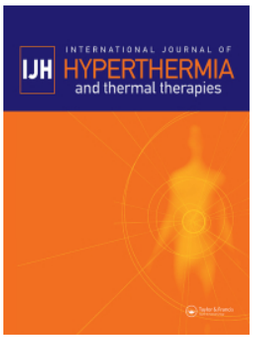

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Effects of heat on embryos and foetuses

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Objectives: This paper reviews the effects of elevated maternal temperature on embryo and foetal development in experimental animals and in humans.
Conclusions: Hyperthermia during pregnancy can cause embryonic death, abortion, growth retardation and developmental defects. Processes critical to embryonic development, such as cell proliferation, migration, differentiation and programmed cell death (apoptosis) are adversely affected by elevated maternal temperatures, showing some similarity to the effects of ionizing radiation. The development of the central nervous system is especially susceptible: a 2.5°C elevation for 1 h during early neural tube closure in rats resulted in an increased incidence of cranio-facial defects, and a 'spike' temperature elevation of 2–2.5°C in an exposure of 1 h during early neurogenesis in guinea pigs caused an increase in the incidence of microencephaly. However, in general, thresholds and dose-response relationships vary between species and even between different strains of the same species, depending on genotype. This precludes rigorous quantitative extrapolation to humans, although some general principles can be inferred. In humans, epidemiological studies suggest that an elevation of maternal body temperature by 2°C for at least 24 h during fever can cause a range of developmental defects, but there is little information on thresholds for shorter exposures. Further experimental and epidemiological studies are recommended, focusing on stage-specific developmental effects in the central nervous system using a variety of sensitive assays.

Key words: Hyperthermia, heat, teratogen, embryo, foetus, malformation embryonic resorption.

1. Introduction

The highly ordered sequences of cell proliferation and differentiation, migration and programmed cell death (apoptosis) that characterize the prenatal growth and development of the mammalian conceptus through to neo-natal and post-natal periods are susceptible to heat. Experimental work has shown that elevated maternal temperatures can result in embryonic or foetal death, in structural and functional defects or in reduced growth depending on the elevation and duration of maternal temperature and the stage of development during which the thermal insult is delivered. Direct heating of the embryo or foetus has shown a similar spectrum of effects suggesting a direct effect of heat on development; changes in maternal physiology may introduce additional factors complicating the overall interpretation. Developmental defects have also been reported in humans following raised maternal temperatures during pregnancy, either through fever or some other form of heating.

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The sensitivity of the embryo and foetus to heat and to other teratogens varies dramatically during development, depending on the sensitivity of the processes taking place. There are various time 'windows' in pre-natal development during which sensitivity to a teratogen is relatively high. Periods of cell proliferation and migration, for example, are especially vulnerable. The pre-natal development of mammals is roughly divided into three periods: the pre-implantation period, extending from fertilization to settling of the embryo into the uterine wall; the period of major organogenesis, characterized by the formation of the main body structures; and the foetal period, during which growth of structures already formed takes place. Generally, embryos exposed to heat before implantation in the uterine wall either recover completely or are lost, either through failure to implant or embryonic death. This can result in increased pre-implantation losses which may pass unnoticed in humans. Clearly, excessive temperature rises may result in death at any stage, but the varied processes involved in organogenesis, viz proliferation, differentiation, migration and cell death, are particularly susceptible to the induction of developmental defects in specific organs or structures. The developing nervous system, for example, seems to be especially sensitive to a variety of insults, including heat and ionizing radiation. However, skeletal, neuromuscular and cardiac defects have also been induced by heat. By the end of the embryonic period of, for example, humans (8 weeks), the embryo possesses ~90% of the 4500 structures of the adult human[1]. The foetal stage is largely characterized by the growth of these structures; heat exposure results mainly in reduced growth, although defects in the developing nervous system can also be induced.

Thresholds, therefore, vary during development, with different stages and organs or tissues showing different levels of heat sensitivity at different times. Consequently, such values are tissue and developmental stage-specific. It follows that there is no one threshold value for the induction of developmental defects, each malformation has its own threshold dose of heat[2], and that an overall minimum value can only be identified by examining the sensitivity of the most vulnerable period. There is in addition a very large variability in the relative duration of developmental periods between different animal species (see table 1), as well as in the total duration of intrauterine life. Also, at any given stage of development, the state of maturation of any one structure, with respect to all others, varies considerably between species. Within a species, the different sensitivities shown between strains can be traced to strain-specific differences in genotype. This variability precludes the quantitative extrapolation across species and particularly from animals to humans[1].

Table 1. Major developmental periods in some mammalian species.

	Major developmental periods (days pc)		
Species	Pre-implantation	Organogenesis	Foetal
Hamster	0–5	6–12	13–16.5
Mouse	0–5	6–13	14–19.5
Rat	0–7	8–15	16–21.5
Rabbit	0–5	6–15	16–31.5
Guinea-pig	0–8	9–25	26–68
Man	0–8	9–60	61–270

A further complication in the interpretation of developmental responses to heat in particular is that different animal species have evolved with their metabolic processes optimized at different body temperatures. The normal temperature of guinea pigs (39.5°C) and sheep (39°C) are in the teratogenic range for humans (with a body temperature of ~37°C) and the normal temperature of rats (38.5°C) is just below the range for human teratogenesis. The lowest temperatures (threshold) at which defects have been caused in various species are set out later. In summary, in the species studied, a threshold elevation of 2–2.5°C above the normal body temperature was found. Because the normal body temperatures of different species varies widely, it is not possible to specify a single teratogenic body temperature for all species. For this, and other reasons, it appears more appropriate to quote teratogenic temperatures in terms of the elevation over normal rather than a single absolute temperature to be applied for all species[3]. It should also be noted that the few studies on this topic have involved small numbers of animals from limited genetic backgrounds and only a small number of screening tests for damage have been used. They could not have detected small, but important changes in the incidence of defects. Very large numbers of animals will be required to establish precise thresholds. It will also be important to use animals from diverse genetic backgrounds to screen for genetic sensitivity or resistance to hyperthermic embryopathy. It is possible that such experiments might show that there are no thresholds for thermal embryonic damage.

2. Pathogenic mechanisms

The known and suspected mechanisms leading to heat-induced developmental defects are cell death (particularly of proliferating cells), the disturbance of cell migration, the disruption of gene expression (particularly of genes involved in the 'induction' processes of organogenesis) and damage to cell membranes. The similarity between some of the effects of heat and the effects of ionizing radiation, particularly in their targeting of proliferating cells, have been reported by Wanner and Edwards[4]. In addition, damage to blood vessels and the placenta will lead to further disruption of developmental processes. These effects can be modified to some extent by the cellular 'heat shock' response, which provides some protection to exposed embryos. A number of factors can lead to abortion and foetal growth retardation.

Death of cells heated during division is a major feature of the heat-damaged embryonic brain and face and can be found soon after a threshold dose of heat. Within minutes of a moderate elevation of temperature (2–3°C) given to guinea pig embryos during early stages of brain development (day 21 post-conception), cells in the brain in mitosis show clumping of chromosomes and die[5–7]. At higher elevations (3–4°C), cells in S-phase (DNA replication phase) die by apoptosis from ~6 h after exposure, even higher elevations causing greater levels of this form of cell death. The distribution of cell death in heated 9.5 day rat embryos is restricted to the rapidly forming head-fold and anterior neuropore regions[8,9] and defects are subsequently found in the structures that develop from these areas. The defects resulting from restricted regions of cell death are usually very deforming and obvious, for example NTD (neural tube defects). Heat at later stages, for example in day 21 guinea pig embryos, causes a wider, more diffuse distribution of cell death and results in a proportional miniature of the normal brain (microencephaly). The consequences of cell death during early neuronal histogenesis on subsequent development of the brain are discussed later.

Although the cell division cycle is delayed by heat, this does not appear to cause obvious defects in brain or facial development unless accompanied by significant levels of cell death. When a small dose of heat (42°C for 10 min) was given to 9.5 day rat embryos in culture, a protective heat shock response was initiated. When this exposure was followed by a larger dose of heat (43°C for 7.5 min), that would normally cause severe defects in an unprotected embryo, only a few cell deaths were found and no defects of the head followed these exposures[8,9]. Both the initial, protective exposure and the subsequent more severe exposure were followed by suspension of normal cellular protein synthesis and suspension of cell proliferation, but did not cause defective development of the neural tube and face. Successful neural tube closure and development of the head, face and other structures from the neural plate might depend, at least in part, on a threshold number of cells being available to form these structures.

Disruption of gene expression without significant cell death might be responsible for vertebral and rib defects in mice[10]. However, some cell deaths were found in the region of the developing vertebral column of rats after a damaging dose of heat[11].

Cell membranes are disrupted by heat during neural tube closure and this might be one of the mechanisms leading to the formation of rosettes (cyst-like structures) which form encephaloceles in the brain[12,13] and syringomyelia (cysts) affecting the spinal cord[14]. Disruption of the ventricular lining of the cerebral hemispheres and breakdown of junctional complexes were also found in the brain of 21 day heated guinea pig embryos[6]. Studies of cells in culture have also demonstrated damage to membranes[15], microtubules and other elements of the cytoskeleton[16,17].

Disturbances of cell migration are believed to result in malformations of the heart and face (neural crest cell migration) and to cause Hirschsprung disease, a defect of the innervation of the intestine[18], and to result in ectopic nests of neurones in the brain. Neural crest cells commence migration at about the stage of neural tube closure with many destinations, including the face and head, heart, intestine, skin, adrenals and elsewhere. Neuronal cells begin to migrate from their origins around the cerebral ventricles at the time of formation of the cortical plate at days 12–13 in rats, 21–22 in guinea pigs and weeks 7–8 in humans, soon after neuronal proliferation commences. The proliferation of neuronal precursor cells was suppressed and their migration to the cortical plate was decelerated significantly in the brains of foetal mice heated on day 13 or 14[19].

