blechschmidt embryo) and embryo (b) is about 1.8 mm long (Carnegie no. 5982; stage 9; about 20 days; Ludwig Da1 embryo). Note the large increase in surface area of the ectoderm of embryo (b), particularly between the levels 0 and *x*, and the division of the single brain bulge in embryo (a) into the right and left brain bulges separated by the neural groove in embryo (b). (c) Schematic diagram of a mid-sagittal section through embryo (a) (enlarged; cranial end at top). 0 indicates tip of notochordal process; *x*: primitive pit; *s*: amniotic sac; *a*: allantois. (d) Schematic diagram of a mid-sagittal section through an embryo at a stage of development between stages 6 (a) and 9 (b); cranial end at top; alignment is in same sense as (c). 0: tip of notochordal process (dark stipple); *x*: primitive pit; ectoderm (in vicinity of neural groove above *x* and in primitive streak region below *x*) is indicated by fine stipple; endoderm is indicated by thin line at right; coarse stipple indicates gliding layer between ectoderm and notochordal process. The curved arrow above *x* indicates appositional growth at the caudal end of the notochord due to the continual increase in surface area of the neural ectoderm cranial to *x*. The dashed arrow indicates the overall direction of spread of the ectoderm relative to the null-point 0 at the cranial tip of the notochordal process. These diagrams have been modified from Blechschmidt (1978) using data from O'Rahilly and Müller (1987). Scale bar represents 0.2 mm.

embryo and, as such, is the natural reference for the analysis of all displacements of cells and cellular ensembles.

In an older embryo (Carnegie no. 7852, 3.7 mm crown–rump length), Witschi identified germ cells lying within 140 to 290 μm of the centre of the notochord in the transverse plane; no information is provided, or can be easily extracted concerning the longitudinal position of these germ cells with respect to the tip of the notochord. However, the next older embryo (Carnegie no. 7889) is described in greater detail: it is 4.2 mm crown–rump length with 32 somites and is classified as stage 13 (O'Rahilly and Müller, 1987). Witschi (1948) provides only a partial reconstruction of the caudal two-thirds of this embryo; the locations of some 1366 germ cells are plotted in a ribbon-like arc. As the cranial portion of the embryo is missing in the diagram provided by Witschi (1948), it is initially difficult to give the position of the most rostral germ cells with respect to the cranial end of the notochord. However, an illustration (minus the notochord) of a full reconstruction of this same embryo is encountered in the frontispiece of a subsequent textbook (Witschi, 1956), with what appears to be the identical population of germ cells superimposed. The essential parts of this reconstruction of the 4.2 mm embryo have been redrawn (Fig. 1, top right). The approximate position of the notochord has been indicated as a thick black line on this drawing, again estimated from published reconstructions and atlases of similar embryos (Blechschmidt, 1960, 1963; O'Rahilly and Müller, 1987). It can be seen that the most rostral germ cells lie about 200 μm ventral to the notochord and at an arc length of about 3.3 mm from its cranial tip (Fig. 1, top right; Table 1).

During the period between stages 11 and 13, the embryo grows into a tight coil, so much so that crown–rump lengths (measured as a chord using vernier callipers) frequently decrease with embryonic age. Therefore, it is better to compare arc lengths rather than chordal distances. Thus, between the stage 11 and stage 13 embryos (Fig. 1), using the tip of the notochord as a reference point, the most rostral germ cells do not ascend but rather descend axially along an arc of length about 1.0 mm. This conclusion is based on the two most accurately reconstructed embryos for which germ cell location is known in relation to the entire embryo (8005 and 7889). It is estimated from the scale of Fig. 1 that the total arc length of the notochord increases from about 2.9 mm at stage 11 to about 7.3 mm at stage 13. On the basis of an average interval of 4 days between these two stages (Table 1), the speed of notochordal elongation by appositional growth at the primitive streak end is about 40–50 μm per h. Relative to the tip of the notochord, the most rostral germ cells appear to descend at the rate of about 1 mm over 4 days, that is, 10 μm per h. The germ cells remain at essentially the same transverse distance from the notochord, the closest being about 150–200 μm from its central axis, during these 4 days.

