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Teratogen Update: Gestational Effects of Maternal Hyperthermia Due to Febrile Illnesses and Resultant Patterns of Defects in Humans

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Hyperthermia was the first teratogen in animals that was subsequently proven to be teratogenic in humans. Animal studies have demonstrated heat to be a significant cause for reproductive problems in a wide variety of mammals. These problems range from embryonic death and abortion to teratogenically induced anomalies, and are heavily dependent on the dose and timing of the exposure (Edwards, '86; Edwards et al., '95). The threshold of effect in many species begins at about 1.5°C over normal core body temperature. In general, higher temperatures and/or longer durations are most likely to cause abortions, while lower elevations cause embryonic death and resorption, or abnormalities of embryogenesis, if exposure occurs at critical stages of development.

The range of defects induced by hyperthermia in experimental animals includes: anencephaly/exencephaly, encephalocele (Webster and Edwards, '84; Cawdell-Smith et al., '92), micrencephaly (Edwards, '69b; Edwards et al., '84; Upfold et al., '89), microphthalmia, talipes, arthrogryposis, abdominal wall defects, and limb reduction defects (Edwards, '86). Such defects (Figs. 1, 2) have been induced by heat in a variety of mammals, including guinea pigs, hamsters, rats, mice, rabbits, sheep, pigs, monkeys, and humans (Edwards, '86), though the confounding effects of the febrile illnesses themselves and their therapies remain problematic in the interpretation of human data. Central nervous system (CNS) defects appear to be the most common consequence of hyperthermia in all species, and cell death or delay in proliferation of neuroblasts is believed to be one major explanation for these effects (Edwards et al., '74; Wanner et al., '75; Upfold et al., '89). Vascular disruption may also be involved in the pathogenesis of some defects of the CNS and other structures (Nilsen, '85; Webster et al., '87).

In the human, heat-induced vascular disruption has been implicated in the pathogenesis of Moebius syndrome (Graham et al., '88; Lipson et al., '89), oromandibular-limb hypogenesis syndrome (Superneau and Wertelecki, '85), and the amyoplasia form of arthrogryposis (Ivarsson and Henriksson, '84; Edwards et al., '90). In this review, we confirm an association between febrile maternal illnesses and offspring born with these conditions, as well as other central nervous system problems. The nature of the specific associated anomalies appears to relate to the extent, duration, and timing of the maternal fever. These associated defects are similar to those induced experimentally in guinea pigs, monkeys, and a wide variety of other experimental animals (Hendrickx et al., '79; Edwards, '86; Edwards et. al., '95).

BACKGROUND IN EXPERIMENTAL ANIMALS

Hyperthermia refers to an elevated body temperature. It has many causes, including febrile infections, hot/humid environments, and heavy exercise (especially in conditions of high heat and humidity), which may affect all species of animals. The normal physiological homeostatic mechanisms, which maintain body temperatures at relatively stable levels, may be altered or overwhelmed by certain conditions or drugs. There is an increased risk of hyperthermia when a combination of causes coincides, e.g., certain drug exposures, fever, or exercise occurring together in a hot environment (Lomax, '87).

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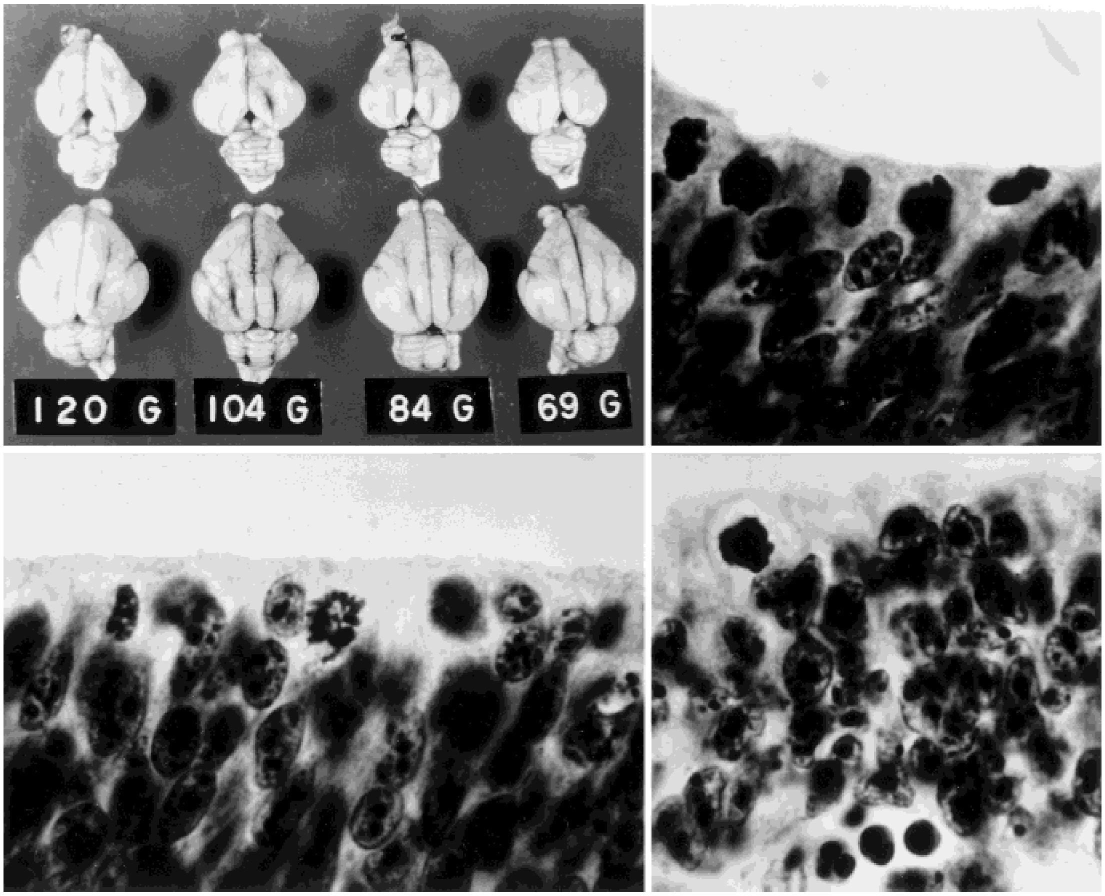

