were nursed by a hypertensive or a normotensive mother, only male SH pups developed severe hypertension when nursed by WKy dams.

These findings suggest that an animal genetically programmed to be normotensive can become hypertensive if it is nursed by a hypertensive mother. Some factor may be transmitted through the milk, triggering the pathogenesis of progressively increasing blood pressure. This factor or factors appear to be specific in genetic transcription, since not all genetically normotensive weanlings nursed by hypertensive dams respond by developing hypertension. SH offspring become inordinately sensitive to extra dietary salt if they are nursed by mothers that were provided with extra salt during gestation (*7*). Cholesterol metabolism in weanling rats favors atherogenesis if they were suckled by mothers who were fed a diet high in cholesterol and fat (*7*).

Alternatively, the hypertension in the offspring may be attributable to the mother's behavior during the 21 days of lactation. SH rats are extremely sensitive to stress; even mild disturbances can cause inappropriate hyperkinetic behavior and marked stimulation of the pituitary-adrenal axis (*4*). If disturbed, SH mothers may eat their young.

A hypertensinogen, a factor that will induce high blood pressure de novo, has been sought in hypertensive humans and animals (*8*). Although this factor causes increased aldosterone secretion, sodium retention, blood volume expansion, and sustained hypertension—all reduced by adrenalectomy—it is not adrenocorticotropic hormone, renin, or angiotensin (*8*). Our investigations of cerebrohypothalamic-pituitary function in various substrains of SH rats (*4*, *9*) direct attention to the possibility that the hypersensitivity of SH rats to stress is mediated by endogenous opiates or cerebropeptides (β-endorphin, enkephalins, and so forth) (*10*). The factor transmitted through the mother's milk or activated by her hyperkinetic behavior may be such a cerebrohypothalamic neurotransmitter. The fact that SD offspring, rather than WKy, were more susceptible to the purported hypertensinogen activated or transmitted by SH dams underscores the belief that individuals genetically destined to become hypertensive will do so eventually if they are exposed to hypertension-inducing conditions.

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6. The disbursement of Okamoto-Aoki SH strain and the WKy rat, its purported normotensive counterpart, has resulted in the dilution of strains. Use of the WKy rat as the only suitable control for SH rats is no longer justifiable. Other investigators encounter loss of elevated blood pressure in their SH strain, but blood pressure in our strain has remained elevated. Other investigators complain that their SH rats are susceptible to respiratory disease, but our strain is resistant. Most SH substrains are short-lived; ours is unusually long-lived. Other investigators report comparatively low aldosterone and corticosterone concentrations and decreased responsiveness to stress in their SH rats; we find high concentrations of these hormones and hypersensitivity to stress. Okamoto and Aoki (*1*) claim that early gonadectomy does not inhibit the pathogenesis of hypertension in SH rats, but we find it to be an effective retardant of hypertension. Okamoto *et al.* (*2*) describe a stroke-prone SH substrain; our descendants of their stroke-prone rats develop exceptionally high blood pressure but do not have strokes. Yamori *et al.* (*3*) describe ringlike fatty deposits in fat-fed SH rats; the same diet caused complete inhibition of hypertension, severe hyperlipidemia, but no atherosclerosis in our SH strain.
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Trisomic Hemopoietic Stem Cells of Fetal Origin Restore Hemopoiesis in Lethally Irradiated Mice

Abstract. *Autosomal trisomy in the mouse is invariably associated with fetal or early postnatal death. Hemopoietic stem cells from fetuses trisomic for chromosome 12 or 19 can be rescued by transplantation into lethally irradiated mice. These trisomic cells restore hemopoiesis, including lymphopoiesis, in the irradiated mice and establish a permanent and almost complete engraftment. There is no evidence that hemopoietic cells with trisomy 12 or 19 are cytogenetically unstable.*

The life-span of mice with autosomal trisomies is limited. Developmental failure occurs during the second or final third of fetal development, depending on the chromosome involved (*1*, *2*). Short postnatal survival is observed only in mice with trisomies 13, 16, and 19 (*3*). In this report, we examine whether hemopoietic stem cells from the liver of trisomic fetuses can reconstitute hemopoiesis in hosts given a lethal dose of radiation, thus enabling trisomic cells to survive in the hosts. Such radiation chimeras provide a tool for studying the stability of chromosomally unbalanced donor cell lines and for analyzing the biological effects of trisomy in hemopoietic cells. It can be shown that a permanent and almost complete reestablishment of hemopoiesis, including lymphopoiesis, is obtained with cells trisomic for chromosome 12 or 19.

