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Adrenoleukodystrophy fibroblast
cholesterol Schilder's disease

Cholesterol Metabolism in Cultured Fibroblasts in Adrenoleukodystrophy

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Extract

The basic biochemical defect of X-linked adrenoleukodystrophy (sudanophilic leukodystrophy, Schilder's disease) is unknown. To investigate reported abnormalities in cholesterol metabolism *in vitro*, we examined cultured skin fibroblasts of four patients and four normal control subjects. The kinetics of retention and accumulation of [^{14}C]cholesterol by these cells was studied. After 3 days of exposure to tracer amounts of [^{14}C]cholesterol, an apparent steady state between the medium and cellular cholesterol was established. The specific radioactivity expressed per mg of protein was similar for both Schilder and control fibroblasts. After labeling the pre-existing cellular cholesterol pool, the rate of loss of label was followed up for a 6-day period. About 23% and 14%, respectively, of the cellular radioactivity in both Schilder's disease and control cells were released into the medium after the consecutive change with fresh nonlabeled medium. No significant differences in [^{14}C] cholesterol rates of uptake or release were observed between control and Schilder's disease fibroblasts. About 44% of the labeled cholesterol was present in an esterfied form after incubation in the presence of unheated serum in both Schilder's and control cultures.

Speculation

These results do not confirm previously reported abnormalities in cholesterol metabolism in cultured fibroblasts. Nevertheless, accumulating evidence from studies of fatty acid composition of brain and adrenal tissues in Schilder's disease indicates the presence of an abnormal series of long chain fatty acids. Studies are now in progress to determine whether these possibly unique long chain fatty acids are evident in fibroblasts from patients with Schilder's disease.

The association of adrenoleukodystrophy (sudanophilic leukodystrophy, Schilder's disease, or diffuse cerebral sclerosis) and adrenal insufficiency has been recognized for over 50 years (17). This disorder has been clearly delineated as X-linked recessive and separated from the heterogenous group of fatal degenerative leukodystrophies characterized by other modes of inheritance (6, 7, 15, 18, 19). Distinct heterogeneity exists in the X-linked form with variable severity of the adrenal cortical atrophy from asymptomatic to overt adrenal crises. The characteristic pathology includes widespread demyelination and gliosis of cerebral and

cerebellar white matter with preservation of subcortical U fibers and a relatively normal cerebral cortex (4).

Evidence for abnormalities in cholesterol metabolism in Schilder's disease has been presented. Eto and Suzuki (3) found cholesterol esters with an abnormal fatty acid composition in the brains of patients with Schilder's disease and other disorders associated with sudanophilic demyelination, probably reflecting nonspecific changes due to abnormal histology. Others reported an abnormal sterol in the white matter of the brain in one patient with sex-linked Schilder's disease (4). Forsyth *et al.* (5) reported an absolute and relative increase in esterified cholesterol in the brains of a number of patients with Schilder's disease. Pathologic studies of the adrenal glands have also revealed distinctive changes in the adrenal cortex (nests of ballooned, nonsudanophilic cortical cells containing cytoplasmic aggregates of birefringent crystals) (16), which have been found in other disorders with other abnormalities in cholesterol metabolism, *e.g.*, Wolman's disease (10).

Most recently Burton and Nadler (1) reported abnormal cholesterol retention and accumulation in cultivated skin fibroblasts in Schilder's disease. In contrast, this study documents our inability to demonstrate any abnormal kinetics of cholesterol metabolism in cultured skin fibroblasts.

CASE REPORTS

CASE 1 (PW 1005): AGE 6.5 YEARS

Growth and development of this patient were normal until 6 years of age, when visual difficulty with associated lateral rotation of the left eye was noticed. Subsequently, bilateral cortical blindness, left hemiparesis, then right hemiplegia and spasticity resulted. EEG, skull x-rays, cerebrospinal fluid, and arteriography were normal. The pneumoencephalogram suggested central atrophy of the left occipital lobe. Fasting morning blood cortisol values were at the lower limit of normal (5.2 μg/100 ml).

Progressive neurologic deterioration occurred over the next 3 years. It was characterized by the development of complete spastic quadriplegia, severe dysarthria, and emotional lability with preservation of consciousness. Now, at 9.5 years of age, the patient remains blind and spastic with fixed decorticate posturing, and responds to simple commands with a barely intelligible voice. There is no evidence of adrenocortical insufficiency.