Fig. 1. When guinea pigs are exposed to hyperthermia (1 hr per day, 2–3°C above core temperature) between 18–25 days of gestation (equivalent to 5–8 weeks postconception in the human), the most common abnormality is micrencephaly. Normal neuroepithelial stem cells can be seen undergoing mitosis in the periventricular layer (bottom left), but 45 min after heating, these cells become pyknotic (top right), with cell death evident within 2 hr (bottom right). The end result is a proportionately smaller brain (top row, top left) when compared with control brains (bottom row, top left).

The average body temperature of most mammalian species falls between 37–40°C, and the usual maximal diurnal variation is approximately 1°C on either side of the average. Various experimental methods have been used to induce hyperthermia in studies of its adverse effects on prenatal development. In chickens, Dareste (1877), Alsop ('19), and Nilsen ('85) used elevated temperatures of incubation. Hot-air incubators were used for rats (Edwards, '67; Kimmel et al., '93), guinea pigs (Edwards, '67; '69a), hamsters (Kilham and Ferm, '76; Umpierre and Dukelow, '77), mice (Lecyk, '66), sheep (Hartley et al., '74), pigs (Done et al., '82), and monkeys (Poswillo et al., '74; Hendrickx et al., '79). Warm-water baths were used for rats (Germain et al., '85; Webster et al., '85) and mice (Webster and Edwards, '84; Finnell et al., '86). Electromagnetic radiation was used in rats (Lary et al., '82, '83; Brown-Woodman et al., '88), and fever was induced in rabbits by injection of milk (Brinsmade and Rubsaamen, '57) or endotoxin (Hellmann, '77). Skreb and Frank ('63) exteriorized the pregnant uterine horn of rats and immersed it in hot water. Hofmann and Dietzel ('53) used diathermy in rats, and Fukui et al. ('92) used microwaves in mice. Cockroft and New ('75, '78), Mirkes ('85), and Walsh et al. ('85, '87) exposed rat embryos in culture to elevated temperatures, and ultrasound was used by Angles et al. ('90).

The interactions between hyperthermia and other teratogens have been examined in a number of studies. Evidence of synergistic or additive effects have been found between low teratogenic doses of heat and vitamin A (Ferm and Ferm, '79), heat and arsenic (Ferm and Kilham, '77), heat and lead (Edwards and Beatson, '84), heat and ultrasound (Angles et al., '90), heat and alcohol (Shiota et al., '88), heat and endotoxins (Hilbelink et al., '86).

EFFECTS OF HYPERTHERMIA IN EXPERIMENTAL ANIMALS

The type of defect caused by heat in embryos is determined largely by the developmental stage at the time of the exposure, while the severity and incidence of defects depend largely on the dose. There is no single or

Fig. 2. In addition to micrencephaly, other defects seen when guinea pigs are exposed to hyperthermia (1 hr per day, 2–3°C above core temperature) between 18–25 days of gestation (equivalent to 5–8 weeks postconception in the human) include: microphthalmia (top left), hypoplastic incisors (top right), exomphalos (bottom left), and brachydactyly (center right) or oligodactyly (bottom right).

simple means of quantifying the dose, which is a function of the elevation of temperature and the duration of elevation. This has made it difficult to define threshold dose levels of hyperthermia, the threshold being the lowest dose demonstrated to cause a given defect in a significant number of exposed embryos. Although the thresholds for temperature elevations causing defects have been determined for a number of species, only a limited number of accurate dose (temperature elevation/duration) thresholds have been published, and they relate mainly to the rat. In addition, within a particular species, different defects have different dose thresholds, and some different inbred mouse strains have different thresholds for the same defect (Finnell et al., ’86). Exposure to higher elevations for a fixed duration, or to a fixed elevation for longer durations, generally increases the severity and incidence of defects. A number of studies in rats have defined the threshold dose by finding the shortest duration of exposure at a given elevation of temperature for the production of a defect. These results showed comparable thresholds, and as the temperature was elevated, the time required to cause a particular defect was reduced logarithmically. In all species there appears to be a threshold elevation capable of causing defects of approximately 2.0–2.5°C above the normal temperature for that species. The threshold duration at a temperature elevation of 2.0–2.5°C appears to be about 1 hr. This dose causes neural tube defects and microphthalmia in 9.5-day rat embryos (Germain et al., ’85) and irreversible micrencephaly in 21-day guinea pig embryos (Edwards, ’69b). Even very prolonged exposures to elevations of less than 2°C do not appear to cause defects, so it appears justified to cite 2°C as a threshold elevation without specifying a duration. Microphthalmia is produced in rats by 10 min of heat at an elevation of 3.5°C, with a spike elevation to 4.5°C. Lary et al. (’83) induced hyperthermia in rats by exposure to electromagnetic radiation and found similar time/elevation thresholds. Shiota (’88) found a similar set of thresholds in mice at 8.5 days for exencephaly (10 min at an elevation of 3.5°C, with 5 min at 4.5°C).

It is important to note that the normal deep body temperatures differ between these species: for guinea pigs, it is 39.5°C (Edwards, ’69a), for rats it is 38.5°C (Germain et al., ’85), and for mice it is approximately 38.3°C (Shiota, ’88). This is important because it indicates that the threshold for damage is related to the elevation of the temperature of the embryo above normal, rather than to the actual temperature achieved.