We induced trisomy by crossing males heterozygous for two Robertsonian (Rb) translocation metacentrics showing homology of one of their arms with females with acrocentric chromosomes only (Fig. 1) (*1*). To induce trisomy 12, Rb(8.12)5Bnr/Rb(4.12)9Bnr males were mated with NMRI females, and to induce trisomy 19, Rb(9,19)163H/Rb(8.19)-1Ct males were mated with C3H/He females. Since the life expectancy of trisomic individuals is limited, it seemed appropriate to remove the fetuses with trisomy 12 on day 14 or 15 and the fetuses with trisomy 19 on days 16 to 19 (day 1 being the day on which the vaginal plug appeared).

To distinguish between trisomic and normal fetuses, we performed cytogenetic analyses on extraembryonic membranes (days 14 and 15) or small pieces of liver (after day 15). The samples were incubated for 45 minutes in tissue culture medium (TCM) 199 containing 20 percent fetal calf serum and 0.05 μg of Colcemid per milliliter. Further processing involved standard techniques. Trisomic fetuses (cytologic marker: two metacentric chromosomes) and control fetuses (cytologic marker: one metacentric chromosome) were chosen from the same litter whenever possible, and suspensions of single cells were made separately from each liver. Adult C3H/He females were irradiated with 1000 R (55 R/min) (*4*), and 1 to 4 hours later were in-

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jected intravenously with 1×10^6 to 10×10^6 nucleated cells from the suspensions (Fig. 1). The number of cells injected depended on the size and age of the donor fetus.

As shown in Table 1, there were no major differences between trisomic and control chimeras with respect to mortality during the first month after irradiation and transplantation. The same was true for the period thereafter. Host death during the first month after irradiation is regarded as a consequence of the still insufficient hemopoietic function of the gradually proliferating donor cells (*5*). Deaths after this period include those caused by delayed graft-versus-host reaction (*6*).

We destroyed slightly more than one-third of each of the experimental and control groups between 40 and 200 days after irradiation (Table 1) and examined the bone marrow cells and spleen lymphocytes in order to determine whether the trisomic cells engrafted completely. Furthermore, we sought to ascertain whether secondary clonal chromosomal anomalies appear in trisomic lines proliferating for periods up to 200 days. The presence of such secondary anomalies would indicate that the trisomic karyotype is unstable.

Upon examining cytogenetic preparations of the bone marrow, we found that 100 percent of the cells in metaphase were of the donor (fetal) type. This was true for all the chimeras 40 or more days after irradiation (Table 2). The spleen lymphocytes were cultured for 3 days in the presence of 10 μg of concanavalinA per milliliter (for T cells) or 50 μg of lipopolysaccharides per milliliter (for B cells) (*7*). With one exception, 90 to 100 percent of the evaluated lymphocytes were of the donor (trisomy or control) type (Table 2). The other cells in metaphase were of the recipient type (cytologic marker: no metacentrics) (Fig. 1). Only 5 percent of these cells contained chromosomes with clearly visible radiation damage.

Chromosomal anomalies were occasionally observed in the donor cells. For example, extra chromosomes (usually one or two extra acrocentrics) were seen in 0.5 percent of metaphase-stage marrow cells from the trisomic mice and in 0.7 percent of marrow cells from the controls. The majority of these anomalies seem to have been caused by preparation artifacts. In spleen cell (lymphocyte) cultures, 1.4 percent of the trisomic cells and 1.3 percent of the control cells had supernumerary chromosomes. In addition, 0.8 percent of the spleen cells had broken chromosomes. In a separate control study, we examined spleen cells from nonirradiated adult mice heterozygous for Rb metacentrics similar to those of the irradiated controls. Of 1175 cells in metaphase, 2.4 percent had supernumerary chromosomes and 0.5 percent broken chromosomes.

These results show that the origin of most anomalies is associated with the culture procedure rather than with the chimeric condition and that the anomalies are independent of the trisomic condition. There is no evidence that clonal evolution of new chromosomal types occurs. Therefore, it can be assumed that the trisomic karyotype is stable under the conditions tested.