CASE 2 (JC 1026): AGE 8 YEARS

The patient had always been in excellent health except for viral meningoencephalitis at 5 years of age. Symptoms began at about 7.5 years of age with deterioration in school work, complaints of diplopia, difficulty in reading, intermittent nausea, and later tinnitus, slurred speech, and inappropriate or confused responses. His gait became increasingly unsteady, he had several falls, and he began to drop food when feeding himself. He had always tanned extremely easily, but had had no salt craving. His 13-year-old brother (*case 3*) had always tanned excessively, but had never had any neurologic symptoms.

Examination showed bronzing of the skin, receptive and expressive aphasia, and perseveration. There were deficits in both recent and remote memory and difficulty in drawing, reading, and calculating, as well as slight slurring of speech. There was subjective diplopia, but cranial nerve examination was objectively unremarkable. Both plantar responses were extensor. Hyperreflexia was present in the legs, but no clonus was evident. Marked bilateral cerebellar signs were elicited. Sensation was grossly intact. The EEG was abnormal with generalized slowing and superimposed low voltage fast activity. A previous EEG at age 6 was reviewed and was normal. A technetium brain scan was severely abnormal because of markedly increased uptake in the midline temperoparietal area. An EMI scan was strikingly abnormal, indicative of gross bilateral loss of cerebral substance superficially with some central atrophic change as well. A pneumoencephalogram also revealed generalized dilation of the ventricular system and an atrophic process. Spinal fluid was mildly xanthochromic at normal pressure and with a protein level of 156 mg/100 ml. Cerebral spinal fluid measles antibodies and spinal fluid γ-globulin were normal.

Fasting early morning blood cortisol was 0.4–0.6 μg/100 ml, well below the normal range of 5–25 μg/100 ml, and there was no rise after adrenocorticotropic hormone (ACTH) stimulation. Electrolytes were normal, renin level was 10.6 mg/ml/hr, and testosterone level was 0.02 μg/100 ml. A third sibling and both parents had normal cortisol levels, although the brother who tanned excessively also had documented adrenocortical insufficiency (*case 3*).

Progressive neurologic deterioration followed with the development of severe aphasia, bilateral optic atrophy, spastic quadriparesis, and, eventually, an unresponsive bedridden state. The patient's adrenocortical insufficiency was well controlled with Florinef and prednisone. The patient died at 10 years of age. Neuropathologic examination (by Dr. H. H. Schaumburg) revealed the typical features of adrenoleukodystrophy, the demyelination affecting large areas of the white matter, especially of the occipital lobes, including lesions of the centrum ovale.

CASE 3 (BC 1027): AGE 13 YEARS

This patient is the brother of *case 2*, who was first examined after the diagnosis of Schilder's and adrenocortical insufficiency. Except for generalized pigmentation, he appeared to be a healthy 13 year old. In particular, there were no signs of neurologic dysfunction. The fasting morning blood cortisol value was 1.6 μg/100 ml (normal lower limit 5.2 μg/100 ml). Cortisol values after the ACTH stimulation test were unchanged at 1.6 μg/100 ml. Treatment with Florinef and prednisone was then started. He has remained clinically asymptomatic and free of any neurologic signs at 15 years of age.

CASE 4 (JS 1121): AGE 10.5 YEARS

Primary adrenocortical insufficiency was diagnosed at the age of 4 years. Replacement therapy with prednisone, initiated then, continues. He remained well until 10 ears of age when decreased auditory perception and left homonomous hemianopsia were observed. Slow progressive neurologic dysfunction has continued. Now, at 12 years of age, he has advanced neurologic degeneration and is spastic, unresponsive, bedridden, and dying. His only two male siblings died at ages 8 and 5 years, respectively, with skin pigmentation and sudanophilic leukodystrophy.

MATERIALS AND METHODS

Fibroblasts from upper arm skin biopsies of the four Schilder patients and four control subjects were employed and subcultured for 1–5 generations. Cells were routinely grown in petri dishes in minimal essential medium (MEM) with Earle's salt solution, supplemented with 15% fetal calf serum at 37° in an atmosphere of 5% CO_2 in air. No antibiotics or antimycotic agents were added.

