ARTICLE

The RSH/''Smith–Lemli–Opitz'' Syndrome: Historical Footnote

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Thirty years after its clinical delineation in humans and its teratologic simulation in rats, a Garrodian error of metabolism was discovered in the autosomal recessive RSH/SLO syndrome, namely defective conversion of 7-dehydrocholesterol to cholesterol due to the mutant 7-dehydrocholesterol reductase. This opened the door to the study of several other defects of sterol biosynthesis in humans and the creation of animal ''models.'' The gross discrepancy between expected and observed birth prevalence suggests high embryolethality. The discovery of the role of cholesterol in the synthesis of the morphogen sonic hedgehog has greatly advanced our understanding of mammalian development. © 2012 Wiley Periodicals, Inc.

KEY WORDS: RSH/''Smith–Lemli–Opitz'' syndrome; autosomal recessive; DHCR7 deficiency; development; rat and mouse models

How to cite this article: Opitz JM, Furtado LV. 2012. The RSH/''Smith–Lemli–Opitz'' syndrome: Historical footnote. Am J Med Genet Part C Semin Med Genet 160C:242–249.

INTRODUCTION

I am afraid that when the annals of "developmental morphology" are written, much foolishness, witting, or unwitting, will also be recorded. As for example: Wrong diagnoses, false splitting or false lumping, mistaken genetic or pathogenetic hypotheses, false or deliberately ignored attributions, wholesale blindness to the past, especially the role of evolution in the morphogenesis of species and individuals, or the role of whole ("alien") cultures (and I quote: "damn Germans", or "damn doctors") in making their own specific contribution to the whole of the science. In over 50 years in the field I have seen it all, and, alas, have also contributed to individual or collective foolishness or prejudice.

One of these I was taught in my youth was the virtually dogmatic conviction that inborn errors of metabolism did *not* cause malformations or multiple congenital anomalies (MCA) syndromes.

This view was held firmly by the late and much admired Professors Harry A. Waisman and David W. Smith in the Department of Pediatrics at the University of Wisconsin, Madison, when I arrived there in 1961 at the age of 26 (a half century ago) to complete a residency in Pediatrics and to begin my fellowship with the Drs. David W. Smith and Klaus Pätau (later Patau, pronounced the same way in the USA). Before "newborn screening" (meaning the biochemical "screening" of newborn infants) we screened the children (and a few adults) in institutions for the "mentally retarded" for inborn errors of metabolism, found many and concluded that those of small molecular weight (i.e., galactosemia) will kill in infancy; those of large molecular weight eventually, but more slowly (i.e., Hurler, Hunter, the GM_1, gangliosidoses...); and a few (PKU) will be compatible with, but a, miserable life, mentally retarded or seizuring, in institutions. When the biochemical results of urine and/or plasma evaluations led to a metabolic diagnosis we closed our eyes,

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DOI 10.1002/ajmg.c.31341

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 9 October 2012

relieved not having to examine the child too closely, especially in those "smelly" places!

Shortly after I arrived in Madison, David W. Smith took me to "Central Colony," on the north side of Lake Mendota, and told me to take over the genetic investigations of the children and a few non-ambulatory adults.

I loved the place, a state institution for the "mentally retarded" up to age 7 and the non-ambulatory over age 7, its patients, staff and the truly outstanding care they gave them; it was a new facility on a beautiful campus, not "smelly" in the slightest, and its clinical director, Dr. Elisabeth G. Kaveggia from Budapest, became my Beatrice leading me through the sad inferno of the damned and abandoned.

During part of my second year of pediatric residency in Madison I was "Chief Resident" in charge, also of admissions. As the old Children's Hospital was being remodeled, in the days of 120% or so bed occupancy, my desk was moved to the hallway of one of the wards with beds immediately to the left of the desk.

Then in March and June 1962, two 10-month-old boys called attention to themselves by incessant screaming and projectile vomiting onto the papers on my desk, even after pylorotomy at other hospitals. Their phenotype was remarkably similar and because of genital abnormalities (hypospadias, incomplete fusion of labioscrotal folds in one) considered a form of pseudohermaphroditism, David W. Smith, then formally the pediatric endocrinologist on the faculty, assigned the cases to Luc Lemli, Belgian and endocrinologist-in-training, for evaluation. Dr. Lemli returned to Belgium and Dr. Smith was off to Zürich to work with Gian Töndury for 1 year, so I completed work and manuscript on these two boys.