The subsequent processes of neuronal proliferation, differentiation and migration are of profound importance in the further development of the brain, especially the cortex, and have been shown to be acutely sensitive to ionizing radiation in large numbers of studies of experimental animals and exposed humans, leading to behavioural deficits and reductions in IQ[1,20,21]. The effect of heat on processes such as corticogenesis (see below) seem to have been less fully explored.

Leakage and interstitial oedema[22] follow vascular disruption with damage to the endothelium of vessels. It has been proposed as the cause of a number of defects, following exposure during late embryonic or early-to-mid-foetal stages including hypoplasia of limbs and toes, exomphalos (umbilical hernia), Moebius syndrome (damage to neurones of a number of brainstem nuclei) and arthrogryposis (damage to motor neurones of the spinal cord with loss of muscular function of limbs or body trunk). Placental infarcts in rats[23] and placental infarcts and intervillous thrombi in monkeys[24] were observed following teratogenic doses of heat.

Abortions could be due to severe embryonic damage that is incompatible with continuing development or to maternal factors including increased uterine activity, placental damage and changes in the endocrine status of the mother. The temperature of pregnant baboons that were close to term was raised to 41–42°C, causing a two-fold increase in uterine activity during exposure, which might initiate abortions soon after exposure[25]. Other changes included severe maternal and foetal acidosis. To test a possible association between maternal fever (temperature of 37.78°C and above) and spontaneous abortion in women, Kline *et al.*[26] interviewed a control group of 1057 women who delivered at or after 28 weeks gestation and a group of 1835 women who had a spontaneous abortion prior to 28 weeks. A number of the foetuses were karyotyped and, among the euploid foetuses (normal chromosome complement), fevers occurred in 18% of the abortions and in 7.1% of the controls. It was concluded that fever during pregnancy is a risk factor for spontaneous abortion.

3. Maternal–foetal heat exchange

Foetal temperature is determined primarily by maternal temperature; the foetus has almost no ability to regulate its own temperature[27]. Calculation reveals foetal heat production of $\sim 3\,W\,kg^{1}\,kg^{-1}$, about twice the resting heat production of the adult. A temperature difference of ~0.5°C is required to bring the rate at which heat is produced in the foetus into balance with the rate at which it is dissipated to the mother. This close coupling of the foetus to the mother may be regarded a heat clamp, which effectively prevents the foetus from independently controlling its body temperature before birth.

Pathways for the transfer of heat are the umbilical circulation and through the foetal skin, amniotic fluid and uterine wall. Of these, the umbilical circulation is generally thought to eliminate the bulk (85%) of foetal heat[27,28]. Because the maternal and foetal blood flows are relatively large and the placenta exposes a large surface area with only a thin barrier, temperature equilibrium is expected to be reached rapidly. Foetal temperatures rise quickly if the umbilical cord is occluded, supporting the importance of this site of heat exchange. However, heat exchange is less efficient than predicted, probably because of counter-current heat exchange in the vessels of the umbilical cord and perhaps in the smaller vessels in the placenta.

Factors which affect the temperature of the foetus, therefore, not only include maternal body temperature *per se* but also placental blood flow. Factors that have been implicated in reduced placental blood flow include heat stress from vigorous exercise[29], short-term exercise-induced heat stress in hypertensive patients[30] and fever[31]. During extended periods of hyperthermia, reduced blood flow to the uterus and placenta is believed to contribute to reduction in placental and foetal body weights[32,33]. The significance of these effects in teratogenesis is not clear, but they might interfere with embryonic/maternal heat exchange, retarding heat exchange and embryonic growth.

4. The heat-shock response

Heat and a number of other toxic agents can induce the 'heat shock' response in the embryo and foetus. The response is a survival mechanism and takes precedence over other cellular activities. Normal protein synthesis and cell proliferation are suspended when the response is activated. The constitutive (chaperone) heat shock genes are involved in many developmental processes, and they are present in greater

amounts in areas of the embryo and at times when organ induction and growth are in progress[34]. It has been proposed that chaperone proteins might offer some limited protection to embryos against the effects of small doses of heat, up to the threshold of 2–2.5°C[3]. The induced response is initiated after a potentially damaging dose of heat. It cannot, however, be induced in mouse and rabbit embryos between the 2-cell and blastocyst stages, prior to implantation[35,36], and embryonic death commonly follows exposure to heat at this stage.

The heat shock response has been studied in rat embryos in culture, particularly by Walsh *et al.*[34,37,38] and Mirkes and colleagues[39–43]. During the early stages of organogenesis (day 9.5), a non-malforming dose of heat (42°C for 10 min) induces a protective response against a subsequent, larger dose (43°C for 7.5 min) which otherwise would cause severe malformations[34]. The protective response, coincident with the synthesis of a number of induced HSP (heat shock proteins), is detectable within 15 min after the inducing dose if embryos are allowed to recover for at least 15 min at the normal culture temperature (38.5°C). If the embryos remain at 42°C, severe defects occur. It is possible that the protective response might be induced at lower elevations of temperature, without a recovery period at normal temperature. For this reason, the rate as well as the extent of temperature elevation might be an important factor in thermal damage. Rapidly achieved (within 15 min) high levels of elevation did not allow an efficient induction of the protective response in rat embryos. In 10.5 day rat embryos in culture, it was shown[42] that synthesis of HSP 72 was detectable within 2.5 h after exposure to 41°C for 15 min, 42°C for 15 min and 43°C for 2.5 min. It persisted in detectable amounts for 24–48 h.

Particularly noticeable during foetal development, prolonged or repeated doses of heat commonly cause reduced weights of the placenta and foetuses that have been attributed to a number of factors, but especially reduced maternal blood flow to the uterus[32]. It is also possible that foetal growth retardation following prolonged periods of hyperthermia might also be due, in part, to repeated induction of the heat shock response with suspension of normal protein synthesis in both the placenta and foetus.

5. Critical periods

As indicated above, a sufficient (threshold) dose of heat can cause defective development only during sensitive periods of organogenesis. Each susceptible organ has one or more sensitive stages that are usually circumscribed and can be brief. A defect can be caused only if a threshold dose is delivered during these stages. There is no apparent carry-over deleterious effect if the dose is given before a susceptible stage of development. Once the susceptible period has passed, the organ is relatively resistant to its effects. The stages of embryonic and foetal susceptibility to heat closely resemble the stages of susceptibility to ionizing radiation[4]. However, susceptibility appears to be greatest just before and just after the commencement of the rapid cell proliferative phase of a developmental event, that is during the stage of organ induction and the early rapid phase of cell proliferation that forms the organ. Larger doses of heat are required to cause a defect during the later active phase of cellular proliferation. After the organ is formed, it is relatively resistant to heat.

The progression of critical stages for the induction of defects in a number of species are set out in table 2 and illustrated in figure 1. As the embryo passes through various stages of development, different structures become susceptible to damage. In pre-implantation stages, before organs begin to form, heat can cause embryonic

Table 2. Teratogenic effects of heat at different developmental stages.

Developmental stages	Pre-implantation	Neural tube, eye, face, heart and vertebral formation—early embryo stage	Neurogenesis, including corticogenesis, neural cell migration and development of body structures—mid-embryo/foetal stage	Glial cell proliferation and myelination; foetal/early post-natal growth
Hamster, days	3–5	6–9	9–14	
Mouse, days	3–6	7–9	12–15	
Rat, days	3–6	9–12	13–18	18 post-natal
Guinea pig, days	4–6	12–15	20–35	35 post-natal
Equivalent stage humans, weeks	1–3	3–7	8–18	18 weeks foetal to 2 years post-natal
Pathogenic mechanisms found in experimental animals		Cell death, disruption of gene induction, cell membranes and neural crest migration (heart defects), over growth of neural tissue (neural tube defects)	Cell death, disruption of gene induction of organogenesis, neural proliferation and migration; placental damage*	Cell death, disruption of gene induction and glial cell proliferation, delayed myelination and vascular disruption
Developmental effects	Resorption, death; no defects	Abortion; Neural tube defects*; Heart defects*; Vertebral defects; Microphthalmia*; Coloboma; Blindness	Abortion*; Stillbirth; Microencephaly*; Learning defects*; Seizures*; Abdominal wall defect*; Clubfoot*; Moebius sequence*; Missing/small toes and teeth; Cataract; Increased/decreased muscle tone*	Abortion; Microencephaly*; Learning defects*; Arthrogryposis*

* Also found in humans.

