PSEUDOCHOLINESTERASES

II. Two Cases of Pseudocholinesterase Deficiency

Alpaslan M. Karahasanoğlu, M.D.,* and Pınar Özand,

Pseudocholinesterase enzymes comprise a group of glycoproteins that constitute a very small per cent (about 0.01 per cent) of the total plasma proteins.[1] The physiologic importance of this enzyme is still unknown. However, it is known that pseudocholinesterase is responsible for the hydrolysis of the cholinesters circulating in plasma. For example, it hydrolyzes the bulk of the injected succinyldicholine administered during an operation or shock treatment before the drug reaches the motor end-plate in normal subjects.[2] If the plasma cholinesterase is deficient or defective in its function, the injected drug leads to a markedly prolonged paralysis, hence the resultant apnea may be lethal.[2] This condition can only be relieved by giving the patient fresh plasma. At times, even this therapeutic measure may prove to be fruitless because a late dual block settles, and the patient may require a long period of resuscitation.[2]

These observations led to an extensive study of the genetics and nature of the enzyme. It is now known that the enzyme can be either completely lacking or defective as a result of inborn errors of metabolism. The formation of the normal enzyme is controlled by a set of allelic genes: E_1^u is the gene that produces the normal protein unit. The synthesis of the normal unit is controlled by a «silent» gene, E_1^s. This locus is responsible for the regulation of the rate of synthesis.

There seem to be two defective variants of the enzyme, each characterized by an entirely different kinetic behavior. These variants are expressed as E_1^a and E_1^f. These abnormal enzymes have a decreased affinity for various cholinesters and for the usual inhibitors of the normal enzyme. The E_1^a variant produces an enzyme that is insensitive to dibucaine, decamethonium, quinidine, etc., and to normal substrates, whereas the E_1^f variant synthesizes an enzyme

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From Ankara University Hacettepe Medical Center Department of Biochemistry.
* Research Assistant in Biochemistry.
** Head, Department of Biochemistry.
Requests for reprints should be sent to Dr. Pınar Özand.

that is fluoride insensitive. The detection of these variants depends on the detection of this decreased susceptibility to the inhibitors. In a heterozygotic defective individual, Karahasanoğlu and Özand made use of the decreased affinity of the abnormal enzyme for butyrylthiocholine as a screening procedure.

Excellent reviews have appeared on the defective enzyme and the interested reader is referred to these.[2-4]

Pseudocholinesterase is only rarely completely missing from the plasma. Four such cases of pseudocholinesterase anenzymia have so far been reported.[1,5,6] Clinically, these patients had trouble only after the injection of succinyldicholine, manifested in a very prolonged apnea. That these patients had no enzyme in their plasma was shown by the greatly decreased hydrolytic activity with benzoylcholine as substrate. Furthermore, immunochemical tests indicated no altered enzyme protein in the blood, and tests on liver biopsy specimens demonstrated no such activity in that organ. Presumably, the disease is caused by the E_1^s «silent» gene.[5]

In this article, we would like to report our preliminary investigations in two cases of pseudocholinesterase anenzymia. Their sera showed only a trace of cholinesterase activity that could not be considered as pseudocholinesterase biochemically. We will present the evidence that this trace of activity was in fact caused by erythrocyte acetylcholinesterase contaminating the assay system. Detailed report of the immunochemical work and enzyme half life studies on one of these patients is still in progress and will be published in the future.

Materials and Methods

Cholinesterase activity was measured by a modification of the Ellman technique.[7,8] Spot test was carried out as described elsewhere.[9,10] Hestrin's method was used for certain control experiments.[11] Initial enzyme rates were taken as the basic unit, as described in the text.

The chemicals used were of C. P. grade and obtained on the market. Acetylcholine, propionylcholine, butyrylcholine, myristoylcholine, benzoylcholine and succinyldicholine were obtained from Mann Research Laboratories, New York City, New York, U.S.A. Acetylthiocholine and butyrylthiocholine iodides were purchased from the Sigma Chemical Company, St. Louis, Missouri, U.S.A. Standard preparations of bovine erythrocyte acetylcholinesterase and of pooled human plasma pseudocholinesterase were used without

further column purification, and were obtained from the Sigma Chemical Company. Dithiobisnitrobenzoic acid was purchased from the Aldrich Chemical Company, Milwaukee, Wisconsin, U.S.A. A Zeiss PQ spectrophotometer with a recorder and tempering cell holder was used for all enzyme initial rate measurements.