When I began my fellowship, Klaus Patau recruited me into his "blind study" of chromosomes of those with mental retardation having previously studied (with David W. Smith) the 13-trisomy and 18-trisomy syndromes. Thus, my frequent excursions into the institutions in Wisconsin, Illinois, and Minnesota to collect blood (then without IRB clearance) from those with MR/MCA syndromes. Hence, Dr. Eeva Patau and I visited the Dixon State School in Illinois in 1963 leading to the ascertainment of a boy, 5 years old, seemingly with the same syndrome and a prodigious amount of green snot; he had had an "identically" affected younger brother who died at 2 months of varicella pneumonia. X-linked inheritance of the condition could not be excluded; all three had apparently normal chromosomes in the Patau study; this lad was added to the manuscript on the original two patients.

Besides mental retardation their condition comprised small stature, failure to thrive, disproportionately small head, ptosis, anteverted nostrils, mild micrognathia, rugose narrow palate, broad maxillary alveolar ridge, hypospadias, facultative cryptorchidism, vomiting, pyloric stenosis, (rudimentary) postaxial polydactyly of hands, high ridge count (whorl) finger dermal ridge patterns, cutaneous syndactyly of 2nd and 3rd toes and strabismus; they were hypotonic asleep, hypertonic awake with metatarsus adductus, hyperactive deep tendon reflexes, persistence of "primitive" reflexes, a propensity to scream, and feeding difficulties [Smith et al., 1964]. Since then, gastroesophageal reflux and sleep disturbance have been added as prominent manifestations in infancy and early childhood.

Patient 1 ("S") in the Smith et al. [1964] report was reascertained by Pauli et al. [1997] at age 34. He had spent his life till then in institutions heavily medicated and in restraints because of severe behavior disturbance characterized as "explosive" episodes leading to destruction of objects and injury to self and others. A seizure disorder was well controlled and general health was excellent. He had severe mental retardation. At 34 years, his height was 170 cm and occipitofrontal circumference was 51.7 cm, hence microcephalic. Other findings were: prominence of supraorbital ridges, marked ptosis, submucous cleft of palate (which I had missed), pectus excavatum, left accessory nipple, brachydactyly, 10 whorls on fingertips, syndactyly of 2nd and 3rd toes with hypospadias and chordee. Dr. G.S. Tint performed biochemical assays: cholesterol 65 mg/dl, 7-DHC 30 mg/dl, and 8-DHC 12 mg/dl, confirming the initial clinical impression. Supplementation of diet with an additional 700 mg of cholesterol per day caused caregivers to describe him as "calmer, happier and more verbal." But, I am getting ahead of the story (Fig. 1).

By the time of the First Conference on the Clinical Delineation of Birth Defects in 1968 there had been seven additional publications on the "RSH" syndrome, so-called after the initials of the surnames of the first three patients in Smith et al. [1964]. At that conference Opitz et al. [1969] reported on 11 additional patients, suggested that this condition might be an autosomal recessive (not X-linked) trait based on Pinsky and DiGeorge [1965], Blair and Martin [1966] and personal studies of affected brother and sister or two affected sisters. In that study three "girls" were, in fact, genetic males.

By the time of the First Conference on the Clinical Delineation of Birth Defects [McKusick] in 1968 there had been seven additional publications on the "RSH" syndrome, so-called after the initials of the surnames of the first three patients in Smith et al. At that conference Opitz et al. reported on 11 additional patients, suggesting that this condition might be an autosomal recessive (not X-linked) trait.