In all experimental studies, hyperthermia has caused a spectrum of effects in pregnant animals, which include embryonic and fetal resorption, abortion, and malformations. Only a 1.5°C elevation of temperature above normal core temperature, during the preimplantation period, can result in increased rates of embryonic death and resorption in a wide range of species (Bell, ’87). After implantation, relatively higher doses can result in malformation, while more severe exposures result in embryonic or fetal death, followed by resorption or abortion. Rigid control of the temperature elevation and the duration of exposure can achieve low levels of resorption and abortion in experimental animals. Nonexperimental or uncontrolled hyperthermia exposures such as those from febrile infections, hot environments, heavy exercise, or dehydration can result in high elevations of temperature, and fetal resorptions or abortions occur frequently. In pregnant domestic animals, abortion is one of the most common early manifestations of a febrile infection. It has been shown that uterine motility is stimulated by induced hyperthermia (Morishima et al., ’75), and it has been suggested that abortion could be the most common adverse outcome (Graham and Edwards, ’89). Induced hyperthermia in pregnant sheep causes the release of prostaglandins in fetal and maternal tissues, which could be the mechanism for such hyperthermia-related abortion (Andriankis et al., ’89).

Experimentally induced malformations after hyperthermic exposures in animals involve many organs and structures (reviewed by Edwards, ’86; Edwards et al., ’95). However, those affecting the central nervous system are most common and include neural tube defects, microphthalmia (in rats, mice, hamsters, guinea pigs, and monkeys), micrencephaly (in rats, mice, guinea pigs, pigs, and sheep), cranial nerve defects (guinea pigs), behavioral abnormalities (guinea pigs and mice), disturbances of muscle tone, talipes, arthrogryposis

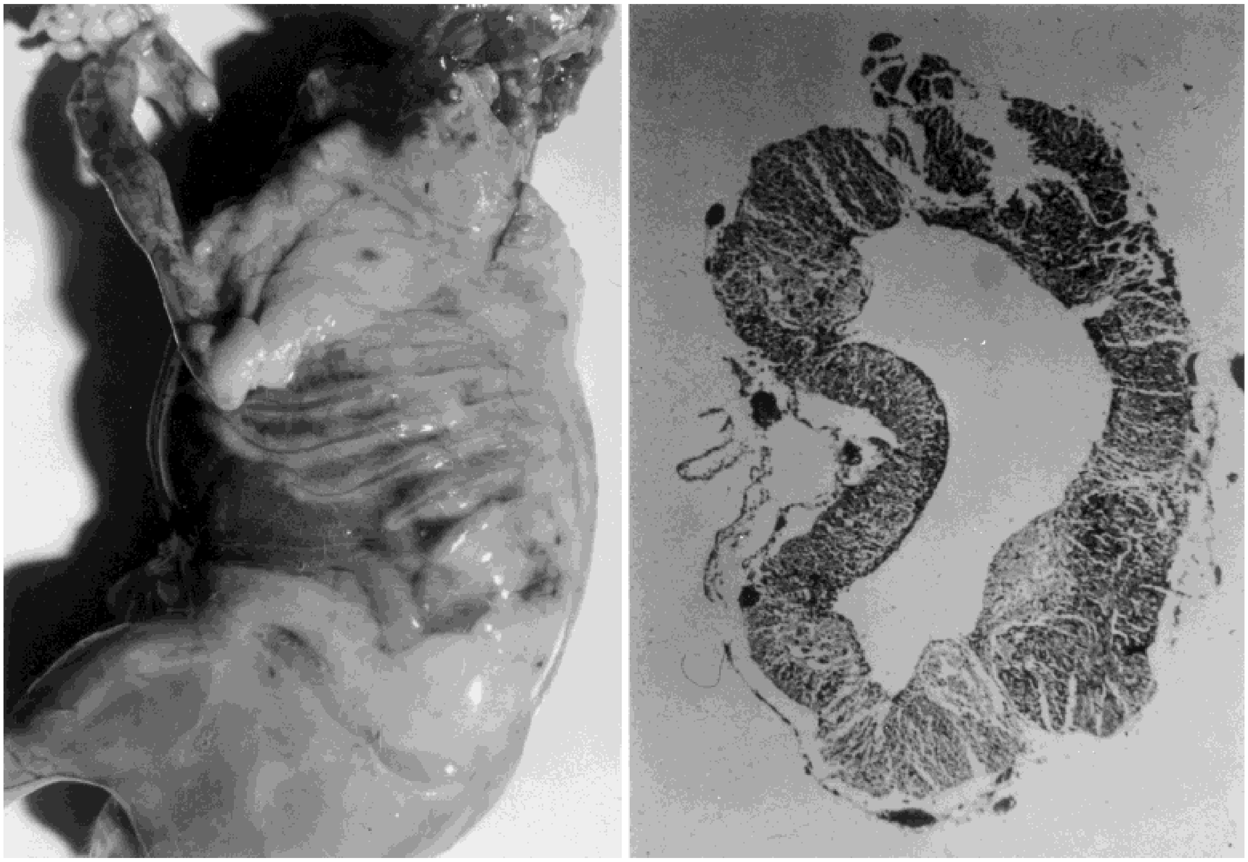

Fig. 3. Forelimb neurogenic arthrogryposis (left) in guinea pigs exposed to hyperthermia on gestational days 26–27, with cervical cord syringomyelia (right) and cavitation of grey matter within the central canal of the spinal cord. Muscles in the affected areas of forelimbs appear wasted, with partial replacement of muscle by adipose tissue.

multiplex congenita (guinea pigs and monkeys), and reduced learning capacity (mice and guinea pigs). Other defects include craniofacial anomalies (rats, mice, guinea pigs, and monkeys), heart defects and hypodactyly (rats, guinea pigs, and monkeys), cataracts and coloboma (guinea pigs), kyphoscoliosis (rats, mice, guinea pigs, and monkeys), renal anomalies (guinea pigs and monkeys), dental agenesis, and exomphalos (guinea pigs). These defects are illustrated in Figures 1–4. The timing for some of these exposures and defects is shown in the table, along with timing for similar defects reported in humans.