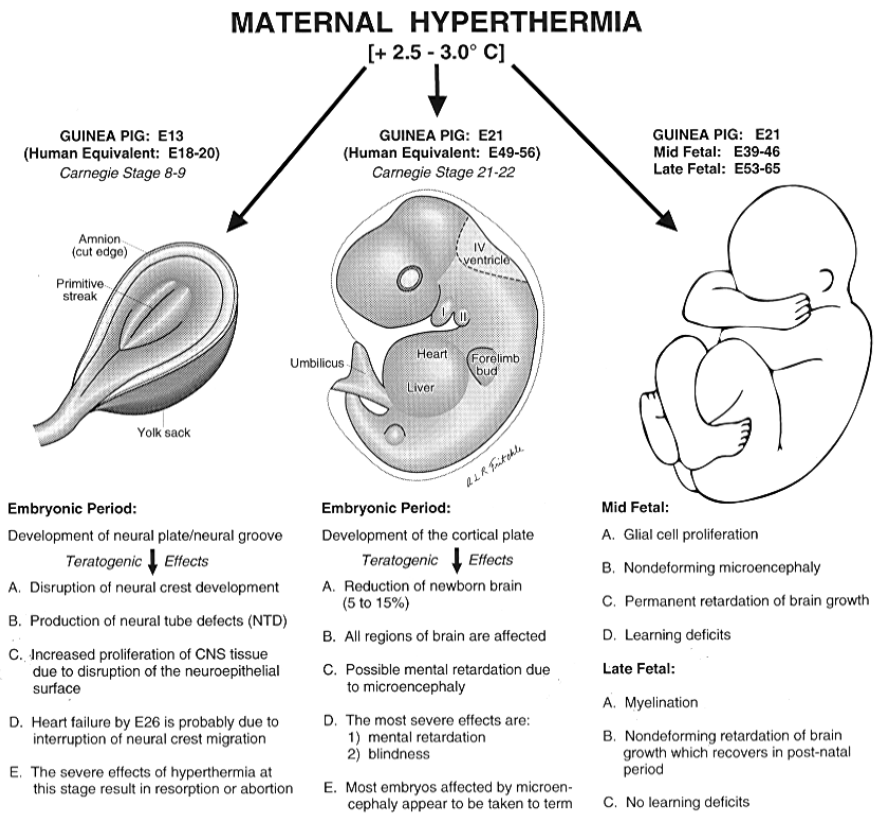

Figure 1. Summary of the effects of hyperthermia at the early and late embryonic period and the mid-to-late foetal period (from Edwards *et al.*[3], reproduced with permission).

death, but malformations are rare. It is important to note that the developmental stages in different species occur at different developmental ages, mostly in the post-implantation embryonic stages. The susceptible stage for neural tube closure occurs at, and soon after, the time the neural plate cells are being induced to cause closure, which is day 8.5 in mice, day 9.5 in rats, days 13–14 in guinea pigs and days 20–28 in humans. At the beginning of organogenesis, the neural plate and neural tube stages, the embryo is susceptible to damage of the brain (defects include anencephaly, spina bifida and encephalocele), eyes (anophthalmia, microphthalmia and defects of the iris), face (small upper or lower jaws, clefts of the face) and heart (table 2). After the neural tube closes to form the brain and spinal cord, the vertebrae and ribs become susceptible to damage. Later, in the mid- to late embryonic stages, neuronal cells that will form the brain begin to proliferate. They are killed by heat at this stage and this loss of cells is irreversible, leading to microencephaly, affecting all regions of the brain. Other defects induced at this stage are clubfoot (talipes) associated with defective spinal cord development, small or missing toes, tooth defects, abdominal wall defects (umbilical hernia), cataract, coloboma (split iris) and functional defects including blindness, learning problems and seizures.

The vulnerability of the developing brain to the induction of deleterious effects has been further explored using ionizing radiation; the observed effect may well be relevant to heat-related effects, as noted above. The extreme vulnerability results

from the limited number and restricted physical location of the cohorts of proliferating cells from which the brain arises and the precision required for the complex neuronal architecture essential to proper brain function[20]. Precise interconnections need to be made between neurons arriving at the proper place and the proper time, apoptotic processes have a critical role in removing excess cells that fail to undergo correct differentiation and/or synaptogenesis. Corticogenesis seems particularly vulnerable[1,44,45]. Here, post-mitotic neuronal progenitor cells migrate from a region adjacent to the ventricular zone along 'scaffolding structures' such as radial glial fibres, forming synapses as they pass layers of previously formed cells, and eventually take up their position at the outer aspect of the cortex, the distances traversed range from mm to cm. Low doses (< 1.0 Gy) of ionizing radiation disrupt these processes, resulting in morphological changes such as reduced dendritic fibre alignment[45] (see figure 2) and decrements in associative-type learning in animals[46] and in school performance and IQ in humans[21] (see figure 3). The possibility that these processes are also susceptible to heat should be further explored.

During the foetal stage, further brain development and glial cell proliferation can be retarded by heat, leading to an irreversible reduction in brain size and function. In particular, granule cells of the cerebellum, olfactory bulb and dentate gyrus of the hippocampal formation show a late proliferative burst. Exposure to low doses of ionizing radiation during late foetal and peri-natal stages results in deficits in spatial memory-dependent behaviour[46–48] associated with the hippocampus. The possible effects of hyperthermia may also be worth investigating. Damage to the spinal cord by hyperthermia during the foetal stage leads to the development of arthrogryposis

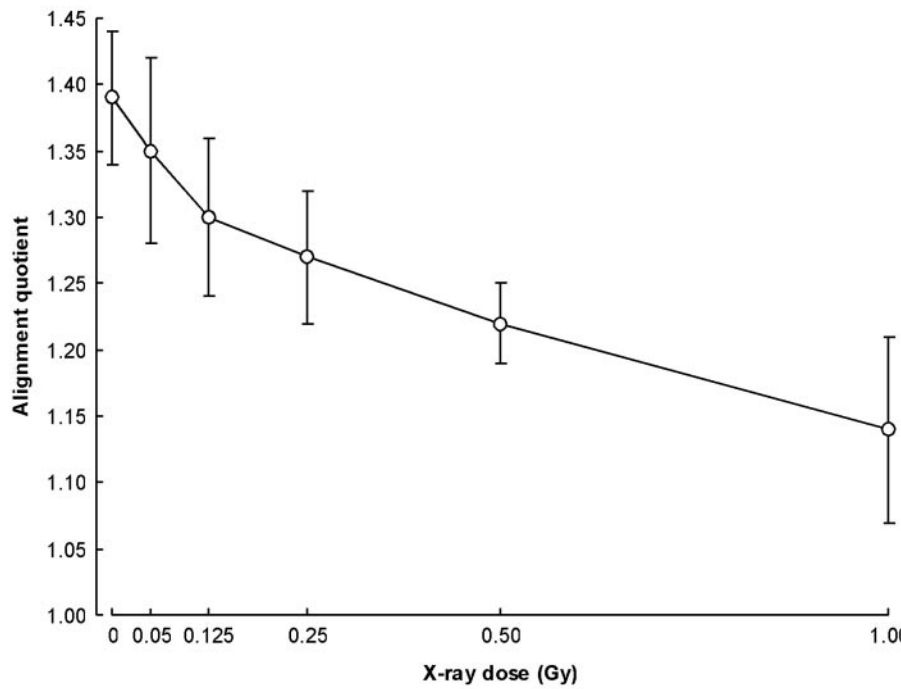

Figure 2. Dendritic alignment in the cortex of adult (approximately post-natal day 40) mice following exposure to X-rays during early corticogenesis (day 12 of gestation) (from Konermann[45]).

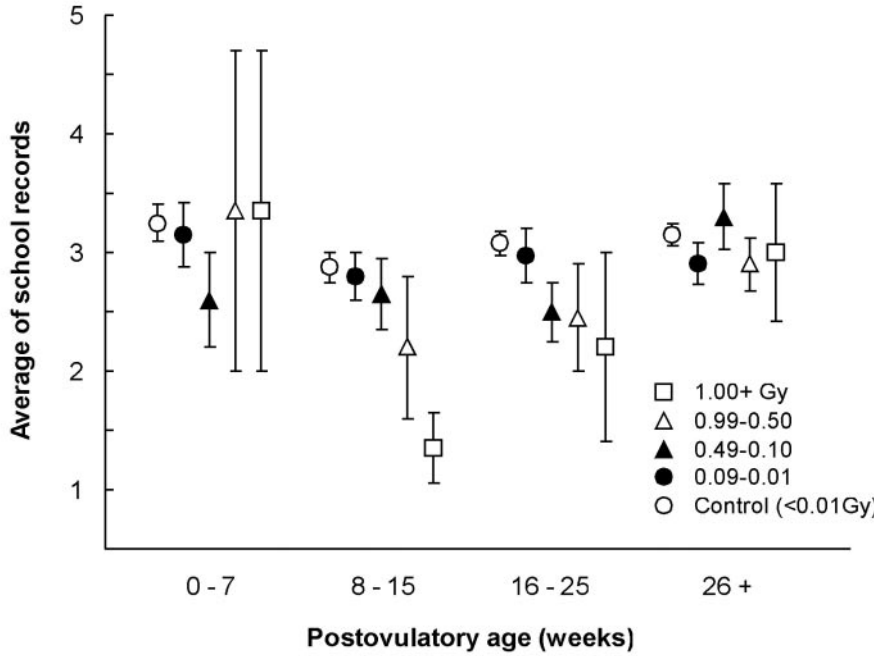

Figure 3. Average school performance and 95% confidence limits in the offspring of atom bomb survivors by absorbed ionizing radiation dose in the uterus and post-ovulatory age. Significant dose-dependent deficits in school performance were seen exposed during corticogenesis (weeks 8–15 gestation) and during subsequent differentiation and synaptogenesis (weeks 16–25) but not during earlier or later periods (from Otake and Schull[21]).

with rigidity of joints of the limbs, spine and atrophy of muscles caused by loss of motor neurones in the spinal cord. Moebius sequence is a neurological disorder with loss of neurones in the brain stem nuclei, leading to paralysis of a number of the cranial nerves.

Birth occurs at different stages of development in different species. In hamsters, mice and rats, it occurs at the end of neurogenesis, during glial cell proliferation (days 16, 19 and 21, respectively). The offspring are relatively immature in development and completely dependent on the mother. In contrast, the gestation period for guinea pigs is ~68 days and neurogenesis is complete, glial cell production is largely complete and myelination well advanced. The pups can walk and feed on solid food within 1 h of birth. In humans, brain development continues after birth[44] into the infant and juvenile periods.

