sera were superior to rabbit, sheep, and chicken sera but inferior to the human serum control. 3. Comparison was made of the multiplication of the 2 cell lines when cultivated in Chang's, Eagle's, and 199 media with added human, horse, or calf serum in 10 and 20% concentration. 4. Conjunctiva cells have been serially subcultivated for 18 passages in media containing horse or calf serum but no human serum. Kidney cells have been carried for 14 passages in such media. 5. Conjunctiva and kidney cells survived for varying periods when stored at 32°C or 5°C. Some sedimented conjunctiva cells survived 10 freezing-thawing cycles and formed a sheet of cells when incubated at 37°C. 6. Three of 11 conjunctiva cultures survived lyophilization and revived when reincubated at 37°C for 5 weeks. No contaminating bacterium or virus could be detected in these cell lines by the injection of nonviable cell extracts into tissue cultures of monkey kidney, embryonated eggs, rabbits, guinea pigs, and mice. 7. A method is described for the treatment of contaminated tissue cultures. 8. The conjunctiva cells gave results comparable to monkey kidney cells when used for isolation of poliomyelitis virus from 2 stool specimens.

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Serial Biochemistry of Serum and Cerebrospinal Fluid in Amaurotic Family Idiocy (Tay-Sachs Disease).* (22272)

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Studies in Amaurotic Family Idiocy (AFI) have thus far been principally concerned with histologic examination and biochemical analysis of brain tissue. These latter investigations resulted in isolation of a new cerebroside-like substance called a "ganglioside" and differing from other cerebrosides by the presence of neuraminic acid(1-3). In contrast to other lipoidoses, it is generally assumed that there are no characteristic serum changes in AFI.

The present report is concerned with serial studies of the neuraminic acid, protein and lipid fractions in serum, and cerebrospinal fluid (CSF) proteins in a group of 7 children with infantile AFI, the diagnoses in most instances confirmed at autopsy. Similar biochemical studies of 2 children with Schilder's disease and gargoylism, serving as controls, were also carried out. Most children in this survey were admitted to this institution shortly after clinical inception of the disease and remained hospitalized until after death. The increased longevity of some patients with AFI in this study(4), afforded greater opportunity for carrying out such serial biochemical

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TABLE I. Serum and C.S.F. Protein Data in Amaurotic Family Idiocy and Related Disorders.

Diagnosis	T.P., g %	Alb., g %	Alpha-1, g %	Alpha-2, g %	Beta, g %	Gamma, g %	A/G, %
	Serum electrophoretic data						
Tay-Sachs disease, 7 p, 20 d*	6.80 ± .35† <.01‡	3.70 ± .33 <.01	.50 ± .09 .40	.98 ± .17 <.01	.96 ± .13 —	.65 ± .21 <.01	1.20 ± 2.10 .15
Schilder's disease, 1 p, 3 d	7.39§	3.50	.59	.95	1.28	1.08	.90
Gargoylism, 1 p, 4 d	7.12§	3.57	.52	.95	1.25	.83	1.00
Normal subjects	7.26 ± .39	4.07 ± .29	.48 ± .09	.66 ± .11	.96 ± .20	1.01 ± .20	1.30 ± .18

Diagnosis	T.P., mg %	Alpha, mg %	Alpha/T.P., %	Gamma, mg %	G.G./T.P., %	Serum neuraminic acid, mg %
	C.S.F. proteins (chemical)					
Tay-Sachs disease, 7 p, 20 d*	41.7 ± 11.32† .10‡	5.9 ± .94 <.01	16.2 ± 3.11 .045	1.7 ± .98 <.01	4.9 ± 2.88 <.01	89.6 ± 7.10 <.01
Schilder's disease, 1 p, 3 d	51.6§	7.9	15.2	9.5	21.3	82.7
Gargoylism, 1 p, 4 d	50.8§	9.5	18.7	3.4	6.6	84.4
Normal subjects	37.5 ± 5.0	8.6 ± 1.2	18.2 ± 2.2	4.0 ± 1.0	9.4 ± 1.8	72.9 ± 7.4

* p = patients; d = determinations. † Mean ± S.D. ‡ P. § Mean.

analyses.