PREPARATION OF [4-^{14}C]CHOLESTEROL-LABELED EXPERIMENTAL MEDIUM

[4-^{14}C]Cholesterol, 50 μCi (56 mCi/mmol (21)) in benzene was evaporated to dryness under sterile conditions. Ethanol, 50 μl, was added followed by a 10-ml solution containing 20 mg/ml albumin (22) in MEM medium. The solution was thoroughly mixed for 2 min and aliquots were taken for radioactivity determination. To these were added 15 ml Aquasol (21) and the samples were counted in a Packard Scintillation counter, model 3380. The stock solution was adjusted to the appropriate concentration (5×10^4 cpm/ml) by using MEM solution containing 5% heat-inactivated fetal calf serum (Rehatuin (23)). The solution was allowed to equilibrate for 48 hr at 37°.

UPTAKE STUDIES

For uptake studies cells were plated on 60-mm Falcon petri dishes (surface area 28 cm^2) at a concentration of $0.18–0.26 \times 10^5$ cells/cm^2 and allowed entrance into the log phase of growth in the nutrient medium containing 15% serum. After 24 hr, the medium was removed and 2–3 ml of the experimental medium containing [4-^{14}C]cholesterol (5×10^4 cpm/ml) were added. Incubation was carried out at 37° in an atmosphere of 5% CO_2 in air. Cultures were examined daily by phase microscopy. At designated times the radioactive medium was removed and cells were washed twice with MEM solution containing 5% fetal calf serum and once with a solution of 0.9% NaCl. The cells were scraped off the petri dish, suspended in 0.9% NaCl, and transferred into glass conical tubes. After a brief centrifugation ($400 \times g$ for 5 min) the supernatant was discarded, and to the cell pellet, 4 ml of a solution of chloroform-methanol (1:2 by volume) were added. Samples were stored at −20° until further analysis.

EXTRACTION AND FRACTIONATION OF CELLULAR LIPIDS

Most of the procedures employed for lipid extraction have been described elsewhere (20). The chloroform-methanol-treated cells were centrifuged for 5 min at $600 \times g$ and the organic solvent supernatant was transferred into another tube. Aliquots were taken for determination of radioactivity. The solvent was evaporated to dryness under a stream of nitrogen, the lipid residue was dissolved in 0.1 ml chloroform-methanol (2:1 by volume), and aliquots were applied on Silica Gel G (24)-precoated plates. Separation of the neutral lipids was achieved by thin layer chromatography in one direction employing the solvent system of hexane-ether-acetic acid (90:10:0.5 by volume). A standard mixture of neutral lipids (25) was routinely employed. The cellular lipid extracts were scanned for radioactivity using a Berthold-Varian Aerograph radioscanner, model 6000. Alternatively, the thin layer plates were exposed to iodine vapor and the corresponding neutral lipid spots visualized, scraped off the plate, and counted (20). Cell protein after lipid extraction was determined according to the method of Lowry *et al.* (9).

[^{14}C]CHOLESTEROL RETENTION STUDIES

Cells, incubated for a period of 72 hr with cholesterol-labeled medium as previously described, were treated as follows. The radioactive medium was removed and cells were washed three times with a MEM solution containing 5% heat-inactivated fetal calf serum, at which point no further radioactivity was detected in the wash media. Cells were further incubated with 3 ml MEM containing 5% heat-inactivated fetal calf serum. At designated times, 50–100-μl aliquots of the medium were removed under sterile conditions and counted in 10 ml Aquasol solution. Alternatively, at specified times during the decay studies, both medium and cellular radioactivity was determined as previously described. Each value is an average of two to three petri dishes. Each experiment was repeated at least twice.

RESULTS

[^{14}C]CHOLESTEROL UPTAKE

Cultured Schilder and control fibroblasts incubated for 72 hr with labeled cholesterol incorporate radioactivity in the cholesterol fraction (Fig. 1). No label was detected in the cholesterol ester fraction in any experiment. About 18% and 25% of the total radioisotope added were incorporated by the cells at 24 hr and 72 hr, respectively (Table 1). There were no marked differences in the rates of uptake between the Schilder's and the control fibroblasts.

[^{14}C]CHOLESTEROL RETENTION STUDIES

The results of the retention of the ^{14}C-labeled cholesterol by the fibroblast cultures are illustrated in Figure 2. No significant differences in the retention of label in Schilder cells was observed as compared with the control cells after 4 days. Between the second and the fourth day, a relative slowdown in the rate of disappearance of label was observed.