The graphical representations of germ cell locations in the next two embryos studied by Witschi (Carnegie no. 1380, 38 somites, 5.5 mm, and Carnegie no. 792, 8 mm) are based on partial reconstructions, which do not give the location of the notochord. However, a lateral photograph of embryo no. 1380 (classified as Carnegie stage 14) and a scaled reconstruction of its digestive system are published in O'Rahilly and Müller (1987). Matching the scales of the photograph, Witschi's partial reconstruction, and the total reconstruction of the digestive system with data on notochordal position from total reconstructions of similar embryos (Blechschmidt, 1960), it is possible to estimate the arc length of its notochord as 10–11 mm and the distance from the notochord tip to the most rostral germ cells as 3.4 mm (Table 1).

After the notochord, the next best fiducial structure with minimal growth and least positional change is the superior mesenteric artery, as it has been shown that all embryonic arteries grow less rapidly in length and change position less markedly than the cellular ensembles in their territories of supply (Blechschmidt, 1978; Blechschmidt and Gasser, 1978). From Witschi's data for embryos 7889, 1380 and 792, it is apparent that the most rostral germ cells remain at approximately the same distance relative to the position of the superior mesenteric artery. Superposition of Witschi's data on reconstructions of embryos of similar stages (Blechschmidt, 1960, 1963) indicates that the most rostral germ cells always descend relative to the cranial end of the notochord; they never ascend.

In summary, during development between 3.0 and 8.0 mm (that is, from stages 11 to 16), during which time Witschi claimed the germ cells are most actively migrating in a rostral direction, the germ cells are descending with respect to a static reference point, the notochordal tip. Using Occam's razor, the simplest interpretation is that the germ cells are embedded in the caudal tissues of the embryo (such as allantois, hindgut and cœlomic serosa) and are simply carried along passively during the curvilinear unrolling of this caudal region. The net displacement of germ cells is proportionately less than the elongation of the notochord because the local tissues containing the germ cells lie more ventral than the notochord and so are dragged through a smaller arc. In turn, the notochord elongates less than the neural tube, which at this time is responsible for the global growth of the embryo. As the tissue sheets containing the germ cells do not keep pace with the curvilinear growth of the nervous system and notochord, the individual germ cells appear to descend as these structures appear to ascend.

From Fig. 1 (top right), it can be seen that the total arc length of the ribbon of germ cells at stage 13 is approximately 2.2 mm, or about half the crown–rump length of the whole embryo. After this stage, all that would be required to produce a ribbon-like gonad

about 2 mm long in older embryos is for the germ cells simply to multiply *in situ*. In fact, in a 30 mm fetus, the length of the ovary can be estimated to be about 2.1–2.2 mm (Blechschmidt, 1963). Thus, between stage 13 of embryonic development and early fetal development, it appears that growth movements gradually cease to play a major role in gonad formation. The gonad grows interstitially in width and hardly at all in length during this period: all the major work of displacement is performed before stage 13.

It therefore appears that human germ cells (i) do not actively migrate at any stage, either up or down, or ventrally from the notochord; (ii) are displaced by growth (morphogenetic) movements along a ventral arc until about stage 13 (that is, about 4.2 mm crown–rump length) when they achieve their final ribbon-like topography (Fig. 1, top right); and (iii) multiply locally within a nascent gonad that remains about 2.0 mm long from stage 13 until the start of fetal life. These findings provide quantitative parameters for earlier, qualitative accounts of the growth movements associated with the displacement of human germ cells, which emphasize the importance of the ventrolateral surface of the dorsal aorta in the biochemistry and biomechanics of their displacement (Blechschmidt, 1960, 1978). It is as though between stages 11 and 13, the germ cells are sequestered in an increasingly tension-free environment, protected from external influences, and nourished directly from the nearby aorta.

Quantitative analysis (referenced to the notochord) therefore indicates that germ cell descent in human embryos is merely a consequence of the failure of the ventrocaudal growth movements to keep pace with the faster dorsocranial movements accompanying the ascent of the neural tube. In human embryos, active, cell-driven displacements of about 50 μm might take place, but these are not migrations in the sense claimed by Witschi and most authors since; they are simply displacements as a consequence of cell division.