Methods. (a) *Serum:* The procedure used for the electrophoretic protein determinations has been described(5,6). Total proteins were determined by biuret procedure(7) with frequent checks against the micro-Kjeldahl method. Total lipids were determined gravimetrically with the Bloor(8) method; phospholipids with the modified colorimetric procedure of Youngburg and Youngburg(9) and free fatty acids by the titrimetric technic of Kaiser and Kagan(10). Total and free cholesterol values were obtained with colorimetric method of Zak, *et al.*(11) and cholesterol esters and cholesterol ester/total cholesterol ratios were calculated from these experimental results. Neutral fats were calculated from the above data by the formula given in the article by Goldbloom(12). Lipase values were determined by the method of Goldstein, *et al.*(13) and neuraminic acid by a modification of the procedure described by Winzler (14). (b) *Cerebrospinal Fluid*: Total proteins were determined by a modification of the Weichselbaum biuret method(15). Gamma globulins were determined by quantitative protein flocculation-ninhydrin colorimetric procedure(16) and alpha globulins with a newly developed turbidimetric method(17) as modified from a previously described technic of Jacox(18) for serum alpha globulins. The simultaneous serial studies on both serum and CSF were generally carried out in duplicate at bi-monthly intervals for periods to 10 months. While the derived biochemical data from the AFI cases were *statistically* analysed (Tables I, II) it was deemed unnecessary to apply such an evaluation to the two control cases because of insufficient number of determinations.

Results. Previous experience with other neurological entities of a degenerative nature such as multiple sclerosis, Schilder's disease, amyotrophic lateral sclerosis, etc.. has shown consistent alterations of both the serum and CSF protein fractions as determined by electrophoretic and biochemical technics. The significance of these changes is not related to alterations in the individual protein fractions, but rather in the entire "protein profile"(19,20). For example, while there is a notable increase in CSF gamma globulin in multiple sclerosis, no corresponding rise in serum gamma globulin is evident, in contrast to many infectious types of disease, *e.g.*, neurosyphilis, wherein both CSF and serum gamma globulin fractions rise above the anticipated normal.

TABLE II. Serum Lipid Data in Amaurotic Family Idiocy and Related Disorders.

Diagnosis	Serum total lipids	Serum neutral lipids	Serum phospholipids	Serum free fatty acids	Total cholesterol	Free cholesterol	Cholesterol esters	Ratio, %	Serum lipase, units
	mg %								
Tay-Sachs disease, 4 p, 15 d*	675.0 ± 137.1† <.01‡	221.0 ± 58.4 <.01	155.5 ± 37.0 <.01	332.0 ± 56.6 .48	192.5 ± 41.75 .018	41.8 ± 12.04 <.01	151.0 ± 32.18 .22	78.4 ± 3.62 <.01	1.08 ± .512 .28
Gargoylism, 1 p, 5 d	702.0§	175.0	182.0	382.0	271.0	55.0	215.0	79.4	1.15
Schilder's disease, 1 p, 3 d	954.0§	302.0	226.0	411.0	287.0	79.0	208.0	72.6	1.18
Normal subjects	861.0 ± 75.0	365.0 ± 59.0	218.0 ± 15.0	320.0 ± 35.0	220.0 ± 20.0	53.0 ± 7.5	162.0 ± 14.0	75.0 ± 2.5	1.23 ± .06

* p = patients; d = determinations. † Mean ± S.D. ‡ P. § Means.

In our series of 7 cases with AFI, a statistical analysis of chemical serum total protein and electrophoretically determined serum albumin showed significant decreases below normal levels. (Table I). In the 2 control cases, with remotely related degenerative diseases of CNS (Schilder's disease and gargoylism), a relatively normal total protein was noted, although serum albumin values were somewhat decreased. Alpha-2 serum globulin fractions were increased in AFI, in contrast to a marked decrease in serum gamma globulin component. Alpha-1 and beta globulin fractions did not vary significantly from normal values. A rather surprising finding was a normal A/G ratio in AFI, since it is frequently depressed in many degenerative diseases, irrespective of etiology. This is exemplified by lowering of this ratio in the case of Schilder's disease (Table I).

The biochemical studies on the CSF samples done simultaneously with the sera, and employing chemical methods, showed a relatively normal total protein but a lowered alpha globulin fraction and markedly decreased gamma globulin values, as well as diminished gamma globulin/total protein ratios. This decrease in CSF gamma globulin levels has not been observed in a wide variety of other progressive, and generally fatal, neurologic diseases and hence cannot be equated with debilitation alone. In most other CNS diseases (including Schilder's disease, gargoylism, multiple sclerosis, cerebral palsy, hemiplegia, etc.) this CSF protein component is either normal or elevated(20,21).

Because of the intimate association of neuraminic acid with AFI, studies of serum level of this complex amino acid were inaugurated. This substance has been identified as one of the abnormal components of the "ganglioside" which constitutes the anomalous dystrophic accumulation within the involved neurons in AFI(1-3). A definite elevation of neuraminic acid in our 4 cases of AFI was observed. This finding is in keeping with the elevation of this substance previously demonstrated in cerebral parenchyma in this disease(1,22,23). Since the 2 control cases also show an elevation in this component, the

specificity of this change in AFI as compared to other neurologic disorders is presently under investigation.