The longevity and restoring capacity of trisomic hemopoietic stem cells was further demonstrated by repeated transplantation of bone marrow from chimeric mice with trisomy 12. After two consecutive passages, complete engraftment was achieved in the third recipient. In these experiments the trisomic cells survived for 17 months.

Two months after irradiation, the healthy-looking chimeras of both the trisomic and control groups showed plentiful cellularity of the hemopoietic and

Table 1. Fate of animals with transplants.

Type of cell transplanted	Number of mice with transplants	Number of mice killed for study	Number of deaths			
			Days after irradiation and transplantation			Date unknown
			1 to 29	30 to 89	90 to 180	
Trisomy 12	115	41	43	20	3	8
Control	117	29	46	19	3	20
Trisomy 19	133	45	44	20	4	20
Control	120	59	26	17	2	16

Table 2. Results of cytogenetic analyses of bone marrow cells and spleen lymphocytes from the radiation chimeras. The numbers in parentheses are numbers of animals studied. Some 25 to 50 cells in metaphase were evaluated in each preparation. Indication of time (months): time after transplantation. Abbreviations: C, concanavalin A; L, lipopolysaccharides.

Type of cell transplanted	Percentage of bone marrow cells of the donor type			Percentage of spleen cells of the donor type					
	1 to 3 months	3 to 6 months	> 6 months	1 to 3 months		3 to 6 months		> 6 months	
				C	L	C	L	C	L
Trisomy 12	100 (15)	100 (8)	100 (5)	91 (5)	100 (4)	100 (3)	98 (5)	86 (1)	100 (1)
Control	100 (12)	100 (7)	100 (4)	96 (5)	100 (4)	100 (3)	100 (5)	100 (1)	100 (1)
Trisomy 19	100 (5)	100 (10)	100 (1)	96 (4)	98 (4)	94 (5)	98 (4)		
Control	100 (9)	100 (7)	100 (1)	99 (6)	99 (5)	98 (8)	100 (5)	98 (1)	

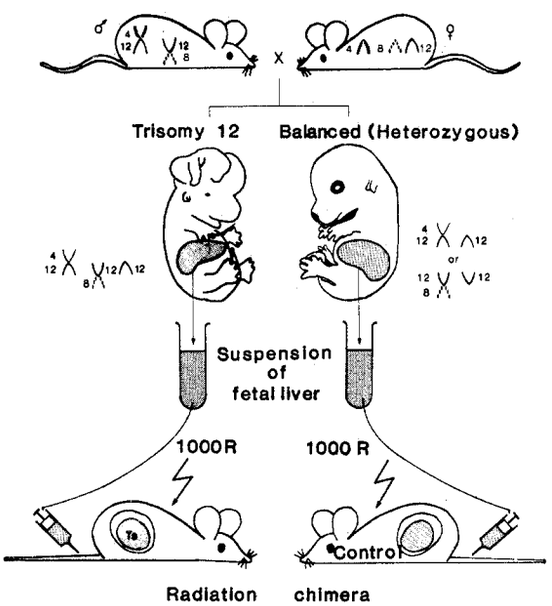

Fig. 1. Transplantation of liver cells from trisomic (trisomy 12) fetuses to adult mice. Fetuses with trisomy 12 usually are exencephalic (*1*), whereas major visible malformations are absent in fetuses with trisomy 19.

lymphopoietic organs. The bone marrow and spleen contained erythropoietic, granulopoietic, and thrombopoietic elements. Lymphoid follicles, some with germinal centers, were observed in the spleen and in the lymph nodes, and the cortex of the thymus exhibited dense lymphoid cellularity.

So far, the ability to restore hemopoiesis has been proven for stem cells with trisomies 12 and 19. Further tests have demonstrated that these cells form almost normal numbers of colony-forming units in spleen tissue and in agar (*8*). However, stem cells with other trisomies may not possess the same survival capacity. In fact, there is evidence that stem cells with trisomy 13 or 16 cannot restore hemopoiesis permanently in lethally irradiated recipients (*9*).