MECHANISMS

Cell death, membrane disruption, vascular disruption, and placental infarction have been implicated in causing embryonic damage. Hyperthermia during the stage of neural tube closure results in neural tube defects. Hyperthermia during later stages of neuronal proliferation results in microcephaly, and causes marked histological changes, especially in the neuroepithelium (Fig. 1). These changes include cessation of normal proliferative activity, marked reductions in mitotic figures for 3–6 hr after exposure, and variable levels of apoptotic cell death. In guinea pigs (Edwards et al., ’74; Wanner et al., ’75; Upfold et al., ’89), mitotic cells are killed by elevations in excess of 2°C, and cells in S-phase undergo apoptosis after temperatures exceeding 3°C, the number depending on the level of elevation. In day 8.5 or 9.5 mouse embryos in vivo (Shiota, ’88), or rat embryos in culture on day 10.5 (Cockroft and New, ’75, ’78; Walsh et al., ’85, ’87; Mirkes, 85), and in 13–14-day rat embryos in vivo (Harding and Edwards, 93), variable but usually low levels of apoptotic cell death were found in the head, and especially within the neuroepithelium between 3–15 hr after exposure. Mirkes et al. (’97) noted DNA fragmentation (a hallmark of apoptosis) as early as 2.5 hr after rat embryos were exposed to 43°C, with a smaller but significant increase in DNA fragmentation noted 5 hr after exposure to 42°C. Using the TUNEL method (Gavrielli et al., ’92), apoptosis-specific DNA degradation was related to the degree of temperature elevation and confirmed in the neural epithelium at the point of neural tube closure and in the optic stalk (Mirkes et al., ’97). Thus, hyperthermia-induced cell death correlates with

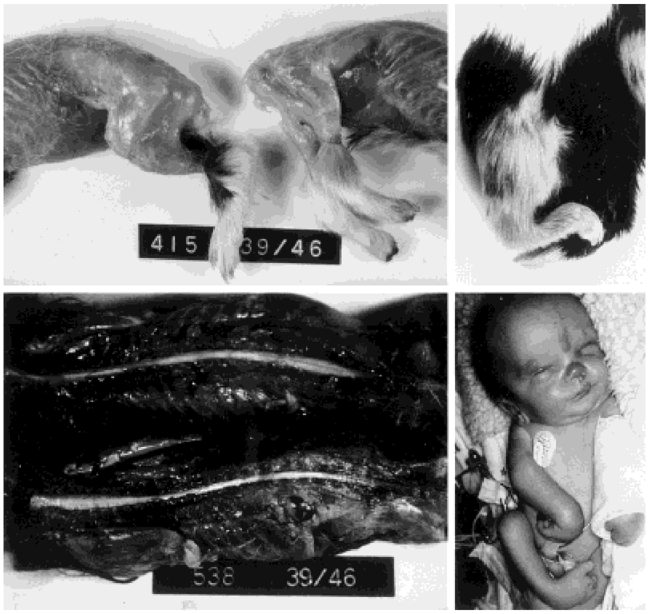

Fig. 4. Hindlimb neurogenic arthrogryposis in guinea pigs exposed to hyperthermia between gestational days 39–46 (equivalent to second trimester in humans). Neurogenic muscular atrophy (top left, control on left with heated fetus on right) is associated with decreased lumbar cord diameter (bottom left, control at top, heated fetus at bottom). Neurogenic arthrogryposis in guinea pig (top right) is similar to that seen in a human infant (bottom right) whose mother experienced intermittent high fevers to 102°F every 2–3 days during the second and third trimesters.

internucleosomal DNA fragmentation, which is characteristic of apoptosis and programmed cell death. In guinea pigs with neurogenic talipes induced by heat, there was also moderate to severe disruption of the basement membrane, which led to disordered architecture of the neuroepithelium, including the subsequent formation of rosettes and ectopic nests of neurons, and abnormal development of the gray-white architecture around multiple or misplaced central canals of the spinal cord (Figs. 3, 4) (Edwards, ’71).

The heat-damaged embryonic brain does not appear to have a capacity for compensatory growth to make up the deficit of cells killed by heat. After heat damage during neural tube closure, an additional division by only a minor proportion of the neuroepithelial population could make up the numbers required for closure. Also, the cells lost due to hyperthermia during early brain histogenesis, leading to microencephaly, could similarly be made up by a few additional divisions by the surviving cell population. It has been proposed that during embryonic brain development, neuronal proliferation terminates at a precise time, even when the target number of cells has not been achieved, possibly because the cells induced to form the brain are programmed for a finite number of divisions (Edwards et al., ’76; Edwards, ’81). In this model, cells lost following heat exposure would not be replaced. Even after supplementation via osmotic minipumps with folate, which may facilitate mitosis, heat-induced exencephaly is not significantly changed in the hamster model (Graham and Ferm, ’85).

Another mechanism of heat damage to embryos is vascular disruption, resulting in microvascular insufficiency due to endothelial damage, with leakage and perivascular and interstitial edema (Nilsen, ’85). Webster et al. (’87, ’88) showed that a number of agents, including hyperthermia, caused embryonic vascular damage with hemorrhage and resulted in hypoplasia of limbs and digits, and other defects. Defects such as gastroschisis, cranial nerve defects, neurogenic arthrogryposis, and hypodactyly could all be the result of this mechanism. Although maternal physiological changes caused by hyperthermia might affect embryonic and fetal growth and development, there is no strong evidence that maternal changes are direct causes of defects. However, it is possible that severe maternal reactions could modify the embryonic or fetal responses.