Patient 1 in that report [Opitz et al., 1969] died suddenly at home at 27½ months. Neuropathologic analysis (still uncommon in this condition) by

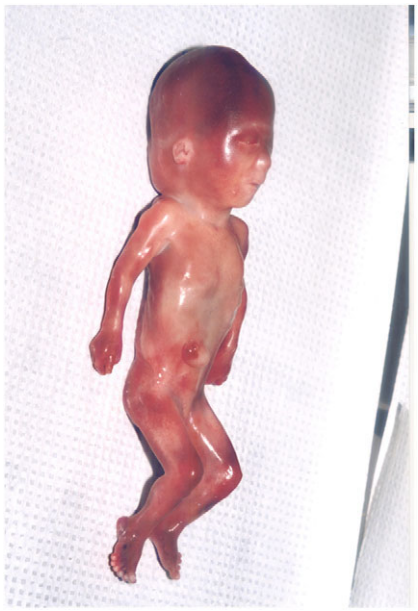

Figure 1. Stillborn male fetus at gestational age (GA) 21–22 weeks from a 36-year-old woman (with previous normal term child). Amniocentesis showed a normal karyotype (46, XY). Ultrasound examination at GA 21 weeks showed congenital anomalies. Autopsy (Dr. Jeanne L Ackerman, Florida) documented postaxial hexadactyly of feet with syndactyly of toes 2 and 3 and 5 and 6 bilaterally, post-axial postminimus of left hand; ambiguous genitalia and cebocephaly with choanal atresia. Biochemical analysis of amniotic fluid (Dr. G.S. Tint) demonstrated: cholesterol level: 6.2 mg/ml (normal range: 8–36). 7DHC: 2.2 mg/ml (normal <0.2 mg/ml), "values... diagnostic for the Smith–Lemli–Opitz syndrome." Case 2 in Opitz et al., [2002]. See also Putnam et al., [2005].

Dr. T.T. Tang at Milwaukee Children's Hospital: Microencephaly, aquaductal stenosis with hydrocephalus, hypoplasia of anterior commissure, incomplete separation of mammillary bodies, short corpus callosum with hypoplasia of splenium, dorsal fusion of the dorsomedial nuclei and pulvinar of thalamus, hypoplasia of cerebral pedundes, platybasic deformity of cerebellum and compression atrophy of the superficial cerebellar folia. Skull: agenesis of anterior fossa, elevation of the posterior cranial fossa, prominent metopic suture and platybasia. Spinal cord: cervical hydromyelia, hypoplasia of spinothalamic, and spinocerebellar tracts. Histologically there was astrogliosis of the pulvinar and gliosis of olivary bodies.

Also noted then: blindness (cataracts with postoperative glaucoma), pallor of optic discs, diffuse retinal hyperpigmentation, foveal depigmentation. And again: pyloric stenosis (requiring surgery), ptosis, epicanthic folds, more or less severe irritability, screaming, severe failure to thrive, developmental delay, foot deformities, cleft palate, pale platinum blond hair in some, and syndactyly of 2nd and 3rd toes. In wondering at the extraordinary severity of the condition in these children, all ascertained in University Hospitals or institutions for the mentally retarded, it must be remembered that these were the early years of delineation of the RSH syndrome leading, generally, to ascertainment of the most severe "cases" and introducing an artificial phenotypic uniformity for two reasons: need to have the phenotype conform as closely as possible to the prototype and to impress editors with apparent non-heterogeneity. Lacking a diagnostic marker "independent" of phenotype decades ago, all we had to estimate or establish the phenotypic limits of a given syndrome was familial occurrence. Now that we can assay easily plasma cholesterol and 7-dehydrocholesterol levels, alleles at the *DHCR7* locus and solutes in the urine of pregnant women (with prior affected child), we can make the diagnosis in those with suggestive phenotype and "normal cognition and behavior" [Mueller et al., 2003] or with "a... mild form of Smith-Lemli-Opitz syndrome" [Langius et al., 2003]. Or, in those lethally affected fetuses and newborn infants that come to autopsy for diagnosis of final instance. Or, to clear up a nosologic point, e.g. "Rutledge syndrome" as a "variant of RSH syndrome" [e.g., Rakheja et al., 2003].

THE METABOLIC DEFECT

The metabolic/biochemical aspects of this Garrodian inborn error have been reviewed several times over the years since its elucidation in August/September 1992.