6. Extrapolation between species

The range of defects caused by hyperthermia is broadly similar for all species, but each species has its own particular susceptibilities to specific defects. Different genotypes within a species can have markedly different sensitivities to certain types of defect and there might be differences between strains in the times in gestation at which the defect can be induced[49,50]. This indicates that genotype can have a strong influence on the type of defect, its incidence and its severity. There are also some differences between genotypes in the dose of heat required to cause certain defects[50,51]. Vacha *et al.*[52], for example, have identified a growth arrest specific

(*gas 5*) gene responsible for the differences in susceptibility of different strains of mice to neural tube defects.

In an analysis of the effects of pre-natal exposure to ionizing radiation, UNSCEAR[1] note that any attempt to extrapolate results obtained in animals to man must take into account the embryological peculiarities of each species, emphasizing that the quantitative extrapolation of animal data to humans is, in principle, unjustified. In addition, as described above, different species maintain their bodies at different temperatures; the physiologic and metabolic changes induced by shifts in body temperature relate to relative changes of temperature rather than absolute values.

7. Thresholds for teratogenesis in various species

Thermal dose is a function of both the temperature elevation and the duration of the elevated temperature, higher elevations requiring shorter durations than lower elevations to cause a defect. The concept of thermal dose is described in depth in Miller *et al.*[53] and Dewhirst *et al.*[54]. As discussed above, thresholds are specific to developing tissues or organs at given stages of development, they are also strain and species specific. In the majority of studies described below, rats, mice or guinea pigs were exposed during early organogenesis. Results are given in table 3.

The few published attempts to find the lowest temperature elevations which result in malformations include studies of rats heated with radiofrequency radiation[55–58] (see figure 4), heated in a warm water bath[2] (see figure 5), heated in warm air[51], with surgically exteriorised uterine horns heated in warm water[59] and with embryos heated in culture[60,61]. Studies of pregnant guinea pigs heated with warm air were carried out by Edwards[62,63], Jonson *et al.*[64] and Wanner and Edwards[4] (see figure 6). These different methods of heating resulted in different temperature profiles. Embryos exteriorized or heated in culture will experience rapid and controlled changes in temperature, such that a given temperature rise can be applied over a known time. However, confounding may be introduced through the use of surgical or culture techniques. Heating of the mother is more natural, but will be of a slower time-course and may induce changes in maternal physiology which confound interpretation. With warm air heating, temperatures may only peak (a temperature spike) at the end of the period when heat is applied.

7.1. *Mice*

Some studies carried out using mice have looked at the incidence of cranio-facial defects following exposure on days 8–9, which includes the susceptible stage for neural tube closure. The formation of the eye, face, heart and vertebrae also take place at this time. Other studies have examined effects on brain and behaviour following exposure on days 12–15, a period of neuronal proliferation, migration and differentiation in the mouse brain.

Finnell *et al.*[49] heated pregnant mice in a water bath on days 8, 8.5 and 9 and found marked differences in sensitivity of certain inbred strains of mice to hyperthermia-induced exencephaly. They concluded that the genetic basis for the sensitivity of the SWV strain involved only a few genes. Webster and Edwards[50] and Chernoff and Golden[65] found a markedly enhanced incidence of cranio-facial defects with two elevations of 5.5°C on day 8.5 at an interval of 6 h. Defects in mice[66] were caused by exposure on day 8.5 of pregnancy (table 3). The time required to cause a malformation was halved for each 1°C elevation over 3.7°C. Hyperthermia at the

Table 3. Thresholds from short exposures for cranio-facial and skeletal defects.

Species	Temperature elevation, (sham) °C	Minimum duration, min	Incidence of defects,% (*n*)		Incidence resorptions, % (*n*)	References
			Cranio-facial	Skeletal		
Mice, day 8.5						
SVW	5	10 (spike)	44 (47/106)		22	49
SVW control	0		0.9 (1/116)		8	
DBA	5	10 (spike)	0 (0/27)		53	
DBA control	0		0 (0/49)		27	
SWR	5	10 (spike)	14 (19/139)		8	
SWR control	0		0 (0/90)		4	
	3.7	10	4 (6/157)		5	66
	3.7	12.5	15 (20/133)		16	
	4.7	5	2 (2/111)		13	
	4.7	8.75	31 (31/99)		32	
Control	0 (38.3)		0 (0/108)		0	
QS (outbred); one exposure	5.5	7.6 (spike)	5 (15/278)		5 (15/293)	50
QS; two exposures	5.5	7.6 (spike)	34 (34/100)		17 (20/120)	
C57Bl (inbred); two exposures	5	7.5 (spike)	52 (29/55)		31 (25/801)	
Control	0		0 (0/122)		13 (18/140)	
Rats, day 9						
	2.9	14–22 (spike) ##	1.5 (4/275)	1.5 (4/275)	10 (28/294)	56
	2.9	134–142 ##	2 (6/294)#*	6 (18/294)#*	9 (30/322)	
	3.9	13–33 (spike) ##	4.5 (12/270)	8 (23/270)	15 (46/312)	
	3.9	28–52 ##	53 (140/263)#**	49 (130/263)#**	22 (77/341)	
Control	0 (38.5)		0.3 (1/258)	1.4 (4/258)	10 (29/287)	
	2.6	10–40 (spike)	0.8 (2/257)	6 (15/257)	8 (22/280)	57
	3.6	10–40 (spike)	8 (19/231)	17 (37/231)	8 (21/253)	
	4.5	10–40 (spike)	60 (104/172)	80 (137/172)	40 (110/227)	
Control	0 (38.5)		0.6 (2/271)	2 (6/271)	6 (18/287)	
Rats, day 9.5						
	2.0	480	0		10 (8/80)	2
	2.5	60	25 (10/40)***		11 (5/45)	
	3.0	20	9 (4/44)*		0	
	3.5	10	36 (17/47)***		10 (5/52)	
	4.0	5	29 (20/69)***		1 (1/70)	
	4.5	2	42 (27/65)***		4 (2/47)	
	5.0	< 1	100 (40/40)***		27 (14/52)	
Control	0 (38.5)		0 (0/64)		1 (1/65)	
	2.5	50	0 (0/38)		17	58
	3.0	20	11 (2/19)		10	
	4.0	3	21 (6/29)		21	
	5.0	< 1	100 (7/7)		74	
Control	0 (38.5)					
Rats, day 10.5						
	1.2–2.6	40–60 (spike)		26 (41/157)		51
	2.5–3.1	40–60 (spike)		21 (29/138)		
	3.0–4.1	40–60 (spike)		93 (96/104)*		
Control	0 (37.8–38)			12 (19/152)		
Sham control				9 (13/147)		
	3.0–3.2	40–60 (spike)		16 (32/194)		
	4.0–4.7	40–60 (spike)		90 (151/168)		
Sham control	0 (37.8–38)			10 (20/189)		
Rat embryos in culture, day 9.5						
	3.5	40	100 (6/6)			37
	4	15	100 (6/6)			
	4.5	7.5	100 (15/15)			

(*continued*)

Table 3. *Continued.*

Species	Temperature elevation, (sham) °C	Minimum duration, min	Incidence of defects,% (*n*)		Incidence resorptions, % (*n*)	References
			Cranio-facial	Skeletal		
Rat embryos in culture, day 9.5 (continued)						
	5	2.5	17 (1/6)			37
	5	5	100 (6/6)			
Controls	0 (38.5)		0 (0/41)			
Rat embryos in culture, day 10.5						
	3	240	11 (2/23)			39
	4	60	45 (13/29)			
	4	120	45 (13/29)			
	5	15	10 (2/21)			
	5	30	72 (13/21)			
	0 (37)		0 (0/166)			
Guinea pigs, days 13–14						
One exposure	3.9	60 (spike)	23 (10/44)	30 (13/44)	31 (20/64)	72
Two exposures	3.4–3.8	60 (spike)	25 (31/124)	16 (20/124)	5 (6/130)	
Control			0 (0/80)	0 (0/80)	2 (2/82)	

Compared to spike group. ## Heating to the designated elevation required 14–22 min for the +2.98C group and 13–33 min for the +3.9°C group. These durations have been added to the plateau elevations of 2.9°C for 120 min and 3.9°C for 15 min.

* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

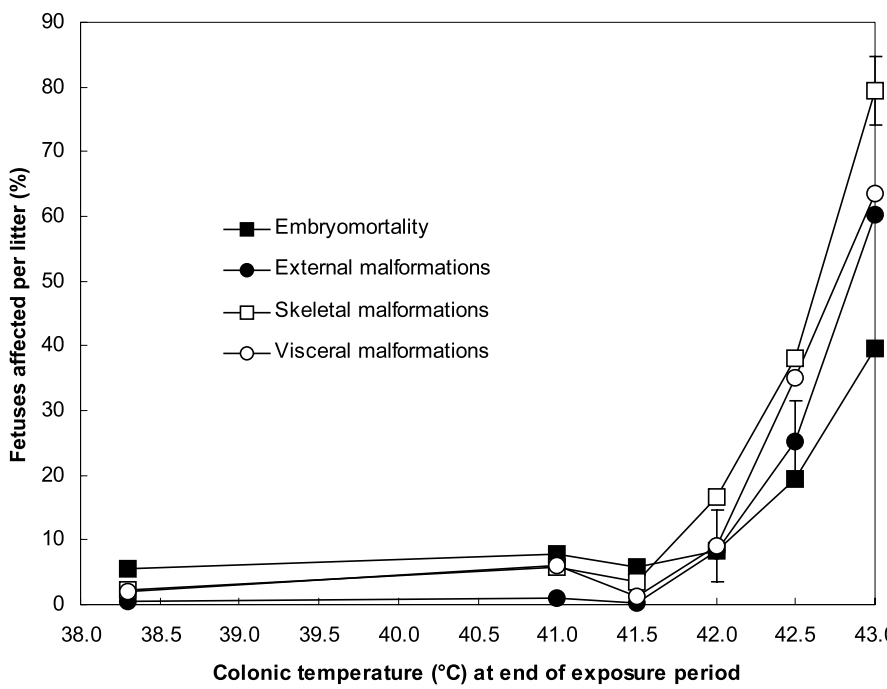

Figure 4. Incidence of embryonic death and foetal malformations (mean se) in rats made hyperthermic by exposure to 27.12 MHz radiofrequency radiation for 10–40 min on gestation day 9 (from Lary *et al.*[57]).