An apparent steady state between the cellular cholesterol and the medium cholesterol was established after 3 days (Fig. 3). If at that time the radioactive medium was removed and replaced with fresh, nonlabeled medium, a new apparent steady state was established within 3 days. This steady state was reached at different levels depending on the amount of radioactivity initially present in the cells. This is further illustrated in Table 2. Cells incubated for 72 hr with [^{14}C]cholesterol retain various amounts of label in their cellular lipids. When uptake is expressed per mg of protein, both Schilder and control cultures exhibit the same specific activity. When further exposed for 3 days to nonlabeled medium, the amount of label appearing in the medium is proportional to the initial radioactivity. Thus, in all cases, a similar percentage of radioactivity was observed in the medium after 3 days. When cells were incubated for 3 additional days, similar

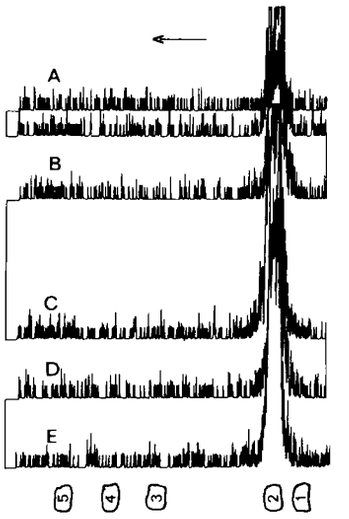

Fig. 1. Radioactive scan of a thin layer chromatogram of lipid extracts after incubation with [4-^{14}C]cholesterol. Cultured fibroblasts from two control lines (*A*, *B*) and three Schilder lines (*C*, *D*, *E*) were incubated for 72 hr in the presence of [4-^{14}C]cholesterol. Cell lipids were extracted and chromatograped on thin layer plates as described in *Materials and Methods*. *1:* origin, also containing polar lipids; *2:* cholesterol; *3:* triglycerides; *4:* fatty acid methyl esters; *5:* cholesterol ester.

Table 1. *Uptake of [4^{14}C]cholesterol by cultured fibroblasts*[1]

Fibroblasts	Uptake, cpm $\times 10^{-3}$/mg protein	
	24 hr	72 hr
Schilder cases		
PW 1005	57.2	84.1
JC 1026	56.8	77.0
BC 1027	66.1	90.4
JS 1121	60.4	84.8
Average uptake	60.1	84.0
Control cases		
1200	62.8	83.0
1201	56.9	78.9
Average uptake	59.8	80.8

[1] Experimental conditions are described under Materials and Methods. Values, expressed as counts per min per mg of protein, are averages of two to three cultures.

rates of appearance of label were observed in both Schilder and control media. The percentage of label appearing in the second incubation period was lower than that of the first incubation period.

CHOLESTEROL ESTER RETENTION STUDES

When fetal calf serum which had not been heat treated was employed for [^{14}C]cholesterol uptake studies, a large proportion of radioactivity was detected in the cholesterol ester fraction. This observation prompted us to study the rate of cholesterol ester disappearance after exposure of cells to conditions in which both cholesterol and cholesterol ester intracellular pools were labeled.

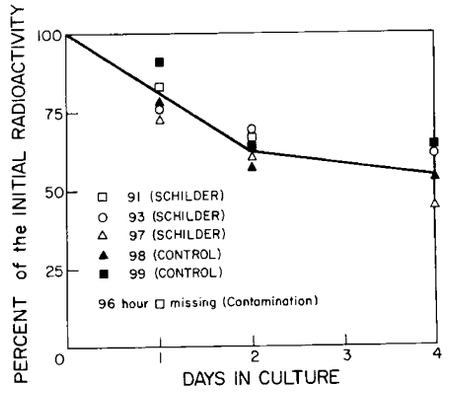

Fig. 2. Retention of ^{14}C-labeled cholesterol by cultured Schilder's and control fibroblasts. Experimental conditions are described under *Materials and Methods*. Cultures were exposed for 72 hr to [4-^{14}C]cholesterol and, after the removal of the medium, were further incubated for designated periods of time. The values are expressed as percentage of the total radioactivity present in Schilder (□, ○, △) and control (▲, ■) fibroblasts at the beginning of the chase study and represent averages of two to three cultures (*91*, *93*, and *97* represent *cell lines 1005*, *1027*, and *1121*).