Case Reports

Case 1 : T. O., a 24 year old male medical technology student, was admitted to Hacettepe Medical Center with a tumor of the right scapula and radiating pain caused by irritation of the fifth and sixth cervical dorsal roots. The history revealed a sudden enlargement of a small tumor within the four months prior to admission. Past history and family history were not relevant. Physical findings were normal except for the presence of a firm, 10 by 10 cm tumor on the right scapula.

Laboratory examination : White blood cells, red blood cells and platelets were of normal number and morphology. Serum proteins and their electrophoresis, cephalin cholesterol flocculation and thymol turbidity were all normal. Sia test was negative, Coombs test and erythrocyte fragility were normal. The results of bone marrow examination were also normal. The patient was taken to surgery for biopsy under general anesthesia and was given a single dose of succinyldicholine. Apnea developed immediately with complete paralysis. The patient was given a unit of fresh plasma in view of the possibility of an abnormal pseudocholinesterase. The apnea lasted for two and a half hours and then muscle function and spontaneous respiration gradually resumed. Samples of the patient's serum were obtained one month after recovery and on four other occasions the following year.

Although blood samples could not be obtained from other members of the patient's family, the family history indicated no abnormality of pseudocholinesterases. The pathology report on the biopsy specimen indicated the tumor was a hemangioma.

Case 2 : B. O., a 17 year old white male, was admitted to Hacettepe Medical Center with the chief complaint of hemiparesis which had appeared 45 days prior to admission. Past history and family history were not relevant. Physical examination revealed atrophy of the thigh muscles and hypoesthesia of both legs. X-ray examination indicated a herniated disc between the third and fourth lumbar vertebrae, and myelograms showed a block below the third lumbar vertebra. White and red blood cells, NPN, and total serum protein were all normal. A laminectomy was performed under general anesthesia and one dose of succinyldicholine was injected. The patient developed apnea which continued for four hours postoperatively and resuscitative measures were taken. No plasma transfusion was given and after four hours, the patient began to breathe spontaneously. Serum samples were obtained 48 hours after surgery.

Biochemical Investigations

In both of these cases, plasma pseudocholinesterase activity was measured with acetylthiocholine and butyrylthiocholine as substra-

tes. The results obtained with acetylthiocholine are presented in Table 1. Neither patient's sera hydrolyzed butyrylthiocholine, and the very light hydrolysis of acetylthiocholine was not caused by plasma cholinesterase activity. The following experiments were designed to prove this point further.

TABLE 1

ACTIVITY OF NORMAL SERA WITH ACETYLTHIOCHOLINE AS PSEUDOCHOLINESTERASE SUBSTRATE

Plasma (obtained with heparin) pseudocholinesterase activity is measured at 30 C in a tempering cell of a Zeiss PQ spectrophotometer. The activities of plasma of normal subjects and the patients are calculated as units from initial rates, and are expressed as milliMoles of acetylthiocholine hydrolyzed per hour per liter of plasma.

The acetylthiocholine was used at 1 mM concentration; and the rate (unit) was calculated within 1.0 per cent hydrolysis of the substrate.

Subject	v (unit)
Normals (8)	145 ± 11
T. O. patient	2.0
B. O. patient	1.0

Plasma pseudocholinesterase is more specific to cholinesters with increased lypophilic side chains.[2] It would, therefore, be expected that the plasma which contains the pseudocholinesterase would react more readily with butyrylthiocholine than with acetylthiocholine, even if the pseudocholinesterase were abnormal.[2] This point is clearly proved by the experiments summarized in Table 2. Both pooled and purified plasma cholinesterase and normal sera react better with butyrylthiocholine than with acetylthiocholine.

Tests performed on blood samples obtained at different times from one of our patients (T. O.), indicated that the very slight activity detected with acetylthiocholine varied from one sample to another. This observation, together with the reversal of the ratio of the rates found for butyrylthiocholine/acetylthiocholine, suggest that the slight activity detected in sera was not a true pseudocholinesterase activity, but was of erythrocytic origin. To test this idea further, the following experiments were done.