Previous biochemical studies on lipoid dystrophies have dealt mainly with variations in the lipid fractions of visceral organs(24). In a few of these diseases such lipid abnormality is also reflected in alterations of such fractions within the serum. No comparable changes of serum lipids have been reported in AFI to our knowledge. For this reason, biochemical studies of serum lipid partition within total lipids of this disease were performed (Table II). When such results were statistically compared to those derived from normal subjects of comparable age, some marked variations were noted. The total serum lipids, neutral lipids and phospholipids all showed a significant decrease. In the one case of gargoylism, directional changes of a similar nature were recorded. In the instance of Schilder's disease, however, normal or elevated values of these lipid fractions were noted. In studies of the cholesterol fractions, a slight decrease of free cholesterol and slight elevation in the cholesterol ester/total cholesterol ratio were seen. No significant changes were apparent in total cholesterol or cholesterol esters however. Serum lipase and free fatty acid levels were normal. Further studies concerned with lipid and protein metabolism in AFI are now in progress.

Summary. Serial biochemical serum and cerebrospinal fluid (CSF) studies in 7 cases of Amaurotic Family Idiocy (AFI) disclosed some *statistically* significant variation from normal values. Two additional control cases of dissimilar degenerative neurologic illnesses of childhood were also included as control cases. In AFI an elevation in serum electrophoretic alpha-2 globulin fraction was found. Decreased values for serum total protein and albumin, together with markedly depressed gamma globulin results were observed. Serum alpha-1 and beta globulins and A/G ratio findings were normal. Chemical studies of CSF proteins showed decreased alpha and gamma globulins and gamma globulin/total protein ratio values. Serum neuraminic acid was consistently elevated in AFI, as well as in the 2 control cases, when compared to normal values established in this laboratory. Decreases in serum total, neutral and phospholipids were recorded in AFI. A decrease was also observed in serum free cholesterol values. The results in AFI differed from the 2 control cases of degenerative central nervous system disease most markedly with respect to lowered alpha and gamma globulin content of the CSF and with regard to decreased serum total lipids and phospholipids.

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Chemotherapy of Leukemia VII. Effect of Substituted Triazenes on Transplanted Mouse Leukemia.* (22273)

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During routine screening of compounds for possible antitumor effects, a member of a new series of compounds not previously known to possess chemotherapeutic activity, 3,3-dimethyl 1-phenyl triazene (Triazene I), was found by Clarke *et al.*(1) to inhibit growth of Sarcoma 180 in mice. Following this Dagg, Karnofsky *et al.*(2) demonstrated teratogenic effects on the chick embryo and inhibition of growth of Sarcoma 180 and certain human tumors implanted on the chorioallantoic membrane. An evaluation of chemotherapeutic effects of this and related compounds against various strains of transplanted leukemia in mice has been undertaken and the results are herewith reported.

Method. The general technic used for evaluating chemotherapeutic activity of a given drug by means of its ability to prolong survival time of mice with transplanted leukemia has been described previously(3). The 60th to 70th transplant generations of leukemia 82(4), which originated as a spontaneous leukemia in a C58 mouse in October 1953, were used for most of these studies. This transplantable leukemia usually kills the mice in 10 to 15 days after inoculation, with an elevation of total leukocyte count to the 50000 to 200000 level, enlargement of liver and spleen, and some lymphadenopathy. A few studies were conducted on the 10th to 14th generation of leukemia 5471, which arose as a spontaneous leukemia in a C58 mouse in July, 1954. Although morphologically these 2 leukemias are very similar, 82 has always been relatively refractory to folic acid antagonists, whereas 5471 is quite sensitive. In these experiments mice of the F1 hybrid generation of C58 male X Bagg albino female cross were each injected intraperitoneally with 0.1 cc of saline suspension of minced spleen containing one million cells per inoculum. The A-methopterin sensitive (L1210S) and A-methopterin dependent (L1210AD) variants of Leukemia L1210 obtained from Law(5,6) were also used. These leukemias were transplanted by subcutaneous injections into dba mice of minced tumor tissue suspended in isotonic saline. Treatment was initiated 24 hours after inoculation and repeated 3 times weekly for 10 doses over a 23-day period. The mice were observed for development of leukemia and autopsied at death. Triazenes that have been adequately tested and their maximum tolerated doses are listed below. Because of low solubility in water, these compounds were either dissolved in peanut oil (PO) or suspended in 0.5% sodium carboxymethylcellulose (CMC) in isotonic saline as indicated:

I.	3,3-dimethyl-1-phenyl triazene	12-25 mg/kg (PO)
II.	3,3-diethyl-1-phenyl triazene	150 mg/kg (PO)
III.	3,3-dimethyl-1-p-nitrophenyl triazene	250 mg/kg (CMC)
IV.	3,3-dimethyl-1-p-tolyl triazene	40-62.5 mg/kg (CMC)

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