Hendrickx et al. (’79) found that the placentas of bonnet monkeys, heated in a hot-air incubator for approximately 70 min daily for 1–4 exposures between 21–46 days of pregnancy, showed damage in addition to being associated with malformed offspring or fetal death with delayed abortion. The four placentas examined were normal in weight, but each showed infarction, and three had intervillous thrombi. Placental damage was also found in rats by Arora et al. (’79) after heat exposure on days 6, 8, or 10 of pregnancy. Affected placentas showed extensive thickening and necrosis of the decidua basalis. In this study, heated offspring showed micrencephaly, microphthalmia, or skeletal defects after exposure at different stages of embryonic development.

Experimental studies with 9.5–10.5-day rat embryos in culture have shown that heat induces a stress response (Mirkes, ’85, ’87; Mirkes and Doggett, ’92; Walsh et al., ’85, ’87, ’89, ’93, ’94). A mild, nonteratogenic dose of heat (42°C for 10 min) confers strong protection against a subsequent, more severe exposure that would otherwise cause malformations (43.5°C for 7.5 min). The protection is conferred within 15 min after the initial exposure if the embryos are returned to their normal temperature, but embryos maintained at the initial elevated level that provoked the heat shock response were not protected. The onset and duration of protection coincided with the appearance and decay of heat shock proteins.

It has been recognized for many years that heat causes protein denaturation (Mirsky and Pauling, ’36), and even at normal body temperatures there is a balanced but significant loss of cells due to heat damage (Johnson and Pavelec, ’72). It is possible that the cell death and other cellular changes found in heat-damaged embryos might be caused by heat denaturation of enzymes and other functional and structural proteins. Recent research on the heat shock response in embryos adds weight to this possibility.

TABLE 1. Clinical findings in experimental hyperthermia compared with observations in exposed humans

	Mouse	Rat	Hamster	Guinea Pig	Monkey	Human
Total length of gestation	20 days	21–22 days	16 days	68 days	166 days	38 weeks
Exencephaly/encephalocele	7–8 days	9 days	7–8 days	13–14 days		3–4 weeks
Vertebral kyphosls/scoliosis	8 days	9 days		13–14 days	26 days	
Microphthalmia	7–9 days	9 days		14–18 days	26 days	4–7 weeks
Maxillary/mandibular hypoplasia		9–10 days			24–27 days	4–7 weeks
Facial clefts	8 days	9 days	9–10 days			4–7 weeks
Microcephaly with seizures				20–30 days		4–7 weeks
Microcephaly with arthrogryposis				39–46 days		8–18 weeks
Microcephaly	12–15 days	13–14 days		53–60 days		18–26 weeks
Cardiac defects		9.5 days		13–14 days	26 days	5–6 weeks
Talipes			9–10 days	18–25 days	24–27 days	7–20 weeks
Hypodactyly	9 days	12–14 days		18–25 days		7–15 weeks
Exomphalos		9 days		20–23 days		6–10 weeks
Moebius syndrome		14–16 days				7–20 weeks
Arthrogryposis			9–10 days	30–46 days		7–20 weeks

HEAT SHOCK RESPONSE

The heat shock response is the cellular response to hyperthermia. It is a highly conserved mechanism, which offers protection against a number of diverse cellular toxins and surgical injury, as well as hyperthermia. This response was initially studied in *Drosophila melanogaster* exposed to heat (Ritossa, ’62), and was termed “heat shock response.” This response is now also known as the “stress response,” because many other toxic agents can also induce it. The response is common to plants and animals, unicellular and multicellular organisms, and vertebrate and invertebrate species, embryos as well as adults. Also, there is a striking homology between the stress response, heat shock genes (hsp), and heat shock proteins (HSP) in all these forms of life, which underlines their importance (Edwards et al., ’97; Walsh et al., ’97).

A number of stress proteins have been identified which fall into two major groups: the HSP, and the glucose-regulated proteins (GRP). The GRP are induced in response to glucose deprivation, hypoxia, and treatment with substances which disturb protein transport and calcium metabolism. The HSP are produced in response to heat, heavy metals, alcohol, metabolic inhibition, and protein denaturants. A number of nomenclatures are used to specify the HSP and their genes. Distinct families occur within each major group, based on their molecular weights, and on their functions, which relate generally to their molecular weights. Studies in mammalian embryos have been made on the HSP90 (molecular weight of approximately 90 kD), HSP70, HSP47, HSP20, and ubiquitin (approximately 8 kD) families. There are usually two or more genes for each HSP, one of which is induced by heat and other damage, while the other is a constitutively expressed cognate (HSC) which is present in unstressed cells and is involved in normal cellular functions, including chaperone activities (Lindquist, ’86; Hightower, ’91; Edwards et al., ’97; Walsh et al., ’97).

The molecular chaperones in normal unstressed cells facilitate the transport of polypeptides and the proper folding of newly synthesized proteins to form their functional tertiary structures, but do not become incorporated into these structures. A major function of HSP appears to be to protect newly synthesized proteins against incorrect folding and against binding to the reactive surfaces of other proteins, to form functionless aggregates. Induced HSPs bind to thermally-damaged proteins (Lindquist, ’86; Schlesinger, ’90; Hightower, ’91), and assist in their reconstitution by attaching to uncovered active sites on the partially unfolded areas, thereby preventing inappropriate binding to other damaged proteins, to form functionless aggregates. Subsequent sequential, orderly disengagement allows the rescued proteins to assume their former structure and function. Chaperone proteins also appear to protect against denaturation heat injury (Edwards et al., ’97; Walsh et al., ’97).

A number of HSCs are present constitutively in embryos from very early stages of development, presumably with chaperone functions, and possibly also having a developmental role. These HSCs are present in relatively large quantities during some critical phases of development. The heat-inducible HSPs are undetectable, or are present in very small quantities in normal unstressed embryos, but they can be induced at certain stages of development. The hsp70 gene can be expressed in response to heat briefly during the cleavage that forms the two-cell embryo, but it cannot be induced between this period and the blastocyst stage in mouse, rat, and rabbit embryos (Morange et al., ’84; Heikkila et al., ’85). This is of interest, given the greater sensitivity of embryos to hyperthermia at this stage. There is a strong response to heat by all inducible HSPs during the period of organogenesis (Walsh et al., ’89, ’93, ’94; Mirkes et al., ’91; Mirkes and Doggett, ’92).