Neither of the patients' sera showed any esterolytic activity with the following esters: benzoylcholine, succinyldicholine, propionylcholine, butyrylcholine and myristoylcholine. Esterolytic activity was tested by Hestrin's method.[2] The patients' sera reacted only with

TABLE 2

Comparison of activities of pseudocholinesterase of various sera with acetylthiocholine and butyrylthiocholine. Rates reported as units were calculated at room temperature, 23 C. Definition of units is same as that of Table 1.

Subject	I units with acetylthiocholine 1mM	II units with butyrylthiocholine 1mM	ratio II/I
Normal (18)	92 ± 6	140 ± 8	1.52
Purified plasma pseudocholinesterase prepared from pooled human plasma (unit milimole substrate hydrolyzed by mgm enzyme per hour).	0.31	0.61	1.96
T. O.	2.6	0	0
B. Ö.	0.3	0	0

acetylcholine and then only very slightly. Pseudocholinesterase reacts readily with the former group of substrates, whereas erythrocyte acetylcholinesterase reacts preferentially with acetylcholine.[2] Hence our test results support the idea that the slight activity detected in the serum of our patients originates in erythrocytic contamination.

Pseudocholinesterase is inhibited by quinidine, dibucaine, decamethonium, etc., at low concentrations of these substances. At much higher concentrations, these compounds are inhibitors of erythrocyte acetylcholinesterase. The 50 per cent cholinesterase inhibiting concentrations of these drugs are even used as criteria in describing the esterases. When the enzyme is defective (either heterozygote or homozygote), its sensitivity to these chemicals also decreases considerably.[2] Quinidine was used in the experiments reported below, as this substance at concentrations that will inhibit normal or even defective pseudocholinesterase has no effect on erythrocyte cholinesterase. Extremely high concentrations of quinidine will slightly inhibit erythrocyte acetylcholinesterase activity, when both normal and defective pseudocholinesterase activity is completely inhibited. Both of our patients' sera were about 1000 times less sensitive to quinidine than normal pseudocholinesterase and was very close to the sensitivity of erythrocyte acetylcholinesterase.[12]

Erythrocyte acetylcholinesterase is selectively inhibited by caffeine, at concentrations which have little or no inhibitory effect on plasma pseudocholinesterase. Some of the caffeine-inhibition experiments are given in Table 3. The inhibition of esterase activity observed in the sera of both of our patients parallels the inhibition observed with their erythrocytes, which was in the range observed for that of normal human and bovine erythrocytes. This finding supports the view that the trace activity observed originated from the patients' erythrocytes.

TABLE 3

CAFFEINE INHIBITION OF PLASMA PSEUDOCHOLINESTERASE OF NORMALS AND THE PATIENTS

Acetylthiocholine was used at 1 mM, caffeine at 5 mM concentration. Experiments were carried on at 25 C°.

Subject	Percent inhibition by caffeine
Pseudocholinesterase from pooled human plasma	1 %
T. O. plasma	67 %
T. O. erythrocytes	67 %
B. Ö. plasma	72 %
B. Ö. erythrocytes	75 %
Normal erythrocye (10)	81.5 $\pm$ 2.8 %
Bovine erythrocyte stroma *	94 %
Purified bovine erythrocyte acetylcholinesterase*	90 %

* In these experiments the assay was carried on at 23 C with 0.05 mM acetylthiocholine and 2 mM caffeine.

Samples of the patients' sera were tested for kinetic behavior of their esterolytic activity towards acetylthiocholine. Both of the patients' sera showed the same substrate inhibition at increasing substrate concentrations (Figure 1). The same type of kinetic curve was observed for normal human erythrocytes and for bovine erythrocytes. An entirely different kinetic curve is found for normal pseudocholinesterase (curve «p» in Figure 1). Defective pseudocholinesterase has a curve similar to the curve «p» in Figure 1.[12]

The data suggest that the trace of activity observed in the plasma of these two patients came from a slight and visibly hard to detect trace of a hemolytic process during the collection of blood. In fact, the rates of hydrolysis per volume for acetylthiocholine are much higher for human erythrocytes than for plasma, as indicated