Mouse

According to Buehr (1997), evidence for the active migration of germ cells in mice was first established by the study of Mintz and Russell (1957). The subsequent study by Ozdzenski (1967), which stated that the spatial distribution of mouse germ cells from the base of the allantois to endoderm was more likely to arise from 'unequal growth of different parts and layers of the egg-cylinder' than by migration, has been ignored. Rather, migration is claimed to occur in mouse embryos because the primordial germ cells (PGCs) are at the base of the allantois at day 7.75–8.0 after coitus, in the primary endoderm and early hindgut at day 8.5 after coitus, just entering the mesentery at day 9.5 after coitus, and in the genital ridges at day 10.5–11.5 after coitus (Mintz and Russell, 1957; Tam and Snow, 1981; Buehr, 1997). However, these reports do not give a reference frame or a velocity for the movement and no data are provided on the distances between these structures that would allow a speed to be computed. Germ-cell counts at various locations and ages (Tam and Snow, 1981) are presented without a spatial co-ordinate, creating the appearance that the bulk of germ cells is migrating from the allantois to the genital ridge. Indeed, Snow and Monk (1983) commented subsequently that the data of Tam and Snow (1981) 'suggestive of active migration may . . . be artefactual' and that 'it seems likely that mouse PGCs get an 'assisted passage' . . . due to the morphogenetic expansion of the tissues through which they travel'. And Buehr (1997) concedes that, from day 7.5 to day 9 after coitus, 'the (germ) cells are probably caught up in the morphogenetic movements of the developing gut', but adds that 'a clear and unequivocal picture of the mechanisms controlling PGC behaviour in the living embryo is still lacking'.

This lack appears to have been supplied by the exquisite time-lapse experiments with confocal microscopy, monitoring the positions of fluorescent germ cells in slices of mouse embryos, performed by Molyneaux *et al*. (2001). Molyneaux *et al*. (2001) present actual velocities for cell displacements and, as the findings are interpreted as definitive proof of germ-cell migration, it is important to analyse them and ask whether there are other interpretations. Several aspects of these experiments, which will now be considered, indicate that their velocity data cannot be interpreted as proof of active migration.

First, over the 8 h of time-lapse cinematography, the reference for evaluating the velocity of germ-cell displacement appears to be the starting position of the cell at the first image capture with the trajectory plotted forwards, or else the position of the cell at the final 19th image capture with the trajectory plotted backwards. Either way, the velocities appear to be referenced ultimately to the video frame, which one assumes remains fixed with respect to the preparation. However, if the slice preparation itself is moving by growth, pulsating normally or abnormally with respect to the video frame, then the cell velocities are not absolute. A comparison of images of transverse slices taken hours apart indicates there has been some translation and possibly rotation of the preparation; certainly the notochord has shifted. In such preparations, the use of an external frame of reference means that velocities alone cannot be construed as indicating active migration. In particular, between embryonic day 9.0 and 9.5, the trajectories of the germ cells within the wall of the hindgut could be entirely passive, resulting from bulk growth movements of the slice combined with the multitude of lateral forces created by the division of adjacent endodermal cells in the wall of a tube with a tense basement membrane. It is known that asynchronous waves of mitotic division pass spirally along the intact gut tube (Carey, 1920, 1921). It

is not known whether the gut tube is growing normally in these slice preparations, but the movement patterns of the germ cells at this time are reminiscent of the Brownian motion of small inert particles buffeted in a fluid that is subjected to a slight thermal gradient.

Second, after embryonic day 9.5, the endodermal gut tube grows and moves ventrally from the notochord with the pair of dorsal aortae gradually fusing to occupy the intervening space. Thus, the apparent dorsal migration of germ cells shown by Molyneaux *et al.* (2001) at this time could also be entirely passive if the germ cells were squeezed out of a ventrally shifting endodermal tube, similar to the way in which cells are expressed from the crests of the neural folds. The germ cells could be simply shed behind the moving gut, multiplying close to the developing aorta. Between embryonic day 9.0 and 9.5, according to Molyneaux *et al*. (2001), the gut is moving ventrally away from the notochord at an absolute velocity of 5–6 μm per h in the transverse plane, relative to the notochord. Between embryonic day 9.5 and 10.5, the rate of ventral displacement of the gut relative to the notochord appears to be even faster, about 17–20 μm per h. This value is consistent with measurements from the Kaufman (1992) atlas where, between embryonic day 9.0 and 10.5, the rate of ventral displacement of the gut is 10–20 μm per h relative to the notochord. Of course, these velocities are mere estimates as it is impossible to compare the movements unless the precise transverse levels in the different embryos are known. However, it is striking that they are similar to the average velocities at which the germ cells are supposed to be actively migrating at these times.