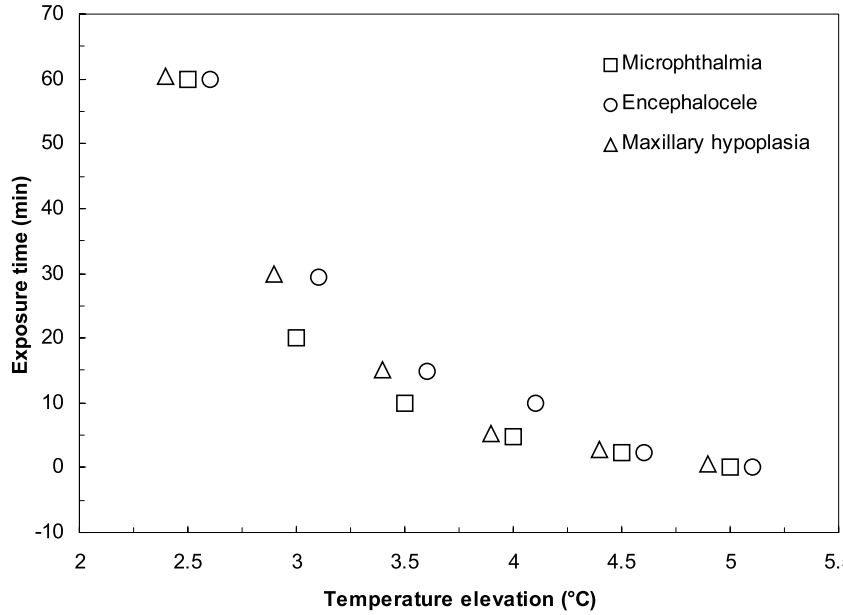

Figure 5. Threshold temperature-time relationships for the induction of abnormalities in rats exposed *in utero* in a waterbath on gestation day 9.5 (from Germain *et al.*[2]). The points indicate the exposure time to the first appearance of the type of abnormality at each temperature elevation.

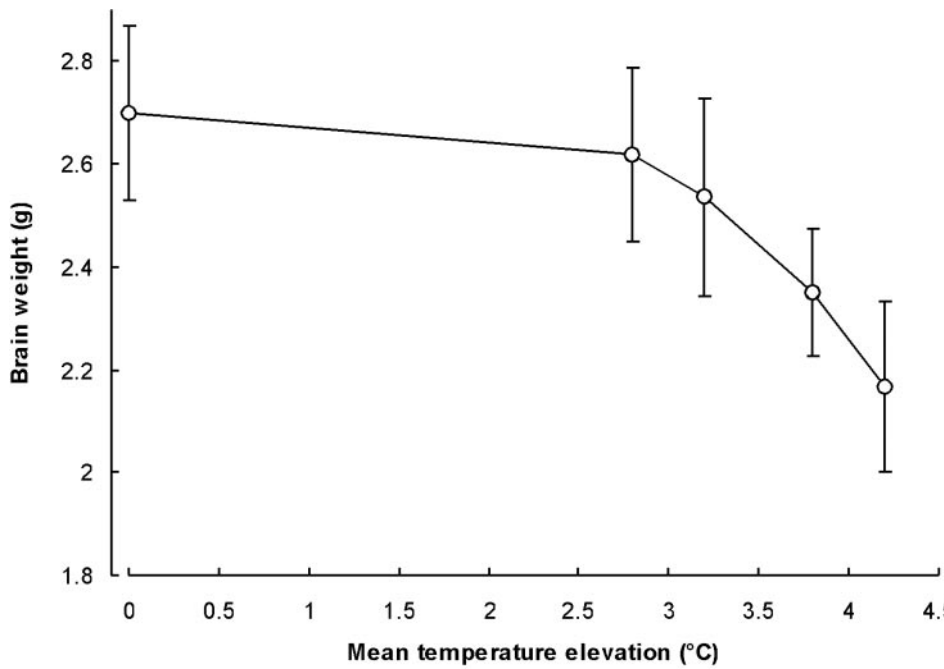

Figure 6. The induction of microencephaly in guinea pigs exposed on day 21 gestation for 1 h to elevated maternal temperatures of which the mean peak elevation, given above (±se), was attained towards the end of exposure (from Wanner and Edwards[4]).

stage of somite differentiation (days 7–9 in mice) caused stage-specific transformations of vertebral identities and concomitant alteration of Hox gene expression domains[10]. They concluded that such vertebral transformations were not due to induced cell death but to a transient arrest of cell proliferation and somitogenesis.

Hinoue *et al.*[19] heated pregnant mice in a water bath at 43°C for 12.5 min on day 13 or 14. The maternal temperature reached the temperature of the water bath in 6–7 min and returned to the resting temperature 10 min after removal from the bath. They found that the brain weight and the thickness of the neopallium in their foetuses were significantly decreased, which might be attributed to the disturbances of proliferation and migration of neuronal precursor cells. Apoptotic cell death increased in the brains of heated foetuses. The results indicated that an elevation of 4.5–4.7°C for 6–7 min caused a reduction in brain weight of 13.4%. Shiota and Kayamura[67] found poor learning performance in mice exposed *in utero* to hyperthermia. They heated pregnant mice in a water bath at 42 or 43°C for 10 min once or twice daily on days 12–15 and observed the behaviour and leaning performance of the offspring. The offspring of heated dams had smaller brains than controls when examined at 11 weeks of age, and were less active in an open field at 5 weeks of age and learned more slowly than controls in a multiple T maze and in a shuttle box at 7 and 10 weeks, respectively. [In contrast, Sienkiewicz *et al.*[47,48] reported the greatest sensitivity of spatial memory tasks (in a radial arm maze) in mice exposed *in utero* to ionizing radiation during days 18 gestation to post-natal day 1, during dentate granule cell proliferation in the hippocampus.]

A comparison was made in pregnant mice, on day 13, of the effects of microwave irradiation (2.45 GHz for 15 or 20 min) with immersion in water at 42°C for 15 min[68]. The highest maternal temperature during microwave irradiation was 42.5°C and the immersion in warm water produced a similar pattern of temperature elevation as microwave exposure. Pyknotic cells in the ventricular zone of the telencephalon were counted at 9 h and showed an incidence of 1.83% and 3.06% following 15 and 20 min, respectively, of microwave exposure. Immersion in water at 42°C for 15 min had a similar effect on cell death (3.51%) in the brain as 20 min of microwave exposure. Some offspring were examined at 6 weeks of age. The brain weights of the group given 20 min of microwave exposure were significantly lower than the control group and the neuronal cell density was much higher.

7.2. *Rats*

All the experiments were carried out on rats during the early stages of organogenesis (days 9–10.5), from when the primitive streak is forming in the neural plate through to the formation of the first (10–12) body somites; the embryo is susceptible to effects on neural tube closure, and developmental abnormalities of the brain, eyes, face and heart occur (see above and table 2). The studies examined primarily craniofacial defects, resulting from incomplete closure of the neural tube, and skeletal defects; specific investigations of nervous system defects and consequent disruptions of behaviour were not examined.

Many studies were of embryos exposed *in vivo*; some were carried out using exteriorized uterine horns or in culture, introducing possible confounding resulting from surgical procedures or the effects of culture conditions. However, there were usually good control procedures that would have uncovered confounding effects. Rat embryos cultured from 9.5–11.5 days at 38°C were indistinguishable in growth (protein accumulation) and differentiation (development) from equivalent embryos

in utero[69], suggesting that any effect of heat is on the embryo and not through maternal factors.

There are, however, indications that maternal factors might modify the embryonic response. A comparison can be made between an *in vivo* study and an *in vitro* study from the same laboratory. Cultured day 10.5 rat embryos were exposed to 42 or 43°C for 10–25 min and examined 1 day later, on day 11[70]. There was a dose-related inhibitory effect on growth in all systems and especially on systems that were in rapid stages of development at the time of exposure. A similar exposure *in vivo*[51] resulted in a similar high rate of skeletal defects but fewer head defects in embryos examined at term. The difference might be related to differences in dose, possible protection from an induced heat shock response during the slower warming period of the *in vivo* study and deletion *in vivo* of embryos with head defects during later gestation.

7.2.1. In vivo *rat studies*. The dose–response relationship between temperature elevation and the incidence of cranio-facial and skeletal defects in radiofrequency-irradiated Sprague Dawley rats at day 9 of pregnancy were studied by Lary *et al.*[55–57] (see figure 4 and table 3). The incidence of skeletal defects increased from a low level at an elevation of maternal temperature of 2.5°C to a maximum (80%) at an elevation of 4.5°C (table 3). The lowest threshold for skeletal malformation estimated by probit analysis was an increase of 2.9°C (CI = 2.6–3.1°C), for external malformations was 3.1°C (CI = 2.9–3.2°C) and for pre-natal mortality was 3.2°C (CI = 2.7–3.5°C).

