complished. Thus, the frequently-voiced suggestion that it is already too late to initiate therapy when signs of disease are present in a virus-infected animal would appear to have been overcome, at least in this system.

Summary. The antiviral activity of 5-iodo-2′-deoxyuridine in treating herpes simplex keratitis in rabbits was confirmed. Another pyrimidine nucleoside, 1-β-D-arabinofuranosylcytosine hydrochloride, was found to be at least as effective as IUDR in treating this infection.

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1. Kaufman, H. E., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 251.

2. Welch, A. D., Prusoff, W. H., *Cancer Chemother. Rept.*, 1960, Issue 6, 29.

3. Evans, J. S., Musser, E. A., Mengel, G. D., Forsblad, K. R., Hunter, J. H., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 350.

4. Renis, H. E., Johnson, H. G., *Bact. Proc.*, 1962, 140.

5. Kaufman, H. E., Nesburn, A. B., Maloney, E. D., *Arch. Ophthal.*, 1962, v67, 583.

6. Chu, M. Y., Fischer, G. A., *Biochem. Pharmacol.*, 1962, v11, 423.

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Biochemical Identification of the Carrier State in Tay-Sachs' Disease.* (27885)

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Abnormally elevated levels of various glycolytic, dehydrogenating and transaminating enzymes have been previously demonstrated in sera and spinal fluids of infants with Tay-Sachs' disease (TSD)(1,2). These elevated concentrations, however, were regarded as reflections of the secondary tissue degenerations inherent to the disorder and were not considered to be an indication of any fundamental metabolic defect. Since the disease has been clearly proven to be a hereditary disorder transmitted through an abnormal autosomal recessive gene, parallel enzyme determinations were also performed upon a group of parents of the afflicted children. No analogous serum enzymatic changes were noted in the parents, although it was observed that levels of fructose-1,6-diphosphate aldolase (F-1,6-DPA) were frequently in the low range of normal. These mildly depressed values suggested the need for more specific analysis of serum aldolase and prompted the current study of a closely related enzyme, fructose-1-phosphate aldolase (F-1-PA).

Fructose-1,6-diphosphate is used as the substrate in determination of serum aldolase. It has been shown that F-1-PA may also catalyze the breakdown of this substrate, but at a diminished velocity(3,4). What is conventionally referred to as F-1,6-DPA concentration therefore is a measurement of both F-1,6-DPA and F-1-PA activity, predominantly the former(5). When fructose-1-phosphate serves as the substrate, only F-1-PA produces cleavage to trioses(5). A postulated absence of circulating F-1-PA might result in a small reduction of serum aldolase (fructose-1,6-diphosphate substrate) but would be unmasked only in a system utilizing fructose-1-phosphate. While F-1-PA is sometimes designated as "liver" aldolase, it is nevertheless present in some measure within all normal tissues and serum(6).

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TABLE I. Fructose-1-Phosphate Aldolase and Fructose-1,6-Diphosphate Aldolase Serum Concentrations.

Group	No. tested	No. with detectable serum F-1-PA	F-1-PA*†	F-1,6-DPA†
A. Tay-Sachs' disease				
1. Patients	15	0	—	15.3 ± 1.5§
2. Mothers	29	0	—	4.0 ± .8
3. Fathers	23	1	.4‡	5.7 ± .9
4. Siblings	12	8	1.0 ± .5	7.2 ± .9
5. Grandparents	19	8	1.3 ± .3	4.9 ± .3
6. Aunts, uncles	17	12	.9 ± .1	5.7 ± .4
7. Other relatives	14	11	.8 ± .1	6.1 ± .8
B. Controls				
1. Children with other neurologic disorders	12	12	1.4 ± .4	8.1 ± 1.3
2. Healthy adults	71	68	1.1 ± .1	5.5 ± .4

* F-1-PA averages represent the arithmetic mean only of those individuals in whom detectable levels were recorded; zero concentrations were not incorporated into the average.
† Sibley and Lehninger units(7,8).
‡ Only one member of this group showed a measurable level of F-1-PA.
§ Arithmetic mean and standard error.

The present report is concerned with the serum levels of aldolase as measured by these 2 substrates in 15 patients with confirmed TSD and 114 parents, siblings and other relatives of these infants. Serum specimens from 12 young children with various forms of degenerative brain disease and from 71 healthy adults were employed for control levels.