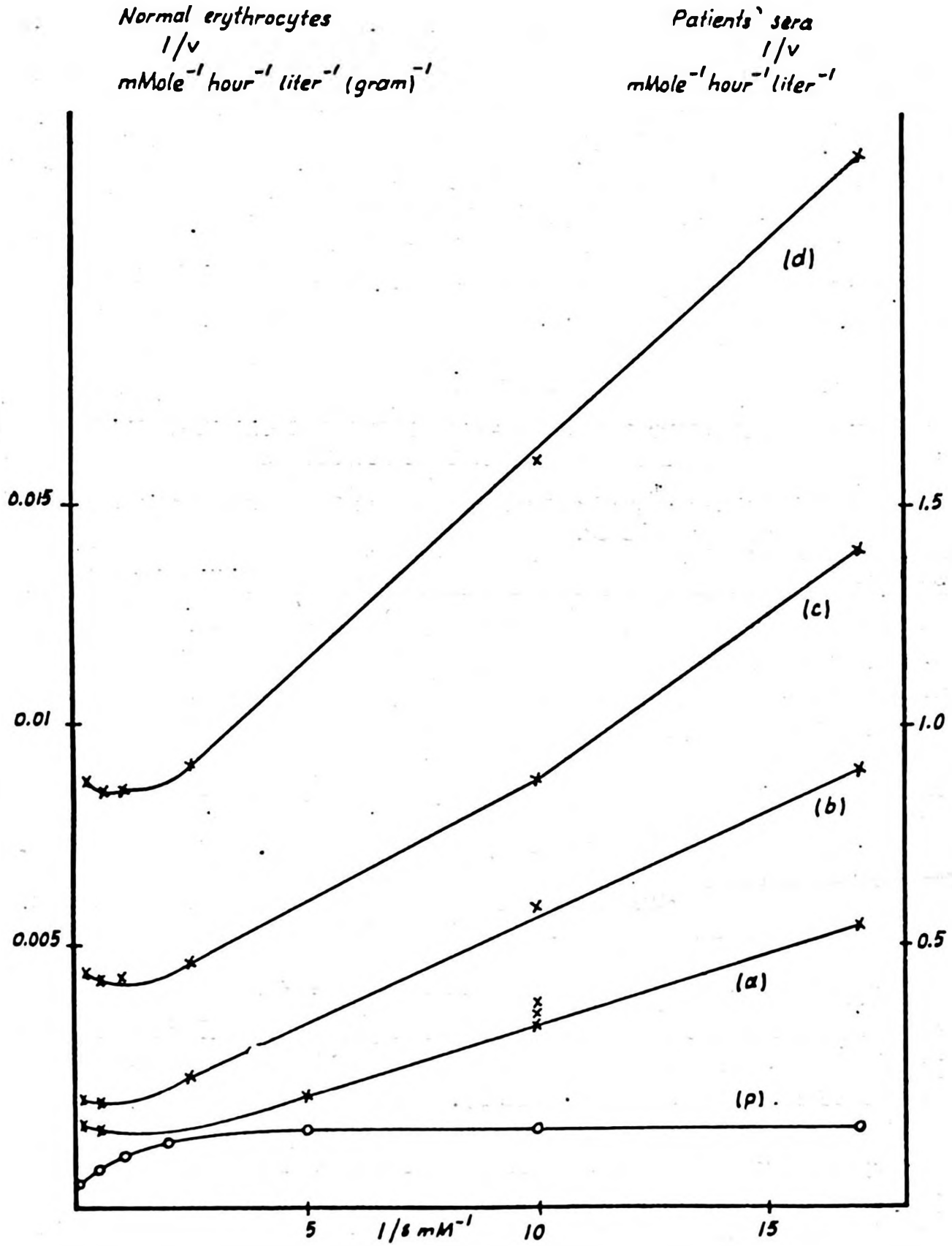

Figure 1. The Lineweaver Burke plots of esterase activity at 23 C. The rates are expressed as : milimoles of acetylthiocholine hydrolyzed per hour per liter of erythrocyte for human erythrocytes, and per gram of enzyme for purified bovine erythrocyte enzyme; milimole of acetylthiocholine hydrolyzed per hour per liter of plasma for plasma activity, or per miligram of purified pseudocholinesterase from pooled human plasma. (a) Bovine erythrocyte enzyme. (b) Average of 5 normal human erythrocyte hemolyzate. (c) T. O. plasma. (d) B. Ö. plasma. (p) Purified pseudocholinesterase from normal human plasma.

in Table 4. On a per volume basis, erythrocytes are about five times more active than plasma, and this characteristic could easily account for the trace activity detected with acetylthiocholine in the plasma of our patients, and also indicates how dangerous it is to use acetylthiocholine for a screening test.