Germain *et al.*[2] defined the minimum elevation and duration of elevation which resulted in cranio-facial defects in rat embryos exposed *in utero* at day 9.5 of gestation in a warm waterbath (figure 5). The maternal temperatures were raised rapidly and then held at the target temperature for a pre-determined duration (the temperature elevation was in the form of a plateau). The lowest temperature to cause cranio-facial defects was 2.5°C applied for 60 min (table 3), an elevation of 2°C applied for 8 h had no effect. As the temperature elevation increased above 2.5°C, the exposure duration to reach threshold for an effect became smaller. The dose-related incidence in treatment groups can be ascribed with some confidence to the treatment, since these cranio-facial malformations were not found in controls in this study or in a parallel study by Webster *et al.*[71]. Microphthalmia showed the lowest thresholds, encephalocoele had slightly higher thresholds while maxillary hypoplasia had the highest thresholds (figure 5).

Brown-Woodman *et al.*[58] carried out a similar experiment to that of Lary *et al.*[57], utilizing radiofrequency radiation (27.12 MHz) to heat rats on day 9.5 of pregnancy. A plateau-type temperature elevation of 2.5°C for 50 and 60 min caused a high rate of resorptions but no malformations (table 3), whereas an elevation of 3°C for 20 min, or a higher temperature for shorter durations, caused an increased incidence of cranio-facial defects, broadly in agreement with the results of Lary *et al.*[57].

Kimmel *et al.*[51] exposed pregnant rats on day 10.5 to warm air (43°C) for 40–60 min (table 3). This stage is later than the most susceptible stage for defects of the head[71]. The authors identified a significant threshold rise in body temperature of ~2–2.5°C for these malformations; interpretation was, however, complicated by comparison with sham-exposed animals whose body temperature dropped slightly during treatment.

7.2.2. In vitro *rat studies*. Cockroft and New[60] incubated day 9.5 rat embryos in culture at 40 or 41°C (2 or 3°C above the control cultures at 38°C), for 48 h. About half the embryos at 40°C had small developmental abnormalities, including microcephaly. Gross abnormalities were found in nearly all the embryos at 41°C. In other experiments[61], embryos were cultured at 38, 40, 40.5 and 41°C for 48 h. The results for cultures at 38, 40 and 41°C were similar to the other experiment. Embryos cultured at 40.5°C (2.5°C above the controls) were retarded, 55% were obviously microcephalic (small head) and one of the 18 embryos had pericardial oedema. Growth of the embryos cultured at 40°C appeared very similar to that of controls. These experiments demonstrated direct effect of heat on the embryo; threshold temperature elevations were 2–2.5°C for 48 h exposure.

Walsh *et al.*[37] exposed day 9.5 rat embryos in culture to various combinations of temperature elevations and duration (table 3). Higher temperature elevations, 3.5–5°C above the temperature of the controls, required shorter periods to induce a similar incidence of cranio-facial defects. However, the thresholds were well in excess of those found *in vivo*. Kimmel *et al.*[70] also found that rat embryos in culture appeared to require a longer time to cause defects than for induction of defects in embryos *in utero*. The reasons for this are not clear. In small quantities of culture medium, in bottles with a relatively large surface area, embryos reach the desired elevated temperature within 2–3 min, whereas the core temperatures of pregnant rats elevate more slowly, which might allow the development of a protective heat shock response. The fast rate of warming *in vitro* would not allow time for induction of this response. However, during maternal heating, a considerable dose of heat is delivered to the embryo during the slower warming up and cooling phases, which should be added to the dose. Rat embryos heated on day 10.5 at elevations 3–5°C above control cultures[39] required much longer durations of elevation to cause cranio-facial malformations.

7.3. *Guinea pigs*

Many studies have been made of the effects of hyperthermia on the pre-natal development of the guinea pig. Abortions and resorptions commonly followed heat exposure between days 11–18. Heat given at about the stage of neural tube closure (days 13–14) has not resulted in offspring with NTD (neural tube defects) at birth, but this did cause a high rate of abortion on about day 33. Subsequent experiments (table 3) showed that heat exposure on days 13–14 caused a high incidence of NTD and skeletal defects in embryos that were examined on days 23 or 24[72]. However, heat during organogenesis clearly caused more damage to growth and development of the brain and spinal cord than to other organs and structures. The most common outcome from heat exposure at this stage of development was a normally shaped, small brain. Because microencephaly was the most common defect and is easy to quantify, it has been extensively used for the determination of threshold doses. The functional consequences of this effect, however, in terms of behavioural performance, have not been fully explored, although a significant relationship between the extent of retardation of brain growth and poor performance in learning trials was reported in guinea pigs that had been heated *in utero* on days 20–24[64].

To determine the threshold temperature causing microencephaly[62,63], pregnant mothers were exposed in incubators on day 21 for 1 h to 38 or 43°C. Maternal temperatures increased from 39.3–39.5°C to 41.2–44°C (mean 42.7 ± 0.7°C) at the end of exposure (table 4). The treatment had very little effect on the body weight of

Table 4. Microencephaly and other defects in guinea pigs.

Species	Temp. elevation, (sham) °C	Minimum duration, min	Incidence of defects, % (*n*)		References
			Microencephaly	Other defects	
Guinea pigs, days 18–25					
Day 21 (×1)	2.5–3.5	60 (spike)	55 (40/73)***		62, 63
Controls	(39.5)		0 (0/35)		
Days, (×2)	3.7–3.8	60 (spike)			
14–15			9 (1/11)		
16–17			46 (6/13)***	Heart 15 (2/13)	
18–19			5 (1/21)		
20–21			73 (22/30)***	Cataract Hypoplasia digits, Exomphalos 10 (3/30)	
22–23			74 (17/23)***	Cataract Exomphalos 4 (1/23) Paralysis 9 (2/23)	
24–25			37 (10/27)**	Cataract 15 (4/27) Hypoplasia incisors 7 (2/27) Exomphalos 11 (3/27)	
26–27			9 (2/23)	Hypoplasia incisors, Talipes 4 (1/23)	
28–29			0 (0/28)	Exomphalos 4 (1/28)	
Controls	(39.5)		0 (0/36)	0 (0/35)	

Species	Temp rise°C mean (range)	Minimum duration, min	Brain weight (g) mean ± se (*n*)	Body weight (g) mean ± se	References
Guinea pigs, day 21					
One exposure	2.8 (2.3–3.0)	60 (spike)	2.618 ± 0.168* (28)	91.5 ± 19.2	4
	3.2 (3.1–3.5)	60 (spike)	2.536 ± 0.193*** (32)	83.4 ± 14.6	
	3.8 (3.6–3.9)	60 (spike)	2.349 ± 0.124*** (34)	86.3 ± 9.7	
	4.2 (4.0–4.4)	60 (spike)	2.167 ± 0.165*** (21)	84.3 ± 16.6	
Controls (sham)	0 (39.5 ± 0.3)	60	2.699 ± 0.168 (81)	90.7 ± 15.9	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the heated offspring at birth, but the brain weight of the heated group was significantly less ($p < 0.001$) than that of the control animals. The incidence of microencephaly in the heated group was 54.6% and there were only three animals (4%) with defects other than microencephaly. A multiple regression analysis of brain weight, body weight and maternal temperature showed significant effects of maternal temperature ($p < 0.001$) and of body weight at birth ($p < 0.001$) on the brain weight of heated offspring. It was estimated that 17.5% of variation in brain weight was due to body weight and 57% due to maternal temperature[62,63]. The regression of brain weight of newborn offspring on maternal temperature indicated that a significant reduction occurred when maternal temperatures exceeded 41.6–41.7°C (an elevation of 2.1–2.5°C). Similarly, the incidence of microencephaly increased at about the same level of maternal temperature elevation.

A significant incidence of microencephaly and reduction of the brain weight at birth were also found in another group of newborn guinea pigs after a single mater-

nal temperature elevation on day 21 (figure 6). An elevation of 2.8°C caused a significant ($p < 0.05$) reduction in brain weight of newborn[4]. In adult guinea pigs that had been heated *in utero* for 1 h daily during days 20–24, Jonson *et al.*[64] demonstrated a dose–response relationship in mature guinea pigs between the weights of the brain and the extent of maternal temperature elevation. A stepwise multiple regression analysis of the effect of temperature, bodyweight and age on the brain weight of heated offspring showed that the maternal temperature accounted for 67% of the variation in brain weight. The brain weight of offspring was reduced when the maternal temperature rose by ~2.1°C and, for each 1°C elevation of maternal temperature above 41.5°C, adult brain weight was reduced by ~10%. As adults, these offspring had learning difficulties in serial discrimination reversal learning tasks, with more errors in original discrimination tasks as well as in the four reversal tasks for both initial and perseverative errors. Their performance was significantly related to the extent of retardation of brain growth.

7.3.1. *Neuronal cell death and subsequent development of the brain.* In guinea pig embryos at 21 days, neuroepithelial cells in mitosis died following a spike elevation of maternal body temperature by at least 2°C[5–7]. At 5–6 h after heating at higher maternal elevations (3–3.5°C), apoptotic cell death was found in cells probably in S-phase at heating. Mitotic activity ceased from the time of heating and persisted for 4–8 h depending on the extent of temperature elevation. An exaggerated burst of mitotic activity resumed when signs of apoptosis were first seen and persisted for ~1 h, but this did not appear to compensate for the loss of neurones. A study of pyramidal cells in the cerebral cortex of newborn guinea pigs after the mothers were exposed to heat on day 21 with temperature elevations of 2.3–3.7°C showed that these cells were reduced in size but retained the basic topology of normal cells[73]. Dendritic arcs were shortened, particularly those of the first order of dendrites, and the differences diminished with distance from the cell body.