Third, Molyneaux *et al*. (2001) note that the rate of displacement of the germ cells in the slice declines with age, with an average speed of 13.2 μm per h at embryonic day 9.5 (when germ cells are being shed from the moving endodermal gut tube), decreasing by more than threefold to 3.9 μm per h at embryonic day 12.5 (when the gonad is well-defined). The average maximum speeds show a similar decline from 34.7 μm per h at embryonic day 9.5 to 10.6 μm per h at embryonic day 12.5. Tam's (1981) detailed analysis of mouse embryonic growth indicates that the speed of the axial (global) elongation of the embryo is also decreasing over this same period, from about 220 μm per h at embryonic day 9.5 to about 30 μm per h at embryonic day 12.5.

Therefore, between embryonic day 9.5 and 12.5, there is an order of magnitude difference between the speed of embryonic axial elongation and the maximum speed of germ-cell displacement; this disparity is even greater when the average speeds of germ cell displacement are considered. Tam (1981) comments that the rate of axial elongation is a reliable index for the rate of tissue incorporation at the caudal end of the embryonic axis, so it can be inferred that some tissues located at the caudal end of the embryo are shifting by at least an order of magnitude faster than the fastest germ cells. Although the measurements of Tam (1981) are based on 15 intact animals, those of Molyneaux *et al.* (2001) appear to be based on just seven cells in explanted slices. Nevertheless, the disparity in the above speeds provides some indication of the small quantum of the total energy in the growing embryo that would be required to displace a few hundred germ cells relative to the notochord. The passive displacement of germ cells appears to be directly related to subcomponent(s) of the global growth of the embryo: when the dynamics of the whole decline from embryonic day 9.5 to 12.5, so too do the partial dynamics of germ-cell displacement.

In summary, the findings of Molyneaux *et al.* (2001) can be re-interpreted as support for the diametrically opposite view that the germ cells are left behind a moving hindgut. As the germ cells are shed, sporadic local forces, such as might arise from cell division and cell death, impinge on them to produce irregular displacements, sometimes faster, sometimes slower than the vectorial displacement of the gut. The trail of germ cells must be associated with local division of both somatic and germ cells, as it is estimated that in the mouse there is more than a 20-fold increase in the number of germ cells between embryonic day 9.5 and 12.5 (Tam and Snow, 1981).

The similarities between mouse embryos and human embryos are striking. As with human embryos, it cannot be claimed that cells in mouse embryos are moving independently if, at the same time, the local environment of the cells is also shifting relative to a less labile structure such as the notochord. A life buoy dropped from a moving ship has a velocity of displacement relative to the ship, but the buoy's movement is not active. Perhaps A. L. McLaren is hinting at this when, in the discussion following a symposium paper on germ-cell migration (Gomperts *et al.*, 1994), the remarkable comment is made that '*in vivo,* the germ cells don't do much active migration at all'. The most parsimonious hypothesis is that differential morphogenetic (that is, growth) movements and divisions of adjacent cells, combined with local germ-cell division, could account for the appearance of increasing numbers of germ cells in different regions of the growing mouse embryo. All that is required to test this hypothesis is a more careful analysis with a reference frame, a ruler and a timepiece.

Movement of germ cells *in vitro*

Imagery of human germ-cell migration based on histology is supposedly complemented by actual images and time-lapse films of moving human oocytes recovered from embryos and fetuses (Blandau, 1969; Blandau and Odor, 1972; Kuwana and Fujimoto, 1983). In the Blandau investigations, the youngest oocytes were prepared from the ovaries of embryos 22 mm in length, well after the period of development studied

by Witschi. Furthermore, the explanted pieces of ovary were subjected to quite abnormal conditions; it is unclear whether the patterns of movements exhibited by these older cells after being squeezed between a glass slide and a coverslip bear any resemblance to the behaviour of the cells *in vivo* at earlier times. Blandau (1969) commented that the 'ameboid movements of individual human oogonia in squash preparations are not as remarkable as those we have observed in the mouse, hamster, rat and guinea-pig' and that the cells did not exhibit 'forward progression' as much as do rodent oocytes. Apart from the unnatural conditions, including the use of xenostimulants such as 10% horse serum to engender survival and movement of oogonia, it is clear that moving oocytes were found only in occasional squash preparations.