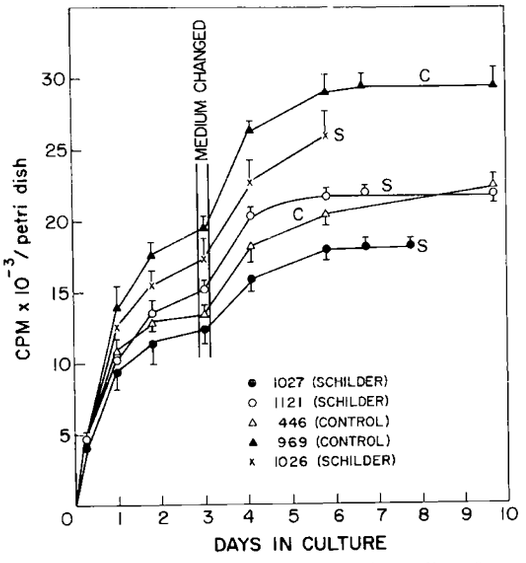

Fig. 3. Rate of [4-^{14}C]cholesterol appearance in medium from Schilder's and control fibroblasts. Experimental conditions are described under *Materials and Methods*. Schilder (●, ○, ×) and control (△; ▲) cultures were incubated with [4-^{14}C]cholesterol-containing medium. After 72 hr the radioactive medium was removed and fresh nonlabeled medium was added. At designated times, aliquots from the medium were taken for determination of radioactivity. After the third day a second portion of fresh nonlabeled medium was added and the appearance of label in the medium was further monitored. Values are averages of three cultures ± standard deviation.

Table 2. *Appearance of* [^{14}C]*cholesterol in medium from Schilder and control fibroblasts*[1]

Cell line	Cellular radioactivity at beginning of chase, cpm/plate	Protein, mg/plate	Specific activity, cpm/g protein	Radioactivity released into medium, % 3 days after first chase	Radioactivity released into medium, % 3 days after second chase
Schilder cases					
1027	44.0	0.21	210	28.4	16.8
1121	72.0	0.32	225	20.8	12.5
1026	80.5	0.37	220	21.7	15.0
Control cases					
969	82.0	0.38	216	23.8	16.0
446	68.4	0.30	238	19.9	13.1

[1] Experimental conditions are similar to those described in Figure 3 except that cells were incubated with 2.5 × 10^5 cpm [^{14}C]cholesterol-containing medium. Values are averages of two to three cultures.

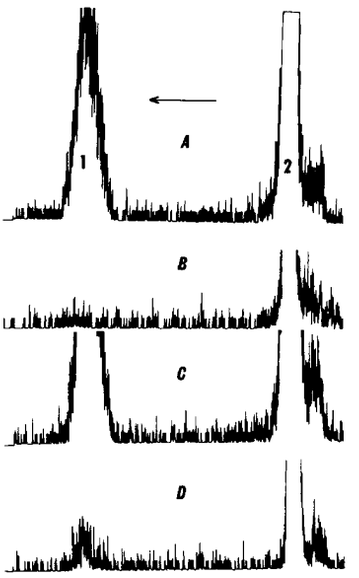

Fig. 4. Thin layer chromatogram scanning of [^{14}C]cholesterol-labeled cellular lipids from Schilder's and control fibroblasts. Experimental details are given in the text and in the legend to Figure 1. Schilder fibroblasts at 3 days (*A*) and 10 days (*B*) in culture; control fibroblast at 3 days (*C*) and 10 days (*D*) in culture. *1:* cholesterol ester; *2:* cholesterol.

After 3 days in the presence of a medium containing 5% nonheated serum and [4-^{14}C]cholesterol, about 40–48% of the total radioactivity incorporated by both the Schilder (Fig. 4*a*) and control fibroblasts (Fig. 4*c*) was detected in the cholesterol ester fraction. After the radioactively labeled medium was removed, cells were further incubated for designated time periods in growth medium containing 5% heat-inactivated serum. After 7 days the relative loss of labeled cholesterol ester was greater than that of cholesterol in both Schilder and control cultures (Fig. 4*b* and 4*d*, respectively). No significant differences in the rate of disappearance of both cholesterol and cholesterol ester were observed between Schilder and control fibroblasts.