The consequences of early neuronal cell deaths on subsequent development of the brain were studied by sequential examinations (from day 30 of gestation to ~90 days of post-natal life) of brains of guinea pig offspring after heating on days 20–23[74,75]. By day 30 of gestation, there was a significant reduction in the weight and DNA (cell numbers) in the brain and its components and the same relative deficits of brain weight and cells persisted throughout neurogenesis until the onset of glial cell proliferation at about days 46–50. In both the control and heated offspring, the rapid stage of neuronal cell proliferation ceased and major glial cell production commenced at about day 50. The extent of glial cell production appeared to be regulated by the number of neurones remaining, because the same relative deficit of cells (and brain weight) was present throughout glial proliferation and myelination, until maturity. The smaller number of oligodendroglia appeared to produce reduced but appropriate amounts of myelin. No compensatory growth was apparent. The concentrations of cells and myelin were similar in heated and control brains throughout development. The net result of the heat exposure was production of a small brain (microencephaly) that was relatively normal in shape, concentration of cells and of myelin in all regions, but was deficient in total number of cells, total myelin and learning function.

It is important to note that only a small number of additional divisions would have been needed to make up the deficit in neurone numbers, but this did not appear to happen. It was proposed that neuronal stem cells are rigidly programmed to a

finite number of divisions and additional divisions by the surviving cells cannot make up any deficit in numbers due to cell death[74].

Heat on days 40–44 (the stage of early glial cell proliferation) caused a persisting microencephaly, but the extent of the deficit was less than that following heat between days 20–24. At maturity, offspring heated on days 40–44 made more perseverative errors in reversal tasks[76]. Guinea pigs heated on days 56—60 (the stage of active myelination) showed no differences from controls in adult brain weight and learning performance.

7.4. *Sheep*

Exposure of pregnant ewes to high ambient temperatures is associated with high rates of early embryonic mortality. Dutt[77] found that exposure of ewes on days 1–2 to 32.5°C, resulting in a maternal temperature elevation of 1.8°C, caused a loss of 78–100% of embryos. A loss of 54–69% followed exposure on days 3–5. Prolonged daily exposures (50 + days) of pregnant sheep to hot air temperatures during the last third or two-thirds of pregnancy resulted in elevation of body temperature of between 1–2.1°C above the normal level of 39°C and retardation of body growth, microencephaly and cavitation lesions of the brains of their lambs[78]. Long-term exposure of pregnant ewes to severe environmental heat during mid (days 50–100) and/or late gestation (days 100–150) caused severe growth retardation of lambs. The weights of the placentas were more severely reduced than the weights of the lambs[32]. Growth restriction of the lambs was attributed to a reduced maternal appetite, diversion of the placental circulation to sites promoting heat dissipation, limitations of the metabolic capacity of the smaller placenta and complex maternal and foetal endocrine changes.

7.5. *Pigs*

Pregnant sows exposed to ambient temperatures of 35–36.7°C on days 1–5[79] experienced temperature elevations to 40.6–40.8°C (a rise of 1.6–1.8°C) associated with severe embryonic loss.

7.6. *Primates*

Hendrickx *et al.*[24] gave bonnet monkeys 3–4 daily warm air exposures of 0.5–1.5 h between days 23–35. The most sensitive period was between days 23–29. During this time, the major events in organogenesis occur. The authors found teratogenic effects in a small number of offspring, including neural tube and umbilical cord defects, midface hypoplasia, anophthalmia, talipes, scoliosis, heart, umbilical artery, adrenal and kidney defects, growth retardation, after maternal temperature elevations of 2.4–4.1°C over the normal of 38.2°C. Resorptions and abortions followed elevations of 3.6–4.4°C and 3.6–3.9°C, respectively. In another study, Poswillo *et al.*[80] elevated the temperatures of cotton eared marmosets by 4.5°C for 1 h daily for 5 days between days 25–50. The mean core temperature of the exposed mothers was 41.5°C, an elevation of 4.5°C. There was a high rate of neonatal deaths in the heated group (46%). There was an exacerbation of skeletal defects (probably of dietary origin) affecting the colony, and one survivor was clumsy in movement, retarded in development and had behavioural problems.

8. Interactions of hyperthermia with other agents or metabolic conditions

The threshold temperature increases observed above for developmental defects might be modified by drugs[81], metabolic conditions or other toxic agents.

There is some evidence that conditions causing an endotoxaemia will also interact with hyperthermia. Niswander and Gordon[82] showed that ~1.5% of women had urinary tract infections with fever in excess of 38°C during pregnancy. Urinary tract infections are usually accompanied by an endotoxaemia. These pregnancies were twice as likely to be associated with a child with neurological problems. This effect was not found in women with urinary tract infections who did not have a fever.

Hilbelink *et al.*[83] showed that *E. coli* endotoxin injected into hamsters caused a high rate of resorptions. Malformations were found in some offspring following maternal temperature elevations to 38.5–38.7°C. The mean temperature of control animals was 37.2°C (with a range of 36.5–38.2°C, the higher temperatures being recorded at night, the active period). The teratogenic temperature elevations were in the order of 1.3–1.5°C.

Ferm and Kilham[84] and Ferm and Ferm[85] showed that minimally teratogenic doses of heat combined with minimally teratogenic doses of arsenic and Vitamin A, respectively, caused a markedly enhanced teratogenic response in hamsters. Edwards and Beatson[86] used small doses of lead to potentiate the effects of heat on pre-natal brain development. Shiota *et al.*[87] found that alcohol potentiated the effects of small temperature elevations. They monitored the rectal temperature of female mice and found that in mice pre-treated with ethanol, a high body temperature, induced by heating in a water bath at 42°C for 10 min, persisted longer (10 min) than controls (6 min) after they were removed from the bath. Angles *et al.*[88] caused abnormalities in rat embryo with small elevations of temperature of the culture medium and exposure to ultrasound. Tiboni *et al.*[89] found that pre-treatment with a small non-teratogenic dose of aspirin potentiated the teratogenic effects of heat in mice. Conversely, Shin and Shiota[90] found that folic acid supplementation of the diet during early gestation gave some protection against heat-induced neural defects in mice.

9. Human studies

There are numerous studies with positive and negative findings on the effects of hyperthermia on human pre-natal development[3,91]. Only the most relevant studies and those defining the temperature elevation associated with defects are included here. In the positive studies, the defects produced generally resemble those found in experimental animals when the hyperthermic episode coincides with the comparable sensitive stage of development, with the central nervous system (CNS) being particularly susceptible. A number of studies are based on the effects of fever, while others include data on other causes of maternal hyperthermia such as exposures to hot tubs, saunas or heavy work in hot environments. It is difficult to separate the effects of heat alone from the confounding effects of maternal metabolic changes occurring in fevers. However, prospective studies by McDonald[92,93] and Milunsky *et al.*[94] include groups exposed to both sources of hyperthermia.

Accurate definition of risks associated with pre-natal hyperthermic exposure requires records of the extent and duration of temperature elevation and precise timing of the exposure. The actual temperatures of many patients do not appear to be taken or recorded regularly. For instance, although Clarren *et al.*[95] reported a documented fever in the first trimester in only 0.3% of 55 000 pregnant women in the

Collaborative Perinatal Study[82], over 5.2% of these women had febrile viral or bacterial infections (influenza, herpes simplex, gastroenteritis, bronchitis, tonsillitis, mumps, measles, chickenpox), during pregnancy[96]. An additional 1.5% of the women had kidney-urinary tract infection with fever.

In most of the epidemiological studies, the stage of gestation studied has been very broad (for example, the first trimester or 5 months of pregnancy) rather than spanning the shorter periods occupied by specific developmental events. Neural tube closure takes place in ~7–10 days, which is only a fraction of a trimester. A study period of 13 or more weeks for NTD significantly dilutes and distorts the estimates of relative risks and prevalence. Another factor to be considered in estimating the risks of an exposure to heat during neural tube closure is that severely damaged embryos are more likely to be aborted than to survive to birth[97], which would also mask the real prevalence of defects.

9.1. *Prospective studies*

Prospective studies with associations between hyperthermia or febrile conditions and birth defects include those by McDonald[92,93], Coffey and Jessop[98,99], Milunsky *et al.*[94] and Chambers *et al.*[100]. A study by Shiota[101] on foetuses from voluntary abortion early in pregnancy was classified as retrospective. However, the obstetric history was taken at the clinic at, or immediately after, the time of delivery and before the foetus was examined. It is included here among the prospective studies.

In a detailed, prospective study of 3144 women, McDonald[92, 93] found significant increases in abortions and malformations in children of mothers who had febrile illnesses during the first 12 weeks of pregnancy. From 148 women with a febrile illness, 4.7% aborted and 6.1% of foetuses had serious malformations compared with 2.1% and 2.8%, respectively, in the controls. Of a group of 27 women who worked in the hot conditions of a laundry, four had a child with a major abnormality. The defects included anencephaly, hydrocephalus, heart defect, cleft lip and palate and hypospadias.

Coffey and Jessop[98,99] prospectively studied the effects of Asian influenza on women at various stages of pregnancy. Although there was a significant 2.4-fold increase in the rate of malformations in the affected group compared with controls, the type of defect (especially of the neural tube) did not correlate well with the stage of pregnancy during infection, in a number of cases.