Kuwana and Fujimoto (1983) studied cultured PGCs obtained from the dorsal mesentery of human embryos of 5 weeks gestational age, at a time when Witschi (1948) claimed the cells were migrating. Whether the PGCs moved depended critically on the type of culture substrate. The authors state that 'many PGCs examined neither adhered to . . . nor moved on these substrates' and that on other substrates 'some PGCs moved slightly'; examination of their Fig. 3 indicates that 'slightly' signifies a displacement of portions of the cell of less than 10 μm in 2 h, while other parts of the cell remain fixed in position. The one cell of Kuwana and Fujimoto (1983) that showed 'the most active motility' over a 48 h period in a special three-dimensional collagenous substrate is illustrated in their Figs 4 and 5. However from these two figures, it can be seen that the cell movements are random, and that the net displacement of the cell after 48 h is only 34 μm from the starting position. For this single 'most active' cell (the long axis of which sometimes extended to 92 μm), such a miniscule net movement can have little relation to the distances (mm) that are supposed to be covered in 2 days by the putative migration of hundreds of germ cells *in vivo*. Furthermore, this sometimes moving, sometimes stationary, sometimes elongating, sometimes rounded, and sometimes phagocytic cell, which Kuwana and Fujimoto (1983) observed, is clearly not a normal germ cell.

It cannot be argued that *in vitro* conditions are a suitable model for *in vivo* growth: cells *in vitro* are freed from the intercellular relationships and growth forces, tensions and pressures that are present in the living embryo. It is not surprising that cells begin to exhibit different properties under such conditions. The fact that 'liberated' former germ cells may crawl actively at rates of up to 133 μm per h across gamma-irradiated mouse fibroblasts in a medium with suitable chemical conditioners (Stott and Wylie, 1986) is irrelevant to what might or might not occur *in vivo*. Nor is it remarkable that older germ cells may behave differently from younger germ cells when removed from embryos of different ages (Donovan *et al.,* 1986). These cultured cells are no longer normal germ cells in a natural environment. Furthermore, in a process reminiscent of sophistry, the unique chemicals and conditions of the culture are selected on the basis that they engender precisely the appropriate cellular performance required to validate the hypothesis of active cell migration *in vivo*.

Electron microscopic studies of germ cells

Evidence for amœboid migration based on electron microscopic investigation of germ cells is also equivocal, insofar that the morphological interpretations generally start from the assumption that Witschi's original conclusion about active migration is valid. In human embryos, fixed germ cells are described as having an irregular appearance and possessing pseudopodia or elaborate cytoplasmic processes during their displacement phase, but a rounded appearance before migration and after settlement in the gonad (Fujimoto *et al*., 1977; Lin *et al*., 1982; Makabe and Motta, 1989; Motta *et al*., 1997a, 1997b). However, this is a circular argument: the cytoplasmic processes are interpreted as evidence of the assumed active cell migration. The processes could equally represent trailing strands of cytoplasm that are left behind as cells are dragged while being held to adjacent somatic cells by desmosomes (Fukuda, 1976; Fujimoto *et al*., 1977), by 'direct' or 'indirect' adhesions (Lin *et al*., 1982), or by 'close contact' (Wartenberg, 1983; Makabe and Motta, 1989; Motta *et al.,* 1997a). The fact that microfilaments and microtubules could be revealed only 'sometimes' in germ cell processes (Miyayama *et al*., 1977) confirms the view that such processes may not always signify active cell locomotion.

In a similar way, the role of fibronectin (Fujimoto *et al.,* 1985; Fujimoto and Yoshinaga, 1986) or the 'unique fibrillar coat' (Pereda and Motta, 1991) on the surface of germ cells at the site where their processes contact somatic cells cannot be taken as unequivocal, independent proof of active cell locomotion. In each of these reports, cell migration is assumed and the nature of the glycocalyx is interpreted as supporting evidence. It seems that precisely the opposite is more likely, that is, the special glycocalyx represents a local anchoring of germ cells to some somatic cells as a partial event in the total morphogenetic movements of the embryo.