Chasalow et al. [1985] reported on "possible abnormalities of steroid secretion in children with Smith-Lemli-Opitz syndrome and their parents" and concluded "that a defect in steroid metabolism may be linked to the... syndrome." They found an "unusual pattern of steroid sulfates in the serum of two... infants with SLO and an exaggerated 17-hydroxyprogesterone response to ACTH in the parents of these children and of three additional unrelated children with the syndrome." Since the technical, biochemical aspects of Chasalow et al. [1985] were unfamiliar to me, I consulted Dr. Richard I. Kelley of Johns Hopkins University, an outstanding expert on RSH/SLO, about the relevance of the Chasalow et al. findings to RSH/SLO. He responded "yes," noting that "[since] 7DHC is a substrate for most of the steroid-metabolizing enzymes, many 7-dehydrosteroids are produced... the basis for the urine pregnancy test and... the relative deficiency of the normal species" (referring to the absence of peaks II and IV from the chromatographs referred to as "Unknown A" and "Unknown B" in Chasalow et al. and identified by Kelley, respectively, as the 7-dehydroform of

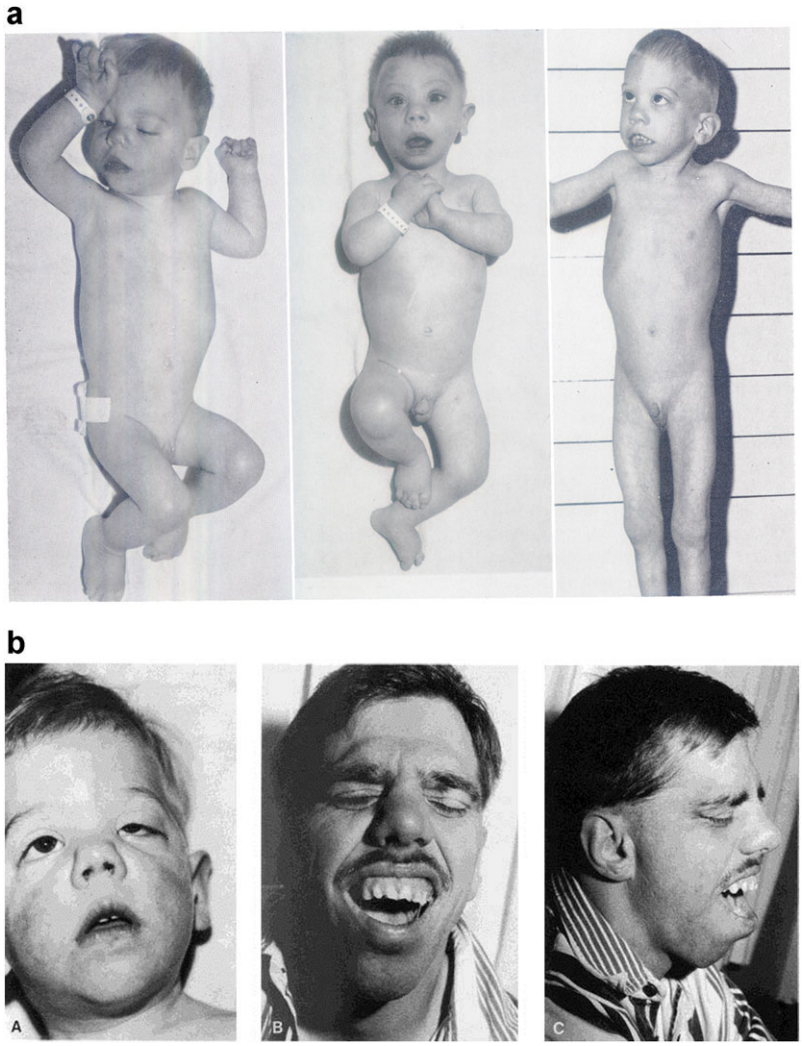

Figure 2. (**a**) From Smith et al. [1964]. Their Figure 2 reproduced with permission, J. Pediatr. (**b**) From Pauli et al. [1997], their Figure 1b,c, reproduced with permission, Wiley-Liss. Patient 1 ("S") of Smith et al. [1964], their Figure 2, their left most patient (WS).

DHEA and as 16-hydroxy-DHEA [personal communication, 7/5/2012). How close they came! In any event, a hint of things to come.