The HSP90 family is present constitutively in relatively large amounts in unstressed cells, is only moderately induced by heat stress, and is believed to have chaperone functions (Buchner, ’97). It interacts with steroid receptors, actin, tubulin, and microtubules. This family includes GRP94, which is a glucose-regulated protein. The HSP70 family includes HSP70–3, which is present constitutively and is moderately induced by heat, and HSP70–1, which is also present constitutively in very small amounts but is strongly induced by heat, binding to damaged proteins (Hightower and Leung, '97). HSP47 is present constitutively, is strongly induced by heat, and binds to damaged collagen (Nagata et al., '88). The HSP20 family in mammals is represented mainly by HSP27, which is also present constitutively and is strongly induced by heat, acting as a molecular chaperone, especially for the actin cytoskeleton (Landry, '97). Ubiquitin is present constitutively, in association with nuclear histones, and is strongly induced by heat, binding to damaged proteins, and assisting in their removal and degradation (Edwards et al., '97; Walsh et al., '97).

Given the existence of these potentially protective mechanisms, it seems reasonable to question why and how embryos can be damaged by heat. German ('84) proposed that the heat shock response might take precedence over normal developmental events and alter programs of gene activity to result in defects, with survival at the expense of normal development. In this hypothesis, the response can be due to diverse environmental agents acting through a common pathway, so that different agents produce similar defects when they operate at similar stages of development. This introduces the question of whether hyperthermia-induced defects are caused by the activation of the heat shock response, or because of its failure to protect, or because of some combination of each. Most damage appears to be caused to embryos in the stage involving induction of the formation of an organ (Edwards et al., '95). The later stage of active cellular proliferation to enlarge the organ is less susceptible to damage by the same amount of heat, and after the period of histogenesis the organ becomes relatively resistant.

A threshold dose of heat causing damage to an unprotected rat embryo in culture can be represented between the extremes of a brief, high elevation of temperature (spike) and a lower sustained, unremitting elevation (plateau). Each teratogenic regime in previously unexposed embryos causes developmental damage but also provokes a heat shock response which generates high levels of hsp mRNA and HSP. This suggests that the response is activated after teratogenic cell damage has occurred. If HSP partially protects against heat, the damage must occur within the first few minutes of an initial exposure, after the threshold heat levels are reached, and before the appearance of the protective response. However, it is doubtful whether the response protects against a very high dose. HSPs in rat embryos appear to be able to promote repair of damage caused by an initial exposure, and to provide a level of defense against a subsequent exposure occurring within the next 6–8 hr (Edwards et al., '97; Walsh et al., '97). Mirkes et al. ('97) present evidence to suggest that the induction of thermotolerance in rat embryos is associated with a significant reduction in internucleosomal DNA fragmentation and associated apoptosis.

The threshold elevation of 2.0–2.5°C required to cause damage might be due to the presence in cells of protective, constitutive, chaperone proteins. In this case, the 2.0–2.5°C threshold would represent an indirect measure of the heat exposure required to titrate out the chaperone proteins (Edwards et al., '95, '97). There is also some evidence that embryos are at increased risk of damage with the decay of the protective response. Embryonic neuroepithelial cells at the stage of mitosis are most susceptible to damage by heat, and during the response, mitotic activity ceases for 6–8 hr (Edwards et al., '74; Wanner et al., '75; Walsh et al., '85, '87; Shiota, '88; Upfold et al., '89), after which it resumes with an exaggerated burst of partially synchronized activity. Additional exposures of embryonic guinea pigs to hyperthermia during this time interval result in severe damage to the embryonic brain (Edwards et al., '84).

In summary, the heat shock response in rat embryos does not protect against an initial threshold dose of heat. After an initial nondamaging exposure, it gives an increased level of protection for 6–8 hr against a teratogenic dose of heat, if the embryos are allowed to recover at their normal temperature for a short period. In guinea pig embryos, a second exposure 6–8 hr after an initial damaging exposure causes more severe damage to brain development than exposure at longer intervals.

HYPERTHERMIA IN HUMANS: DEFECTS AND MECHANISMS

During the past two decades, a series of retrospective and prospective epidemiological studies in humans has confirmed observations in experimental animals that suggested that hyperthermia could cause neural tube defects. The types of defects observed included spina bifida, encephalocele, and anencephaly. The sources of hyperthermia included febrile illnesses, sauna use, and hot tub use (Miller et al., '78; Chance and Smith, '78; Fisher and Smith, '81; Halperin and Wilroy, '78; Layde et al., '80; Hunter, '84; Shiota, '82; Milunsky et al., '92). Among these studies, the proportion of neural tube defects associated with first-trimester hyperthermia ranged from 10–14%. Milunsky et al. ('92) noted that the relationship between exposure and neural tube defects was stronger with hot tub use than with sauna use. They noted that exposure to multiple heat sources was associated with an even greater risk for neural tube defects.