On the other hand, Jeon and Kennedy (1973) found only smooth contours and absence of pseudopodia in germ cells from embryonic mouse tissue processed for serial section electron microscopy; they argued that this supported the concept of passive germ-cell translocation as a consequence of the morphogenetic movements of other cells. It therefore seems that, according to the selection of the processing protocol for electron microscopy, it is possible to produce a variety of cell appearances indicative of migration, or non-migration.

Discussion and conclusion

Whether in physics or biophysics, mechanics or biomechanics, the proof of movement requires a frame of reference and a velocity. Until these features of an investigation are established, the discipline will remain immature, as proven by the history of mechanics. The many claims in biology for the active migration of germ cells rarely supply a reference frame or a velocity: both are indispensable if cell migration is to be considered as a hypothesis. In the case of human embryos, it is argued above that as soon as a single reference frame over the appropriate period of development is established, the justification for the concept of active germ-cell migration evaporates.

It appears that Witschi (1948) took cell migration for a fact and ignored contrary evidence. He did not mention alternative views based on substantial studies that postulated the passive carriage of germ cells by 'the forces of growth' (for example, Okkelberg, 1921; Richards and Thompson, 1921; McGosh, 1930). This is somewhat surprising as Witschi had been working on this problem for over 30 years (Witschi, 1914, 1948). He supported his 1948 claim for the amœboid movement of human germ cells by their appearances in paraffin wax-embedded histological sections. Features that Witschi cited included the presence of pseudopodia on the surface of the germ cell, gaps in basement membranes, spaces between the endodermal cells and spaces between mesodermal cells. In his 1948 paper, the imagery of active movement is strong, for example, a germ cell 'turning to the right in order to dodge the artery'; germ cells 'forcing their way' using 'proteolytic enzymes which ... prepare a path through the surrounding mesenchyme'; germ cells behaving 'very much like parasitic micro-organisms' and proceeding 'with little regard to obstacles'. The possibility of shrinkage and other histological artefacts contributing to these appearances in human embryonic tissues is not mentioned. In the discussion (Witschi, 1948), the reader is left in no doubt that the germ cells seen in histological sections are the remains of cells migrating in life. The power of Witschi's imagery has schooled his successors, who write 'PGCs *in vivo* actively migrate to the gonad, pushing other cells out of their way as they do so' (Donovan *et al*., 1987).

The appearance of an active ascent of germ cells from the allantoic region to the dorsal mesentery and cœlomic angles is an artefact arising from a failure to define the co-ordinates of cells with respect to a reference frame within the embryo during the period of the putative migration. Rather than an active ascent of germ cells in the mammalian embryo, there is a passive carriage of multiplying cells in a caudal direction. Such carriage of cells accompanies the normal development of the caudal part of the embryo, the so-called embryonic unrolling. The vectors associated with this unrolling are no less defined and the global growth movements of the embryo are no less exact than the presumed precision and directionality claimed for active germ-cell migration.

The illusion of cell migration arises when attention is focused on the cellular aspects of development rather than on the global aspects. Actually, cell migration is a *deus ex machina* that obscures our comprehension of the whole embryo: Bronner-Fraser's large, mysterious driving force is simply the growth of the whole embryo and especially the growth of the neural tube, which is the main impetus for development during this early period. For the human embryo, these growth movements were analysed comprehensively between 1948 and 1978 by the Göttingen embryologist Erich Blechschmidt, with the aid of large, three-dimensional total reconstructions. Until we return to a holistic approach to the embryo and make quantitative assessments of the growth dynamics of cellular ensembles rather than cells, our understanding will remain restricted and our interpretation of events, such as cell displacement, incorrect. One is reminded equally of Plato's words: 'That which comes to be always does so as a whole; so that if one does not count the whole among the realities, one ought not speak of substance, or of coming-to-be, as real' (Smuts, 1936) and of Robert Burns' 'best-laid schemes'.

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References

Key references are identified by asterisks.