A causal relationship between NTD (exencephaly and/or myeloschisis) and maternal febrile illness was investigated in embryos from induced or spontaneous abortions in the Embryological Research Centre of Kyoto University[101]. The maternal medical and obstetric history was obtained before or immediately after the foetal loss and before the foetus was examined, so the time span between the interview and a reported event in the pregnancy was short. All data relating to hyperthermia was derived only from the interview records. Three groups of controls from the same collection were used, one normal control matched by maternal attributes with each NTD embryo, another group of 107 embryos with holoprosencephaly and the third group was of 121 embryos with polydactyly. Over 50% of NTD embryos showed signs of intrauterine death at the time of abortion. Among the 113 women with an NTD embryo, 14.2% had a febrile illness during the 35–115 days of pregnancy. This was significantly higher than for the combined controls and the holoprosencephaly controls. There was also a significant excess of febrile illnesses in the cases with exencephaly myeloschisis in all control groups. In 50% (eight of the 16) NTD

cases, the febrile illness occurred during the clinically determined period of neural tube closure. The incidence of febrile illness in the control groups varied between 7.4% for polydactyly and 3.7% for holoprosencephaly.

Milunsky *et al.*[94] conducted a prospective follow-up study of the effects of exposure to hot tubs, sauna, fever or electric blankets during the first 3 months of pregnancy in a group of 23 491 women. Complete outcome and exposure information was available in 97%. A total of 5566 women were exposed to at least one source of heat, 1865 had a fever, 1254 used hot tubs, 367 used saunas and 2883 used electric blankets. There were 17 188 women who did not have exposure to any of these heat sources. Women who had exposures to hot tubs, sauna or fever had a crude risk for NTD of 2.2 times that of women who were not exposed (CI = 1.2–4.1). The relative risk for exposure to two of these sources increased to 6.2 (CI = 2.2–17.2). Exposure to three sources did not cause an increase, but the number of subjects in this group was small. The actual temperatures of the patients were not available.

The prospective study by Chambers *et al.*[100] found that defects were caused by a temperature elevation during fever to at least 38.9°C (~2°C above normal) for a minimum of 24 h but not by a lower elevation for less than 24 h. The defects included anencephaly, hernias, cardiovascular defects and digital, ear and face anomalies. The study indicates that for humans, teratogenic temperatures of 38.9°C and above require a duration of at least 24 h, whereas maternal temperatures below 38.9°C and for less than 24 h did not cause defects.

9.2. *Retrospective studies*

Early retrospective studies[102–106] drew attention to the association between NTD in early pregnancy and an episode of hyperthermia mainly due to fever with temperatures exceeding 38.9°C for at least 1 day. However, the threshold for human teratogenicity is not well defined and needs detailed study. The study by Layde *et al.*[104] at the Birth Defects Branch, Centre for Disease Control, of a group of 229 mothers of children with anencephaly or spina bifida, used 458 mothers of children with Down syndrome or clefts of the lip or palate as controls. These control groups were intended to eliminate possible maternal recall bias. However, it should be noted that associations between fever and clefts of the lip and palate have been found in a number of studies[107,108]. Using the Down's syndrome group as a control, a significantly higher incidence of fever or febrile illness was demonstrated for all NTD, but comparison with the clefts group was not significant. When both groups were used as controls, there was a significant association with spina bifida. Similar results were found in the prospective study by Milunsky *et al.*[94] and in the study by Shiota[101].

In all these studies, the proportion of children with NTD associated with first trimester hyperthermic exposure ranged between 10–14%.

Pleet *et al.*[108] identified a group of children whose mothers had experienced a fever, with temperatures exceeding 38.9C for at least 1 day between 4–14 weeks gestation. The episode of hyperthermia was pinpointed within a 2-week period in each case. The causes of hyperthermia were viral or bacterial infections or exposures to sauna or hot tub baths. All 28 children exposed between 4–14 weeks gestation had mental deficiency, and most had altered muscle tone. [It is interesting to note in this context that decrements in school performance were maximal following *in utero* exposure between weeks 8–15 of gestation[21].] Other findings in these children were

seizures, neuronal heterotopias, facial defects including midface hypoplasia, cleft lip and cleft palate, and microphthalmia and micrognathia were found among those exposed between 4–7 weeks. In an additional study of nine children subsequently reported by Graham and Edwards[109], neurogenic contractures (arthrogryposis) and altered muscle tone were detected from exposures between 4–20 weeks. Defects of brain morphology found by CT scans included mild atrophy and agenesis of the corpus callosum. One child had Moebius sequence (damage to neurons of a number of brainstem nuclei).

Erickson[110] published data from the Atlanta birth defects retrospective case-control study. In a comparison between 4929 babies born with major birth defects and 3029 babies without defects, he found 50 highly significant associations ($p < 0.001$) between certain exposures in pregnancy and serious birth defects. The rate of defects in these associations was 16 times more than could be attributed to chance and, of the 50 positive associations, a total of 27 exposures were to 'any fever' and 'flu' (itself a febrile illness). Approximately 10 of these associations overlapped, but these febrile conditions were by far the most prevalent of all recorded exposures.

Tikkanen and Heinonen[111] investigated the effect of conditions that might cause hyperthermia on the occurrence of cardiovascular malformations in a group of 573 children with defects and 1055 control children. They found a significant ($p < 0.01$) association between first trimester fever ($> 38^{\circ}C$) and atrial septal defects, highly significant ($p < 0.001$) associations between first trimester fever and hypoplastic left heart and between upper respiratory tract infection and all heart defects. Spragget and Fraser[112], Graham and Edwards[109] and Erickson[110] have also reported an association between heart defects and fever.

It has been suggested that foetal loss is probably the most common adverse outcome of hyperthermia in early pregnancy in women[109]. Animal studies have shown that exposures of one to a few days duration to mild hyperthermia (elevations of 1.6–1.8°C) during the pre-implantation stages in sheep[77], pigs[79] and mice[113] caused heavy embryonic loss. Similar losses would probably not be detected in women.

Despite the diversity of fevers caused by viral or bacterial infections and non-infectious causes of hyperthermia in women, their effects on pre-natal development are similar, with the CNS being the major site for damage. The common factor in each agent is elevated temperature. The metabolic changes associated with fever might modify the embryonic response.

10. Summary and conclusions

The pre-natal growth of mammals is characterized by highly ordered sequences of cell proliferation and differentiation, migration and programmed cell death (apoptosis) that are susceptible to heat. Sensitivity varies dramatically during development; various time 'windows' of vulnerability are present, principally during organogenesis when the organ systems of the body are forming but also during the foetal period, when some new structures appear. Thresholds are strain and species, developmental stage and tissue specific, precluding rigorous quantitative extrapolation between species, especially from experimental animal studies to humans, although general comparisons may provide valuable mechanistic insights.

Hyperthermia during organogenesis induces various developmental defects which can be related to the amount by which maternal body temperature is elevated; the normal body temperatures on some animals are in the teratogenic range for

humans. The CNS, which continues to develop in the foetal period and early post-natal life, seems especially vulnerable. Various threshold studies have been carried out in experimental rodents. These have examined principally the incidence of cranio-facial and skeletal defects in mice exposed during early neural tube closure (day 8.5) and microencephaly, reduced cortical thickness and learning defects in mice exposed during neurogenesis, including corticogenesis (days 13–14); the incidence of cranio-facial and skeletal defects in rats exposed during early and mid-neural tube closure (days 9–10.5); and microencephaly and other nervous system defects including learning disorders in guinea pigs exposed during early neurogenesis and formation of the cortical plate (days 20–24). Threshold temperature elevations and dose–response relationships have been identified for cranio-facial and skeletal defects in rats and microcencephaly in guinea pigs. Generally, statistically significant increases in the incidence of heat-induced abnormalities are seen in the laboratory at maternal temperature increases of ~2–2.5°C or more, mostly following exposure for tens of minutes up to 1 h or so. Higher elevations, up to ~5°C, were required for shorter durations.

Some work has been carried out using other species. Long-term exposure of pregnant ewes to severe environmental heat during mid-to-late gestation caused severe growth retardation, attributed to reduced maternal appetite, placental circulation and maternal and foetal endocrine changes. Few studies of the effects of heat on the development of primates has been carried out; a number of teratogenic effects have been reported following repeated elevations of maternal body temperature by ~2.5–4.5°C.

The developing CNS is considered to be the system most sensitive to raised maternal temperature. Microencephaly requires the smallest dose of heat, which, in guinea pigs, is a spike elevation of 2.0–2.5°C, rising and falling over a 60 min period. It can be induced during neural tube closure and early neurogenesis and, to a lesser extent, during glial cell proliferation and during neuronal myelination, although in the latter case the deficit found at birth is made up during post-natal growth. Interestingly, behavioural deficits were seen in mice and guinea pigs exposed during corticogenesis and additionally in guinea pigs during glial cell proliferation. Generally, however, microencephaly is a marker of gross CNS toxicity and would not necessarily account for minor lesions in specific brain regions, nor abnormal neuronal migration and subsequent abnormalities in dendritic arborization. There is clearly scope for more studies that rigorously identify quantitative CNS lesion thresholds, for example by quantifying cortical dendritic alignment and by utilizing a wider range of tests to quantify behavioural change. The possibility that other agents may reduce the threshold for potentially deleterious effects of heat could also be further evaluated.

With regard to the effects of maternal hyperthermia on human *in utero* development, several studies suggest a threshold maternal temperature elevation to ~39°C, a rise of ~2°C above normal, for a significant increase in the incidence of heat-induced defects. Various teratogenic effects have been reported, including neural tube defects such as cranio-facial abnormalities. Where possible, future epidemiological studies should aim to partition gestation into sensitive periods for the endpoints considered in order to avoid the potential dilution of any developmental-stage-specific effect. Such partitioning should be based on developmental sensitivities established in biological experimentation. Since animal studies suggest that the development of the CNS is particularly sensitive to hyperthermia, there is perhaps scope for examining

further the potential adverse effects in humans. In this context, it is notable that IQ and school performance have been examined in children exposed *in utero* to ionizing radiation at Nagasaki and Hiroshima; these and related data on the incidence of microencephaly and severe mental retardation provide important guidance in radiological protection.

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