Dr. Tint's account of the seminal events and discoveries is as follows [Tint et al., 1994]:

"8/17/92: Blood drawn from a 6-month-old female (LM) with suspected Smith–Lemli–Opitz syndrome by pediatrician Roger Frieden, and sent to our laboratory by Mira Irons, M.D., Genetic Counseling Center, New England Medical Center, Boston, MA.

8/19/92: Received sample and began processing for routine neutral sterol analysis.

8/25/92: Sample of extracted plasma injected into capillary gas chromatograph (GC). Technologist noted an unusually low plasma cholesterol level (8 mg/dl) and two unknown compounds in concentrations greater than that of cholesterol.

8/26/92: Injected sterol extract onto GC-mass spectrometer and obtained spectra from the two unknown compounds. Both were found to be C_{27} sterols with a single hydroxyl group and two double bonds in the nucleus.

8/27/92: Suspected that one of these compounds might be 7-dehydrocholesterol (7-DHC) since this is a naturally occurring compound. Checked mass spectrum reported in literature and found a good match.

8/31/92: Ordered authentic 7-DHC from the Aldrich Chemical Co.

9/2/92: Injected 7-DHC standard into gas-chromatograph and GC–mass spectrometer. Retention times and mass spectra of authentic material identical to that of one of the large unknown GC peaks seen in plasma—structure of 7-DHC confirmed. At this point the metabolic defect in the Smith–Lemli–Opitz syndrome (SLOS) became evident as a block in the reduction of the 3β-hydroxysterol $\Delta_{7,8}$ double bond during cholesterol biosynthesis." [Tint, abstract in Opitz and de la Cruz, 1994].

I rather suspect that there were events and circumstances before 8/17/92 not recorded here and that the other CG-MS peak was 8-DHC.

To quote once more from that same conference report: "The senior author... [JMO] treasures a small slip of paper handed him by his secretary (9:05 AM) during case conference on Thursday 11 February 1993 stating: 'John, Dr. Tint, E. Orange, NJ, 201-676-1000 × 2287, thinks they have nailed the cause of SLO' " [Opitz and de la Cruz, 1994].

And to think that I once talked a mother (a registered nurse, no less!) of an RSH syndrome girl out of her concerns about her daughter's low cholesterol level as the least of the child's problems and that, don't you know! high cholesterol is bad for you, and the lower the better.

Historical records on the biochemical aspects of the RSH syndrome are in Irons et al., [1993], Opitz and de la Cruz [1994], Tint et al., [1994], and Kelley [1997]. In retrospect, we should perhaps have been more sensitive to the hints of "disease" in at least the severely affected children with this condition, namely failure to thrive, a neonatally obtunded/semicomatose state in those with very low cholesterol levels,

cataracts, seizures, liver disease with more or less severe cholestasis, rare adrenal insufficiency, testicular insufficiency/failure, and the more or less dramatic reduction in estriol excretion later in pregnancy, a sterol of fetal origin [Donnai et al., 1986; Opitz et al., 2002].

Excellent reviews on the RSH syndrome (and related disorders) over recent years include (but are not limited to): Porter and Herman [2011], Irons [2008], Yu and Patel [2005], Porter [2003], Herman [2003], Kelley and Hennekam [2000], Porter [2002], Kelley and Herman [2001], Nowaczyk and Waye [2001], Herman [2000]. Most of these reviews also address the genetic aspects of the RSH syndrome including:

- Confirmation of autosomal recessive inheritance with negligible parental consanguinity and occasional occurrence in other relatives.
- An approximate 4% carrier state (2pq in the Hardy–Weinberg equation, 2% gene frequency) in the Caucasian population, carriers being apparently normal and no heterozygote advantage known.
- Discrepancy between expected and observed homozygote frequency (1/2,500 vs. 1/101,000) suggesting a high (>95%?) homozygote death rate during (early) gestation.
- Surprising non-heterogeneity (considering so many other highly heterogeneous MCA syndromes, e.g., Fanconi, Meckel, Bardet-Biedl, Joubert syndromes) with only one locus known at *HSA11q13* with loss-of-function mutations in the 3β-hydroxysterol-Δ^7reductase (*DHCR7*) gene; by 2005 Correa-Cerro and Porter [2005] and Yu and Patel [2005] were able to list respectively 105 and 121 known *DHCR7* mutations.
- Elucidation of the structure of the human *DHCR7* gene (highly homologous to the corresponding *A. thaliana*, mouse and rat genes) then made it possible to create mice with corresponding mutations but neonatally lethal phenotypes [Fitzky et al., 2001; Wassif et al., 2001].