Baker TG and Eastwood J (1983) Origin and differentiation of germ cells in man *Bibliotheca Anatomica* **24** 67–76

Blandau RJ (1969) Observations on living oogonia and oocytes from human embryonic and fetal ovaries *American Journal of Obstetrics and Gynecology* **104** 310–319

Blandau RJ and Odor DL (1972) Observations on the behavior of oogonia and oocytes in tissue and organ culture. In *Oogenesis* pp 301–320 Eds JD Biggers and AW Schuetz. University Park Press, Baltimore

***Blechschmidt E** (1960) *The Stages of Human Development Before Birth. An introduction to human embryology.* Karger, Basel

Blechschmidt E (1963) *Der Menschlichen Embryo. Dokumentation zur kinetischen anatomie.* Schattauer-Verlag, Stuttgart

Blechschmidt E (1978) *Anatomie und Ontogenese des Menschen.* Quelle & Meyer, Heidelberg

***Blechschmidt E and Gasser RF** (1978) *Biokinetics and Biodynamics of Human Differentiation. Principles and applications.* Thomas, Springfield

Book KJ, Howard R and Morest DK (1991) Direct observation *in vitro* of how neuroblasts migrate: medulla and cochleovestibular ganglion of the chick embryo *Experimental Neurology* **111** 228–243

Bronner-Fraser M (1982) Distribution of latex beads and retinal pigment epithelial cells along the ventral neural crest pathway *Developmental Biology* **91** 50–63

Buehr M (1997) The primordial germ cells of mammals: some current perspectives *Experimental Cell Research* **232** 194–207

Carey EJ (1920) Studies in the dynamics of histogenesis *Anatomical Record* **19** 199–235

Carey EJ (1921) Studies on the structure and function of the small intestine *Anatomical Record* **21** 199–215

Donovan PJ, Scott D, Cairns LA, Heasman J and Wylie CC (1986) Migratory and postmigratory mouse primordial germ cells behave differently in culture *Cell* **44** 831–838

Donovan PJ, Scott D, Godin I, Heasman J and Wylie CC (1987) Studies on the migration of mouse germ cells *Journal of Cell Science Supplement* **8** 359–367

Fujimoto T and Yoshinaga K (1986) The role of fibronectin in the interstitial migration of primordial germ cells in amniotes *Congenital Anomalies* **26** 187–196

Fujimoto T, Miyayama Y and Fuyuta M (1977) The origin, migration and fine morphology of human primordial germ cells *Anatomical Record* **188** 315–330

Fujimoto T, Yoshinaga K and Kono I (1985) Distribution of fibronectin on the migratory pathway of primordial germ cells in mice *Anatomical Record* **211** 271–278

Fukuda T (1976) Ultrastructure of primordial germ cells in human embryo *Virchows Archiv. B: Cell Pathology* **20** 85–89

Gasser RF (1979) Evidence that sclerotomal cells do not migrate medially during normal embryonic development in the rat *American Journal of Anatomy* **154** 509–524

Gomperts M, Wylie C and Heasman J (1994) Primordial germ cell migration *Ciba Foundation Symposium* **182** 121–139

Hilscher W (1983) Problems of the Keimbahn *Bibliotheca Anatomica* **24** 1–21

Jeon KW and Kennedy JR (1973) The primordial germ cells in early mouse embryos: light and electron microscopic studies *Developmental Biology* **31** 275–284

Kaufman MH (1992) *The Atlas of Mouse Development* Academic Press, London

Kuwana T and Fujimoto T (1983) Active locomotion of human primordial germ cells *in vitro*. *Anatomical Record* **205** 21–26

Lin T-H, Ukeshima A, Maeyama M and Fujimoto T (1982) Scanning electron microscopic study on human primordial germ cells during the migration period *Okajimas Folia Anatomica* **59** 305–320

McCosh G (1930) The origin of the germ cells in *Amblystoma maculatum*. *Journal of Morphology and Physiology* **50** 569–611

Makabe S and Motta PM (1989) Migration of human germ cells and their relationship with the developing ovary: ultrastructural aspects. In *Developments in Ultrastructure of Reproduction* pp 41–54 Ed. PM Motta. Alan R. Liss, New York (*Progress in Clinical and Biological Research* **296** 41–54)