Harvey et al. ('81) measured vaginal temperatures of 20 nonpregnant women while in hot tubs and saunas. It took 10 min in a 41.1°C hot tub, and 15 min in a 39°C hot tub, for vaginal temperature to reach 38.9°C, and none of the 20 women were able to remain in the 81.4°C sauna long enough for their temperatures to reach 38.9°C. In contrast to this, a study of 50 Canadian women indicated that 20 women were able to remain in a sauna set at 93.3–98.8°C for 20 min, resulting in their mean oral body temperatures reaching 38.9°C (Spragget and Fraser, '82a). A study of 24 Australian women revealed they were also able to remain in a hot tub set at 40°C until their temperatures reached 39°C, with 54% of the subjects not feeling uncomfortably hot (Ridge and Budd, '90). This suggests that the subjective feeling of being "overheated" may not be enough to protect all women from teratogenic exposures to heat in saunas and hot tubs. For potentially pregnant women using hot tubs set at 40°C, exposure ought to be limited to no more than 10 min, while exposure in saunas set above 90°C ought to be limited to a maximum of 15 min. These limits appear to be commonly respected in countries such as Finland, where sauna-bathing is a way of life. Such countries demonstrate no excess of congenital defects that might be attributed to sauna-induced hyperthermia (Saxen et al., '82). It is only when these limits are not respected that the sauna might cause hyperthermia-induced defects (Lipson et al., '85: Edwards et al., '95).

Chambers et al. ('97) followed a cohort of 301 pregnant women who called the California Teratogen Information Service with concerns regarding a fever during pregnancy. The high-fever group contained 126 women who reported a fever of 102°F or above for at least 24 hr. The low-fever group reported a fever of less than 102°F for any length of time, or a fever of 102°F or above for less than 24 hr. Compared to a control group of 273 similarly ascertained women exposed to nonteratogens, women in the high-fever group demonstrated a significantly increased rate of major malformations (6/38 or 15.8%) in comparison with the control group (11/242 or 4.5%). These malformations included one case of transposition of the great vessels and two cases of anencephaly, defects which had also been seen in previous retrospective studies of the effects of maternal hyperthermia (Miller et al., '78; Pleet et al., '81). Among 25 liveborn children in the high-fever group, 6 had 3 or more minor malformations, including cleft uvula in 3, short palpebral fissures in 2, and preauricular pit or tag in 2. This supported the hypothesis that hyperthermia at or above 102°F for more than 24 hr in the first 4 weeks after conception may affect both brain and facial morphogenesis in the human.

Among 28 dysmorphic infants exposed to hyperthermia between 4–14 weeks of gestation, all survivors had mental deficiency, and most had altered muscle tone (usually hypotonia with increased deep tendon reflexes). Those exposed at 4–7 weeks had an increased prevalence of facial defects, and in 3 of the 28 pregnancies the hyperthermia was due to a long stay in a hot tub or sauna (Pleet et al., '81). The types of facial defects observed included midface hypoplasia, cleft lip and/or palate, micrognathia, microphthalmia, and external ear anomalies (Fig. 5). Microphthalmia has also been associated with febrile first-trimester illnesses in a study by Fraser and Skelton ('78), and confirmed in a second more recent study, which also noted an association with hypospadias and cardiac defects (Spragget and Fraser, '82b). The association of hyperthermia with cardiac defects was also noted by Graham and Edwards ('89) and demonstrated experimentally by Cockroft and New ('78). Epidemiological studies have confirmed a relationship between congenital heart defects and maternal hyperthermia (Erickson, '91; Tikkanen and Heinonen, '91). In addition to heart defects, Erickson ('91) also noted associations between fever and/or flu and the occurrence of neural tube defects, nervous system defects, gastrointestinal defects, cleft lip and/or palate, defective cardiac valves, and diaphragmatic hernias. Little et al. ('91) confirmed an association between the occurrence of abdominal wall defects and maternal report of fever of 101°F or higher for 24 hr or more during the first trimester, and Lipson ('88) reported an association between first-trimester hyperthermia and Hirschsprung disease.

In a prospective study of 3,144 pregnancies, McDonald ('58, '61) reported that the prevalence of major congenital anomalies and abortions was significantly increased in women who experienced a febrile illness or pulmonary tuberculosis during the first 12 weeks of pregnancy. Of particular note, she found that among 27 women who worked in a hot laundry environment during pregnancy, 4 had children with a major defect (anencephaly, hydrocephalus, congenital heart defect, and hypospadias). When the febrile illness occurred between weeks 5–8, she noted the greatest incidence of congenital anomalies. Kline et al. ('85) confirmed a significant association between fever during pregnancy and spontaneous abortion, with some febrile episodes resulting in fetal death and expulsion 6–8 weeks later, while other febrile episodes resulted in immediate uterine contractures with expulsion of a previable fetus. Viral infections are a common cause of fever, and epidemics of influenza have been associated with an increased occurrence of malformations, most of which affect the central nervous system, particularly neural tube defects (Coffey and Jessop, '59, '63; Saxen et al., '82).

Fetal vascular disruption in the wake of febrile maternal illnesses has been implicated in the pathogenesis of Moebius syndrome (Graham et al., '88; Lipson et al., '89; Govaert et al., '89), oromandibular-limb hypogenesis syndrome (Superneau and Wertelecki, '85), and amyoplasia-type arthrogryposis (Ivarsson and Henriksson, '84; Edwards et al., '90). Each of these defects is thought to be the consequence of vascular disruption, and gestational hyperthermia is only one of many ways in which such vascular disruption might be induced.

Moebius syndrome consists of congenital facial palsy (seventh cranial nerve) combined with lateral rectus palsy (sixth cranial nerve), in association with other cranial nerve, brain-stem, and musculoskeletal problems (Fig. 6). Bouwes-Bavinck and Weaver ('86) and St. Charles et al. ('93) hypothesized a vascular basis for

Fig. 5. This profoundly retarded microcephalic 11-year-old girl (top left and center) was exposed to 3 days of high fever (39–40°C) during the fifth week of gestation due to maternal Hong Kong flu. She also had neurogenic talipes (top right). Bottom: This mentally retarded 12-year-old girl was exposed to 2 weeks of sustained fever (38–39°C) between weeks 6–8 of gestation due to maternal viral laryngotracheitis. She had hypotonic diplegia with neurogenic talipes, ear anomalies, congenital heart defect, cleft palate, and micrognathia.