DISCUSSION

The diagnosis of adr oleukodystrophy rests on clinical assessment helped by consideration of the family history. Except for *case 3*, a clinical diagnosis of adrenoleukodystrophy could be made on

the basis of neurologic signs and laboratory tests without significant doubt. This patient (*case 3*), now 15 years of age, has shown no manifestations of neurologic degeneration, but has classic adrenocortical insufficiency. Uniformity in the pathologic picture has been shown by Schaumburg *et al.*, who demonstrated characteristic histologic abnormalities consistently present in the adrenal cortex and testis, even in those patients without clinical signs of adrenal insufficiency (11, 12, 15).

In the present study we have been unable to demonstrate any significant difference in the kinetics of uptake and release of labeled cholesterol between Schilder and control fibroblasts. Uptake and release of cholesterol are determined by many factors such as cell type, source of serum employed, age, and metabolic conditions (13, 14). Moreover, variation in growth rates and the degree of cell density of cultured skin fibroblasts may affect enzymatic activities and other metabolic functions (2). Morphologic observations of the various cell lines employed in this report revealed that most of the cells attained a state of confluency after 72 hr. Some lines, not necessarily related to the origin of the donor, exhibited a different morphologic appearance as compared with others. They consisted mainly of large cells with extended cytoplasm rather than densely packed cells. This could indicate a possible difference in maximal cell densities attained by the various lines which will result in a different number of cells per surface area. We therefore preferred to express the retention values of [^{14}C]cholesterol as percentages of the initial radioactivity rather than to express them per mg of protein. Under those circumstances Schilder and nomal control fibroblasts exhibited similar kinetics (Figs. 2, 3; Table 2). Neither have we found any differences when uptake values were expressed per mg of protein in nonconfluent, actively dividing cells (Table 1). In contrast, Burton and Nadler (1) reported a significant retention and accumulation of cholesterol in Schilder fibroblasts as compared with normal control fibroblasts.

Our inability to reproduce those findings may well be related to a different rate of growth and degree of cell density existing between various cell lines. Uptake and release of cholesterol may be an indication of the metabolic state of cells. Thus, cells reaching a state of maximal packing density may sooner exhibit faster equilibrium when incubated with a given radioisotope. Retention of labeled cholesterol will be affected by its rates of influx and efflux, which in turn could be dependent on the metabolic state of the cells. It is of primary importance to correlate kinetic data with parameters such as DNA turnover and content or cell number, otherwise conflicting interpretations may arise. In our view, expression of the retention studies data as a percentage of the initial radioactivity is justified.

Our tissue culture methods, sample preparation, and measurement of radioactivity in the kinetic studies were not identical with those used by Burton and Nadler (1). Our uptake data suggested that a steady state condition for [^{14}C]cholesterol was attained after 3 days of exposure to the isotope for both Schilder's disease and controls with fibroblasts reaching confluency. Moreover, by labeling the cells for a 3-day period and following the rates of loss of label for 7 days (Fig. 3; Table 2), under steady state conditions any significant imbalance between the rates of influx and efflux of cholesterol should have been noticeable. We therefore do not believe that uptake studies done over periods beyond the steady state conditions provide any further critical information.

Although distinct clinical heterogeneity does exist in adrenoleukodystrophy, it would seem rather unlikely that such a possibility could satisfactorily explain the differences noted in this study and that of Burton and Nadler (1).

Our observations were similar to those of Burton and Nadler in that we did not observe significant differences between Schilder's and control cells in the uptake and release of cholesterol ester. At the present time we cannot provide any explanation for our observation of cholesterol esterification when unheated sera were used. It is possible that a heat-labile cholesterol ester transferase activity was present in the serum, a finding which requires further study.

Our inability thus far to demonstrate significant abnormalities of cholesterol metabolism *in vitro* does not exclude the possibility that these patients have some disturbance in lipid metabolism. Indeed, Igarashi *et al.* (8) have recently studied the fatty acid composition of individual lipids of white matter and adrenals of four patients with Schilder's disease. They determined that substantial portions of fatty acids in cholesterol esters from both brain and adrenal had chain lengths longer than 22 carbons, whereas cholesterol ester from normal tissues contained essentially no fatty acid longer than 20 carbons. In some of the cases they noted that the longer chain fatty acids constituted more than two-thirds of the total fatty acids. The most prominent was C_{24-26}, but even C_{30} was present in significant amounts. These unusually and possibly unique fatty acid abnormalities were not found in any of the serum lipid fractions.