Mintz B and Russell ES (1957) Gene-induced embryological modifications of primordial germ cells in the mouse *Journal of Experimental Zoology* **134** 207–237

Miyayama Y, Fuyuta M and Fujimoto T (1977) Ultrastructural observations on the origin, amoeboid features and associations of human primordial germ cells (Japanese) *Acta Anatomica Nipponica* **52** 255–268

***Molyneaux KA, Stallock J, Schaible K and Wylie C** (2001) Time-lapse analysis of living mouse germ cell migration *Developmental Biology* **240** 488–498

Morest DK (1970) A study of neurogenesis in the forebrain of opossum pouch young *Zeitschrift für Anatomie und Entwicklungsgeschichte* **130** 265–305

Motta PM, Makabe S and Nottola SA (1997a) The ultrastructure of human reproduction. I. The natural history of the female germ cell: origin, migration and differentiation inside the developing ovary *Human Reproduction Update* **3** 281–295

Motta PM, Nottola SA and Makabe S (1997b) Natural history of the female germ cell from its origin to full maturation through prenatal ovarian development *European Journal of Obstetrics & Gynecology and Reproductive Biology* **75** 5–10

Nichols DH (1986) Formation and distribution of neural crest mesenchyme to the first pharyngeal arch region of the mouse embryo *American Journal of Anatomy* **176** 221–231

Nieuwkoop PD and Sutasurya LA (1979) *Primordial Germ Cells in the Chordates. Embryogenesis and phylogenesis* Cambridge University Press, Cambridge

Okkelberg P (1921) The early history of the germ cells in the brook lamprey, *Entosphenus wilderi* (Gage), up to and including the period of sex differentiation *Journal of Morphology and Physiology* **35** 1–151

O'Rahilly R and Müller F (1987) *Developmental Stages in Human Embryos* Publication 637 Carnegie Institution, Washington

Ozdzenski W (1967) Observations on the origin of primordial germ cells in the mouse *Zoologica Poloniae* **17** 367–379

Pereda J and Motta PM (1991) A unique fibrillar coat on the surface of migrating human primordial germ cells *Archives of Histology and Cytology* **54** 419–425

Poelmann RE (1981) The formation of the embryonic mesoderm in the early post-implantation mouse embryo *Anatomy and Embryology* **162** 29–40

Richards A and Thompson JT (1921) The migration of the primary sex-cells of *Fundulus heteroclitus. Biological Bulletin of the Marine Biological Laboratory* **40** 325–348

Smuts JC (1936) *Holism and Evolution* 3rd Edn Macmillan, London

Snow MHL and Monk M (1983) Emergence and migration of mouse primordial germ cells. In *Current Problems in Germ Cell Differentiation. The 7th Symposium of the British Society for Developmental Biology* pp 115–135 Eds A McLaren and CC Wylie. Cambridge University Press, Cambridge

Stott D and Wylie CC (1986) Invasive behaviour of mouse primordial germ cells *in vitro*. *Journal of Cell Science* **86** 133–144

***Tam PPL** (1981) The control of somitogenesis in mouse embryos *Journal of Embryology and Experimental Morphology Supplement* **65** 103–128

Tam PPL and Snow MHL (1981) Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos *Journal of Embryology and Experimental Morphology* **64** 133–147

Vermeij-Keers C and Poelmann RE (1980) The neural crest: a study on cell degeneration and the improbability of cell migration in mouse embryos *Netherlands Journal of Zoology* **30** 74–81

Wartenberg H (1983) Germ cell migration induced and guided by somatic cell interaction *Bibliotheca Anatomica* **24** 93–110

Witschi E (1914) Experimentelle Untersuchungen über die Entwicklungsgeschichte der Keimdrüsen von *Rana temporaria*. *Archiv für mikroskopische Anatomie* **85** 9–113

***Witschi E** (1948) Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds *Carnegie Institute Contributions to Embryology No. 209* **32** 67–97

Witschi E (1956) *Development of Vertebrates*. Saunders, Philadelphia

Zuckerman S and Baker TG (1977) The development of the ovary and the process of oogenesis. In *The Ovary* Vol. 1 pp 41–67 Eds Lord Zuckerman and BJ Weir. Academic Press, New York