This insight into sterol involvement in the RSH syndrome initiated a rich area of investigation into related defects in the path to cholesterol, namely: Antley–Bixler syndrome, Greenberg HEM dysplasia, CHILD and Conradi–Hünermann syndromes, lathosterolosis, desmosterolosis, and methyl sterol oxidase (MSO) deficiency [Furtado and Opitz, 2012].

One final confession: The infant (C.H.) reported by Opitz and Howe [1969] probably did not have the Meckel but rather the RSH syndrome.

TERATOLOGY

Until they began publishing in English in 1979, the pioneering work of Charles Roux and his coworkers in Paris was virtually unknown or unquoted in the Anglo-American literature. Apparently begun as early as 1961 it continues the truly glorious tradition of French teratology begun early in the 19th century by Étienne Geoffrey St-Hilaire, one of Napoleon's naturalists in Egypt [q.v. his *Philosophie anatomique* of 1822] and his son, Isidore Geoffrey St-Hilaire, who coined the term *tératologie* in his magisterial *Traité de Tératologie* [1837,Greek *teratologia*: "récit de choses extraordinaires"]. Dareste [1877] and Wolff [1948] have documented warmly progress of their own and that of other French investigators continued by such giants as Paul Ancel, Nicole Le Douarin, H. Tuchman-Duplessis, Alfred Jost, Nathalie Josso, Charles Roux, and others.

The latter (Roux) published early work on the teratogenic effect of the anticholesterolemic drug triparanol in Wistar rats (more sensitive than Sprague–Dawley rats) producing fetal death, and in surviving offspring, multiple anomalies including anencephaly/craniorachischisis, an- or microphthalmia, pituitary agenesis, abnormal subdivision of telencephalon as indication of holoprosencephaly, and multiple gross vertebral abnormalities, testicular and renal ectopia, horseshoe kidneys, ureterohydronephrosis, abnormally cystic lungs, oligodactyly, talipes, and ventral (abdominal) wall defects. In 1964 (the same year as Smith et al.) Roux asked whether these severe defects of development were a direct teratogenic effect of triparanol or due cholesterol deficiency perhaps with an excess of desmosterol.

Roux and Dupuis [1966] reported on the teratogenic effect of triparanol in the mouse, requiring a much stronger dose than in the rat, and producing (with 200 mg/kg from the 1st to the 14th day) fetal loss (40.2%), almost 64% malformation rate, including "monorhyny" (single HPE-like proboscis), eye abnormalities, craniorachischisis, and cleft palate (almost 30%). These authors concluded stating that among other studies underway they intend "above all" to investigate cholesterol in the treated animals." Roux and Aubry [1966] reported on the teratogenic action in the rat by AY9944, producing at various doses high fetal mortality, intrauterine growth retardation, monorhiny, anophthalmia, cleft lip, talipes, ureterohydronephrosis, and ectopic testes.

Dietert and Scallen [1969] using triparanol, 20,25-diazacholesterol and AY9944 produced identical electron microscope changes in liver, adrenal, and testes consisting of increased numbers of pleomorphic unit-membrane-limited opaque cytoplasmic inclusions (lysosomes); 14–64% of sterols in these treated animals was desmosterol or 7-DHC indicating that Δ^7reductase was blocked by these three compounds. These authors seemed unaware of the work of Roux. Roux [1969] concluded that triparanol blocks cholesterol synthesis at the level of desmosterol, hence, must affect the Δ^{24}reductase, and AY9944 at the level of 7-DHC, hence must affect the Δ^7reductase. Cholesterol supplementation to the triparanol- and AY9944-treated pregnant rats at a dose of 1 g/kg/day prevented "completely" the cephalic malformations without affecting fetal death or growth retardation.