Igarashi *et al.* (8), although postulating a defect in fatty acid metabolism, cannot yet explain how such an abnormal series of long chain fatty acids could result from a specific abnormality in the known pathways of fatty acid metabolism.

SUMMARY

The kinetics of uptake and release of [^{14}C]cholesterol in cultured fibroblasts from four cases with Schilder's disease and four normal control subjects was investigated. There was no evidence for an accumulation of labeled cholesterol in cells from Schilder's disease as compared with control cultures. In addition, no significant differences in the rates of formation and retention of cholesterol ester were found.

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Methionine Adenosyltransferase and Transmethylation in Fetal and Neonatal Lung of the Human, Monkey, and Rabbit

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Extract

Optimal conditions for the assay of methionine adenosyltransferase in crude extracts of human fetal lung were determined. Maximal activity was obtained with 36 mM ATP, 20 mM L-methionine, 240 mM Mg^{++}, and 160 mM K^+. The pH optimum was 6.2–6.4, which is the same as that for adult human lung but lower than that for human liver. In human fetal iung, there was an increase in specific activity of methionine adenosyltransferase with increasing gestational age ($r = 0.87$; $P < 0.01$) up to 25 weeks of gestation, after which time no fetal specimens were obtained. The specific activity of 5-methyltetrahydrofolate-homocysteine methyltransferase of human fetal lung was 2.51 ± 0.88 nmol/mg protein/hr, which was higher ($P < 0.001$) than the activity found in newborn lung (0.14 ± 0.01). Activity of serine hydroxymethyltransferase and of betaine-homocysteine methyltransferase was absent from human fetal lung. Activity of cystathionine synthase was absent from fetal, neonatal, and mature human lung. Activity of cystathionase in fetal and newborn human lung was present only in trace amounts. In rhesus monkey lung, beginning 15 days before term, the activity of methionine adenosyltransferase increased 6-fold to reach a maximum before term (165 days), and during the first weeks of life the activity gradually diminished. 5-Methyltetrahydrofolate-homocysteine methyltransferase activity in fetal (100–145 days) monkey lung was higher (6.57 ± 0.95 nmol/mg protein/hr) than in newborn lungs (1.91 ± 0.97) ($P < 0.001$). In fetal rabbit lung, the activity of methionine adenosyltransferase decreased 2.5-fold during the last third of pregnancy, whereas a 2-fold increase occurred during the first 48 hr after term birth.

Speculation

The increasing specific activity of methionine adenosyltransferase in the lung of the human, monkey, and rabbit around the time when extrauterine survival is possible in each species suggests that this enzyme may be important in the synthesis of surface-active lecithin and/or in methylation reactions related to detoxification of the constituents in the blood perfusing the breathing lung.

S-Adenosylmethionine, a major donor of methyl groups in mammalian tissues, is formed from L-methionine and ATP in the presence of Mg^{++} and K^+ by methionine adenosyltransferase (ATP:L-methionine *S*-adenosyltransferase, EC. 2.5.1.6) (2). Biosynthesis of *S*-adenosylmethionine is also the first step on the transsulfuration pathway, which transfers the sulfur atom from L-methionine to the 3-carbon skeleton of L-serine to form L-cysteine. In human fetal liver and brain, but not in kidney, the transsulfuration pathway (see Fig. 1) is incomplete (12, 24, 28) as a result of the absence of cystathionase (L-cystathionine cysteine-lyase (deaminating), EC. 4.2.1.1), the enzyme which cleaves cystathionine to cysteine and α-ketobutyrate.

In the human, the transsulfuration pathway apparently becomes active after birth (30). In the mature human, 90% of the methionine sulfur goes down this pathway (26); therefore, we examined some of the enzymes of transsulfuration and related reactions in human fetal tissues. Investigation of the remethylation of homocysteine to methionine by the vitamin B_{12}-dependent 5-methyltetrahydrofolate-homocysteine methyltransferase (5-methyltetrahydropteroyl-L-glutamate:L-homocysteine *S*-methyltransferase, EC. 2.1.1.13) demonstrated that the specific activity of this enzyme was much greater in fetal human liver and brain than in mature human liver and brain (14). The alternative enzyme for remethylation of homocysteine to methionine, betaine-homocysteine methyltransferase (EC. 2.1.1.5), was much less active (14). In addition, the apparent K_m for 5-methyltetrahydrofolate-homocysteine methyltransferase is of the order of 10^{-5} M, whereas that of