Moebius syndrome, and Webster et al. ('87, '88) provided an experimental model that supports this hypothesis. In their rat model, abdominal trauma, uterine vessel clamping and handling, or hyperthermia caused bilateral brain-stem and distal limb reduction lesions following induction of hemorrhages in these regions (Webster et al., '87). The brain-stem calcifications and injury in Moebius syndrome are attributed to prenatal ischemic injury as a consequence of vascular disruption (Govaert et al., '89; Harbord et al., '89; Fujita et al., '91). In addition to hyperthermia, humans with Moebius syndrome have been exposed to a variety of other adverse events during pregnancy which have included abdominal trauma, attempted abortion, alcohol abuse, glue sniffing, electric shock, early chorion villus sampling, and unsuccessful attempted abortion with misoprostal and ergotamine-induced uterine contractions (Lipson et al., '89; Firth et al., '94; Gonzalez et al., '93; Graf and Shepard, '97). Moebius syndrome is frequently associated with distal limb reduction defects such as oromandibular-limb hypogenesis (sporadic distal limb, tongue, and jaw hypoplasia), Poland syndrome (sporadic unilateral distal limb and pectoralis major deficiency), and/or neurogenic equinovarus foot deformation (Kumar, '90). Examples of hyperthermia-related Moebius syndrome in the human are shown in Figure 7.

Superneau and Wertelecki ('85) reported 2 children with oromandibular-limb hypogenesis syndromes whose mothers had febrile illnesses at either 8 weeks or 10–11 weeks of gestation. In the study by Lipson et al. ('89), 2 children with Moebius syndrome and hypoglossia with terminal transverse hemimelia were exposed to febrile maternal illnesses between 7–8 weeks gestation. The case of Moebius syndrome which was reported by Govaert et al. ('89), with prenatal ischemic necrosis and brain-stem calcifications, was associated with a flu-like first-trimester illness. Graham et al. ('88) reported 5 cases of Moebius syndrome associated with various febrile illnesses occurring between 8–22 weeks of gestation, as well as 8 other children with cortical atrophy, microcephaly, and/or abnormalities of the corpus callosum, whose mothers gave histories of febrile second-

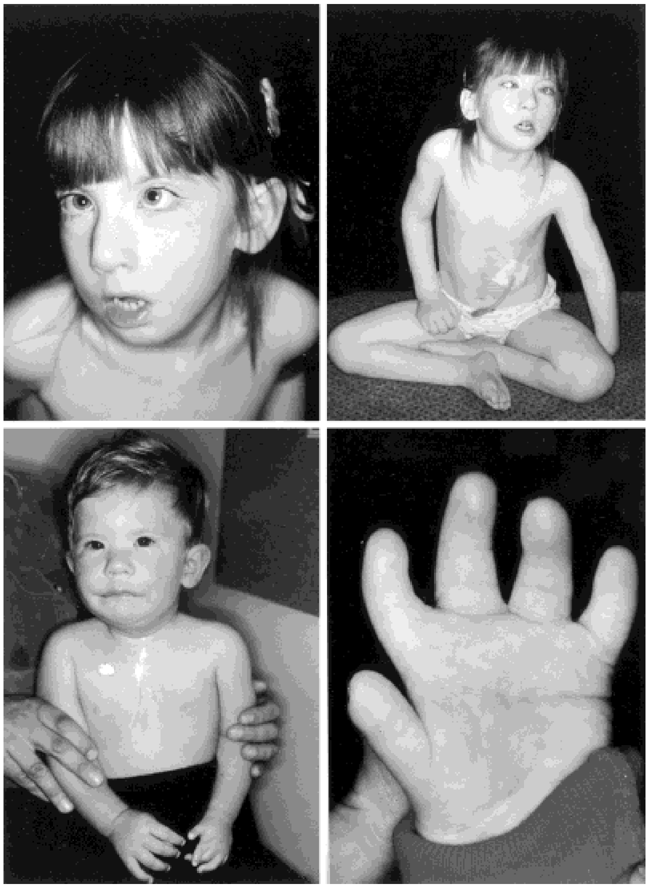

Fig. 6. These 2 children both manifest Moebius sequence, with bilateral sixth and seventh cranial nerve palsies resulting in paralysis of lateral gaze and immobile facial musculature. Top: The girl also has neurogenic swallowing problems and neurogenic talipes. She was exposed to fever of 102°F for 3–4 days at 18 weeks postconception. Bottom: The boy also has left Poland sequence, with hypoplasia of the left hand and pectoralis major muscle. He was exposed to 3 days of high fever at 15 weeks postconception. Both mothers defervesced after being treated with penicillin for a presumed streptococcal infection.

trimester illnesses. Edwards et al. ('90) reported 2 children with the amyoplasia type of arthrogryposis and maternal spikes of fever and chills between 8 weeks and term at about 2-week intervals (Fig. 4). Amyoplasia is thought to result from ischemia to the fetal spinal cord, with injury to anterior horn cells (Reid et al., '86).

These observations suggest that adverse effects from febrile maternal illnesses are not limited to first-trimester exposures, and that second-trimester exposures may trigger hemorrhages in vital fetal structures, causing vascular disruption and loss of fetal structures on this basis.

SUMMARY

This review has covered the pertinent literature concerning the teratogenic effects of hyperthermia in man and experimental animals. This is the first teratogen that was initially discovered in animals and then subsequently found to be a cause for concern in humans when similar patterns of defects were observed. Hyperthermia is a physical agent with a dose-response curve for abortions and malformations, but these effects can be mitigated in some circumstances by the heat shock response (HSR). We have reviewed the known functions of HSR and provided some insight into why embryos have some protection following an initial dose of heat, if it is sufficient to initiate the response. Thus, by reviewing the effects of hyperthermia in experimental animals, as well as malformative and protective mechanisms of teratogenesis, we have attempted to understand the effects of human hyperthermia teratogenesis.

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