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Larvae of Air-Breathing Fishes as Countercurrent Flow Devices in Hypoxic Environments

Abstract. *Larvae of the air-breathing teleost fish* Monopterus *are frequently exposed to periods of critical hypoxia, which they can survive because they have (i) dense capillary networks in the skin, (ii) a small blood-water barrier, (iii) an active pectoral fin mechanism that generates a posteriorly directed respiratory water current originating from the oxygen-rich surface layer, and (iv) a principal flow of blood that runs countercurrent to the water stream. Experimental data show that the larva as a whole is a functional analog of a fish gill lamella and that similar adaptive mechanisms are present in larvae of ancient fishes and some modern teleosts inhabiting permanently or periodically hypoxic waters.*

Adaptations in the gas exchange apparatus accompanying the transition from aquatic to terrestrial vertebrate life have been studied mainly in adult air-breathing fishes (*1*). Yet the most critical phase in the life history of fishes occupying periodically or permanently hypoxic environments is the larval stage before gills or air-breathing organs begin to function (*2*). I now present experimental evidence of a countercurrent flow mechanism by which larvae of some air-breathing fishes can cope with critically low oxygen concentrations in the water. The outer surface of the larva, along its entire length, takes up oxygen from the water. An adaptive advantage is gained by an arrangement that makes the stream of water over the larva and the stream of blood in the larva flow in opposite directions. Thus the larva as a whole is a functional analog of a fish gill lamella.

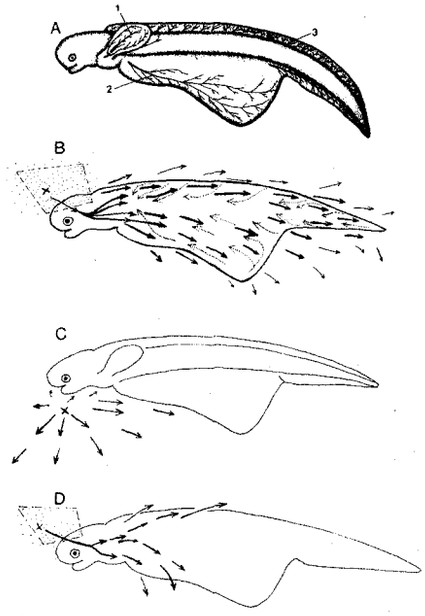

Fig. 1. (A) Larva of *Monopterus albus*, 4 days after hatching, with (1) large vascular pectoral fins, (2) well-developed respiratory capillary networks on the yolk sac, and (3) unpaired (median) fins. (B) Diagrammatic representation of water currents generated by pectoral fins (not included in the drawing) as indicated by heavy arrows. The stippled area is the region of influence of the pectoral fins; × indicates the placement of the dye (methylene blue); dotted arrows show the principal directions of blood flow; this pattern occurs when air equilibration of the water drops below 40 percent. (C) Fate of tracer dye when placed at X, outside the sphere of the pectoral fins (which are shown in this drawing). (D) Illustration of water currents generated by the pectoral fins as indicated by dye movements when air equilibration is above 40 percent; the major current takes a ventral direction after passing the hepatointestinal capillary network, while the dorsal current is only weakly developed so that the tracer dye is diffused (pectoral fins are not shown).

Members of the circumtropical Synbranchiformes are eel-shaped, air-breathing, amphibious teleost fish inhabiting swamps, rice fields, and permanent or temporary ponds, which become permanently or periodically hypoxic. When the larvae of the synbranchiform *Monopterus albus* of southeast Asia hatch (Fig. 1A), they have large muscular and vascularized pectoral fins that function as external gills (*3*). Dye tracer experiments (*4*) show that the active movements of the pectoral fins propel water from a well-circumscribed area anterodorsal to the head posteriorly along the length of the entire larva and its yolk sac (Fig. 1B). This ventilating mechanism, which draws the layer of water anterodorsal to the head posteroventrally, enables the buoyant larva to exploit the thin surface layer of water in which diffusion provides sufficient oxygen in an otherwise depleted water column. Dye tracer that is introduced anteroventral to the head does not get caught in the respiratory current because it is in an area outside the hydrodynamic sphere of the pectoral fins (Fig. 1C). The concentration of oxygen in the water has a strong effect on the frequency and nature of movements of the pectoral fins (*4*) and consequently on the velocity and extent of the respiratory water stream (Fig. 1, B and D). Ten to 13 days after hatching, the fish begins branchial and air respiration and the pectoral fins shrivel and drop off the body (*3*).

The larva of *Monopterus* has an extensive respiratory capillary network just below the epithelial surfaces of the un-

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