Methods. Serum aldolase (F-1,6-DPA) was determined by the method of Sibley and Lehninger(7) based upon a colorimetric determination of the produced trioses. F-1-PA was evaluated by Schapira's modification of the original Sibley and Lehninger technic, employing fructose-1-phosphate as the specific substrate(8).

Results and discussion: F-1-PA activity was absent in the sera of infants with TSD, using Schapira's procedure. In contrast, the sera of the 12 infants and young children with other neurologic disorders (including an infant with Niemann-Pick's disease) were invariably characterized by determinable levels, averaging 1.4 units (Table I).

A lack of serum F-1-PA was also apparent in 61 of the 114 clinically healthy relatives of the TSD infants (Table II). Absent F-1-PA activity was evident in but 3 of the 71 control adults.

TABLE II. Frequency of Absence of Serum Fructose-1-Phosphate Aldolase.

Group		No. tested	No. with absent serum enzyme	Frequency of absence	
				Observed	Calculated*
A. Tay-Sachs' disease	1. Patients	15	15	1.00	1.00
	2. Mothers	29	29	1.00	1.00
	3. Fathers	23	22	.96	1.00
	4. Siblings	12	4	.33	.67
	5. Grandparents	19	11	.58	.50
	6. Aunts, uncles†	17	5	.29	.50
	7. Other relatives	14	3	.21	—
B. Controls	1. Children with other neurologic disorders	12	0	0	.003–.02‡
	2. Healthy adults	71	3	.04	.003–.02

* Expected carrier frequency, assuming an autosomal, recessive gene.
† Only siblings of parents.
‡ Based upon previous genetic surveys(9,10,11).

In a rare, lethal, recessive disorder such as TSD, it is possible, in accordance with basic Mendelian laws, to calculate the anticipated carrier (heterozygote) frequency in individuals, based upon the proximity of their relationship to the affected child. Thus, both parents, two-thirds of siblings and one-half (*i.e.*, one maternal and one paternal) of the grandparents may be expected to be carriers of the particular abnormal gene. The probability of the carrier state in more remote relatives is still less.

Fifty-two parents of children with TSD were tested; in 51, F-1-PA was not detected in the sera and in the fifty-second parent, a low value (0.4 unit) was obtained. The number of pairs of parents in the present survey exceeds the number of affected infants since many parents of deceased TSD patients volunteered to participate in the study. The sera of 19 grandparents were also tested, and in 11, the enzyme was not demonstrated. Six grandparent couples were available for analysis. In 5, one grandparent showed the absence and one the presence (in normal concentration) of circulating F-1-PA. In the sixth pair of grandparents, the enzyme was absent in both members. Biochemical analysis of serum was undertaken in 4 generations of a family constellation containing a patient with confirmed TSD. F-1-PA was absent in the sera of the patient, both parents, one maternal grandparent and one paternal grandparent and in one great grandmother (the only one tested at this level).

Among the 12 otherwise healthy siblings of patients with TSD tested, 4 exhibited an absence of serum F-1-PA. Data relative to aunts, uncles, cousins and more distant relatives are summarized in Table II. It was interesting that in this study there is no example of absence of circulating F-1-PA unless there was a similar absence in the parents.

The frequency of the recessive gene for TSD in various ethnic groups has been approximated previously by various statistical procedures, these estimates commonly based upon an extrapolation derived from incidence of the disorder in extensively analyzed populations(9,10,11). The disease exhibits a characteristic clinical presentation (*viz.*, commencement during the first year of life, hyperacusis, arrest of development, amaurosis with macular degeneration, progressive paralysis, and death generally before the age of 3 years) permitting proper identification in the vast majority of cases. All 15 children in the present series fulfilled these diagnostic criteria. The carrier frequency among Jews derived from eastern Europe (the ancestry of most cases of TSD) has been estimated to be between 0.022 and 0.035 (*i.e.*, one out of every 29-45 in this ethnic population). Among the non-Jewish population of the United States, the carrier frequency has been estimated to be 0.003 (*i.e.*, one out of every 333). It was legitimate, therefore, to inquire into the religious and geographic background of the individuals submitted to this procedure. All of the present patients with TSD and their relatives were Jewish. In the control group of 12 children with diverse but unrelated neurologic diseases and the 71 healthy adults, 36 were of Jewish ancestry and within this group, F-1-PA was undetected in the sera of 2 (a frequency of 0.06). This group, parenthetically, contained 4 parents of children with Niemann-Pick's disease, a similar but genetically distinct sphingolipidosis. Normal concentrations of F-1-PA were demonstrated in all 4. In the remaining 47 non-Jewish control individuals, the serum of one adult male demonstrated absence of this enzyme (frequency of 0.02).