Smith and Hasinoff [1970] still not quoting Roux, studied myelin formation postnatally in suckling rats during the time (age 15–22 days) of rapid myelination using AY9944 and 20,25 diazacholesterol. With the latter compound up to 50% of myelin sterol consisted of desmosterol, with the former at least 1/3

was 7DHC. In brain and spinal cord there was decreased uptake of glucose into all myelin components. The authors conclude that myelin composition is fixed and that a deficiency of one of the lipid components limits the assembly of the *whole* lipid portion of the membrane.

The 80 Wistar rat pups treated with AY9944 as of birth or day 5 all developed weakness of hind limbs, wobbling gait and tremors around 18 days with progression to day 25 and subsequent regression [Suzuki and De Paul, 1971]; these symptoms were accompanied by swelling of oligodendroglial cytoplasm and nuclei, sponginess of white matter and accumulation of numerous cytoplasmic osmiophilic inclusions of neurons.

Roux et al. [1979a] interpreted isolated absence of pituitary in AY9944-treated rat pups as a "lesser form" of holoprosencephaly. Roux et al. [1979b] also showed that a hypercholesterolemia-provoking diet was "completely effective" in preventing holoprosencephaly in AY9944-treated rats but only partially so in preventing the urogenital malformation and fetal mortality [see also Barbu et al., 1988].

Roux et al. [1980] showed an inverse correlation between fetal holoprosencephaly and maternal cholesterol level, and that, in Wistar rats, a cholesterol level <0.3 g/L was the threshold below which the malformations are produced. Repetto et al. [1990] (Roux's group) demonstrated in fetuses cultured in vitro that AY9944 teratogenicity is initiated prior to day 10 of GA.

In 1995, after the discovery of the basic defect in the RSH syndrome, Xu et al. in Tint's group demonstrated that BM15,766, a competitive inhibitor of DHCR7, fed by gavage to rats, can induce a dramatic decrease of plasma cholesterol with corresponding increases in 7DHC. This was confirmed by Wolf et al. [1996] working in the group of Roux in Paris. A new hypocholesterolemic agent, YM9429, was also found to be a potent teratogen inducing cleft palate and skeletal abnormalities in rats, inhibiting cholesterol biosynthesis with concomitant rise in 7DHC in cultured cells [Xu et al., 1995; Honda et al., 1996].

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In 1997, there were at least two important publications from the laboratory of Dr. Kathy Sulik at the University of North Carolina [Dehart et al., 1997]. Pregnant Wistar rats were treated with BM 15,766 producing deficiency in the midline region of the upper face, narrowing of forebrain hemispheres and of the cerebral aqueduct, and micrognathia. Histologic studies on day 11 showed abnormal cell populations at the rim of the developing forebrain and in the alar plate of the lower midbrain and the hindbrain.

Lanoue et al. [1997] took an ingenious approach to the teratogenesis of BM 15,766. Mice have a relatively high basal cholesterol level making them more resistant than rats to cholesterol deficient teratogenesis. Therefore, Lanoue et al. treated an apolipoprotein deficient mouse with BM15766 increasing malformation rate from 48% to 83% in $apob^{-/-}$ and from 1% to 20% in $apob^{-/+}$ animals. Resulting brain and facial anomalies were interpreted as analogous to those of humans with RSH syndrome and as "mild manifestations of holoprosencephaly." These fetuses also had genital and limb defects, suggesting altogether a defect of *Shh* function.

Fitzky et al. [2001] were able to show in $Dhcr^{-/-}$ mice normal mRNA levels for HMG-CoA reductase but a direct proteolytic effect of 7DHC on HMG-CoA reductase suppressing sterol biosynthesis post-translationally.

TREATMENT

Since cholesterol does not cross the blood–brain barrier its use for the CNS manifestations in RSH/SLO syndrome does not make sense. Dietary cholesterol with or without bile acids to enhance intestinal absorption will increase serum cholesterol in some patients without much change in 7DHC or 8DHC levels [Irons et al., 1997; Ullrich et al., 1996]. Jira et al. [1997] used a combination of exchange transfusion and simvastatin in a 3-month-old girl over 190 days; this led to a "substantial" decrease of 7DHC and 8DHC concentrations and increase in and finally normal levels of erythrocyte membrane and plasma cholesterol. Further studies by Jira et al. [2000] with simvastatin (which does cross the blood–brain barrier) treatment in two patients led to improvement in plasma and red cell membranes cholesterol levels superior to the change observed in cerebrospinal fluid.