TABLE 4

COMPARISON OF ACTIVITIES OF HUMAN ERYTHROCYTES AND HUMAN PLASMA WITH ACETYLTHIOCHOLINE

The units are expressed as milliMoles of acetylthiocholine hydrolyzed per liter of plasma (or erythrocyte) per hour at 23 C and 1 mM acetylthiocholine concentration.

Human erythrocytes (10)	485 $\pm$ 42
Human plasma (17)	92 $\pm$ 6

Spot tests of our patients' sera using butyrylthiocholine as a substrate gave a result only after more than 13 hours; well beyond the upper limit of four minutes for the normal population, and the same as that of spontaneous hydrolysis of the substrate.

That neither of the patients' sera had any inhibitors was confirmed by adding the patients' plasma to normal plasma and pooled plasma cholinesterase (Table 5).

TABLE 5

STUDIES ON THE PRESENCE OF INHIBITORS IN PATIENTS' PLASMA

Experiments were conducted at 37 C and with acetylthiocholine at 1 mM concentration. Experiments were run in duplicate, results are expressed as the mean value.

Subject	Disease	Unit
T. O.	Absence of enzyme	1.5
B. Ö.	Absence of enzyme	0.3
C. A.	Normal	163
C. A. + T. O.	Plasma mixed 1/1	165
C. A. + B. Ö.	Plasma mixed 1/1	155
Purified plasma cholinesterase+T. O.	Mixture	No change in activity

Discussion

The two cases reported here most probably represent the pseudocholinesterase anenzymia variety of inborn errors of metabolism. Immunochemical studies could prove this point beyond dispute, but it can definitely be stated that neither of the patients had cholinesterase activity in his plasma. The trace activity detected originated from the *in vitro* or *in vivo* hemolysis of the erythrocytes. Hence, we strongly advocate the use of butyrylthiocholine in lieu of acetylthiocholine for plasma pseudocholinesterase assessments and screening tests.

Neither of the patients have inhibitors in their sera that would account for the absence of pseudocholinesterase activity. We do not know at present whether the sera contained an altered protein that has no enzyme activity but retains its antigenic determinants, but we are testing the sera of one our patients (T. O.) to clarify this point.

A second point worth investigating is the half life of injected pseudocholinesterase of our patients. This could shed more light on the metabolism of the enzyme.

Summary

Two cases of pseudocholinesterase anenzymia are reported and the biochemical investigations on sera obtained from the two patients are discussed.

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REFERENCES

1. Hodgkin, W. E., Giblett, E. R., Levine, H., Bauer, W., and Motulsky, A. G.: Complete pseudocholinesterase deficiency : genetic and immunologic characterization, J. Clin. Invest. 44 : 486, 1965.
2. Kalow, W.: Heredity and the Response to Drugs, London, W. B. Saunders Co., 1962, pp. 69 - 93.
3. Kalow, W.: Cholinesterase types in biochemistry of human genetics, Ciba Foundation Symposium, edited by Wolstenholme, G. E., and Connor, C. M. 1959, pp. 39 - 59.
4. Harris, H., Hopkinson, D. A., Robson, E., and Whittaker, M.: Genetic studies on a new variant of serum cholinesterase detected by paper electrophoresis, Ann. Hum. Genet. 26 : 359, 1963.

5. Liddell, J., Lehman, H., and Silk, E.: A silent pseudocholinesterase gene, Nature (London) 193 : 561, 1962.
6. Doenicke, A. T., Guiter, G., Krenzberg, I., Demes, W., Speiss, W., and Steinbreitner, K.: Serum cholinesterase anenzymia : report of a case confirmed by enzyme-histochemical examination of liver biopsy specimen, Acta anaesth. Scand. 7 : 59. 1963.
7. Karahasanoğlu, A., and Özand, P.: Manuscript in preparation.
8. Ellman, G. C., Courtney, K. P., Andres, V. Jr., and Featherstone, R. M.: A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 : 88, 1961.
9. Karahasanoğlu, A., and Özand, P.: Manuscript in preparation.
10. Karahasanoğlu, A., and Özand, P.: Pseudocholinesterases I. Preliminary report on a simple spot test for plasma pseudocholinesterase activity, Turkish J. Pediat. 8 : 1, 1966.
11. Hestrin, S.: The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application, J. Biol. Chem. 18 : 249, 1949.
12. Özand, P.: Unpublished observations.
13. Karahasanoğlu, A., and Özand, P.: Manuscript in preparation.