When F-1-PA was present in the serum of TSD relatives or in control patients, no notable group differences in concentration were detected. Serum levels of F-1,6-DPA were also determined (Table I). Moderate elevations were present in the TSD children, in conformity with prior observations. The mothers of the affected patients showed slightly but not significantly lowered serum concentrations of this enzyme.

Comment. An absence of fructose-1-phosphate aldolase has been noted in the sera of children afflicted with Tay-Sachs' disease. A similar absence was demonstrated in the sera of 52 of 53 parents and in an appreciable

fraction of other relatives of children with this disease. That the incidence of this biochemical change diminishes in proportion to the remoteness of relationship to the affected child in approximate accordance with Mendelian ratios, suggests that this test may serve as a biological tool in identification of the carrier state. Interpretation of these findings in terms of the pathogenesis of TSD awaits clarification of the metabolic role of the various aldolases.

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1. Aronson, S. M., Saifer, A., Kanof, A., Volk, B. W., *Am. J. Med.,* 1958, v24, 390.

2. Aronson, S. M., Saifer, A., Perle, G., Volk, B. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 331.

3. Hers, H., Jacques, P., *Arch. Int. Physiol.,* 1953, v61, 260.

4. Schapira, F., Dreyfus, J.-C, Schapira, G., *Compt. rend. acad. Sci.,* 1957, v245, 808.

5. Leuthardt, F., Wolf, H. P., in *Methods in Enzymology,* S. P. Colowick, and N. O. Kaplan, Ed., Academic Press, 1955, v1, 320.

6. Schapira, F., *Bull. Soc. Chim. Biol.,* 1961, v43, 1367.

7. Sibley, J., Lehninger, A., *J. Biol. Chem.,* 1949, v177, 859.

8. Schapira, F., *Rev. franc. d'études clin. et biol.,* 1960, v5, 500.

9. Kozinn, P. J., Weiner, J., Cohen, P., *J. Pediat.,* 1957, v51, 58.

10. Myrianthopoulos, N. C., in *Cerebral Sphingolipidoses: A Symposium on Tay-Sachs' Disease and Allied Disorders,* S. M. Aronson and B. W. Volk, Ed., Academic Press, 1962, p359.

11. Aronson, S. M., Volk, B. W., *ibid.,* Academic Press, 1962, p375.

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Potentiation of Striated Muscle Contraction by Piperidylmethylandrostane.* (27886)

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Several compounds have been described which potentiate the indirectly induced twitch response of skeletal muscle. Among such compounds are some of the adrenergic amines (1), certain phenolic quarternary ammonium ions(2), physostigmine(3) and certain organic phosphorous anticholinesterase agents. Recently this laboratory reported on the potentiation and blockade of indirecly induced twitch and tetanus in the intact (rabbit) and isolated (rat) phrenic nerve diaphragm preparation as produced by the steroid, 17 (2 piperidylmethyl) 3β, 17β, androstane diol (PMA)(4). Doses of PMA which produced potentiation of the indirectly induced twitch response prolonged carbachol induced neuromuscular blockade, did not reverse curare induced neuromuscular blockade, and did not influence serum esterase activity. This report is concerned with some effects of PMA on the surface and transmembrane potentials of skeletal muscle of the rabbit.

Methods. The intact anterior tibial muscle preparation of the rabbit was used throughout the study. Mature stock rabbits were lightly anesthetized with sodium pentobarbital (30 mg/kg, IV). Bipolar, platinum, shielded electrodes were placed in the mid portion of the anterior tibial branch of the right sciatic nerve. The entire sciatic nerve was then transected proximal to the electrodes. The tendon of the right anterior tibial muscle was tied with thread, cut near its insertion, and the thread was attached to a force displacement transducer for measurement of isometric contractions. Steel drill pins were inserted into the distal ends of the

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