Kelley [2012] has shown that impaired cholesterol synthesis is due to oxidative conversion of 7-DHC "to a potent inhibitor of HMG-CoA reductase" postulated to cause oxidative damage in mitochondria. Thus, Kelley and coworkers treated "several" SLOS patients with vitamins E and C and

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coenzyme Q10, effectively normalizing the total sterol level in all patients treated. In further studies Kelley showed that "all but the most mildly affected SLOS patients have significant mitochondrial dysfunction, which, when treated with all three antioxidants, leads to increased muscle bulk and strength, reduced peripheral neuropathy, improved behavior, and fewer infections."

SUMMARY

Holoprosencephaly (HPE) may occur in the RSH syndrome in 5–6% [Weaver et al., 2010]. The autosomal recessive non-heterogeneous RSH syndrome is now known to be due to homozygous mutations in the gene coding for 7-dehydrocholesterol reductase (*DHCR7*) with more or less severe impairment of cholesterol synthesis. Cholesterol is required to produce the sonic hedgehog ligand for the receptor *patched* active during early morphogenesis of many organ primordia including the central nervous system. Knock-out of the mouse *sonic hedgehog* gene and agents inhibiting DHCR7 or impairing cholesterol synthesis (jervine in *Veratrum californicum*, AY9944, etc.) may lead to HPE. Thus, the malformations seen in the RSH syndrome probably reflect defective morphogenesis due to impairment of sonic hedgehog synthesis and function.

Since *all* cell membranes of the RSH embryo are also cholesterol deficient, *any* malformation is to be expected including limb defects since sonic hedgehog is a major morphogen in the zone of polarizing activity at the posterior edge of the limb bud. Embryo lethality and intrauterine growth deficiency were demonstrated early [Roux and Dupuis, 1961a,b; 1964] using triparanol to inhibit the sterol-Δ^{24}-reductase responsible for the conversion of desmosterol to cholesterol.

Since the biosynthesis of cholesterol requires 11 atoms of oxygen it probably was absent from the cell membrane of early life forms existing before the "Great Oxygenation" of the world some 2.4 billion years ago. This (photosynthetic) event made possible not only cholesterol synthesis as a major component of cell membranes but also the evolution of eumetazoans.

Finally—let it be remembered how this all started: March 1962: Clinical documentation of a phenotype in *one* patient (apparently sporadic or chance-isolated syndrome); June 1962: Observation of an apparently identical condition in a 2nd patient—hence, formal genesis syndrome, presumed identical *pathogenesis* without any guarantee of causal homogeneity; finally a 3rd patient with affected brother, hence, *causal genesis* syndrome—either X-linked or autosomal recessive.

At last, 30 years later, discovery of a genuine Garrodian inborn error of metabolism in this condition confirming identical pathogenesis, supported by simulation of the condition in rats and mice; and case 1 of Smith et al. [1964] biochemically confirmed as actually having the RSH syndrome. When cause was identified it came as a surprise that this condition, complexity nothwithstanding, was non-heterogeneous, a true causal genesis syndrome, not a phenocopy or genocopy. While it is becoming fashionable to scoff at non-molecular diagnoses it is salutary to remember what perceptive clinical geneticists could accomplish nosologically decades ago—namely delineation of pure syndromes.

While it is becoming fashionable to scoff at non-molecular diagnoses it is salutary to remember what perceptive clinical geneticists could accomplish nosologically decades ago—namely delineation of pure syndromes.

ACKNOWLEDGMENTS

To Dr. Malgorzata J.M. Nowaczyk, McMaster University, Hamilton, Canada for enthusiastic encouragement and outstanding research contributions to the RSH/SLO syndrome; to Lindy Stokes for expert document preparation, literature, references, and efficient logistics; to Dr. Larissa Furtado, my gifted collaborator in sterol diseases for invaluable support; and to all who have worked hard and effectively over the last 50 years to elucidate the biological nature of RSH/SLO syndrome.

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