Isozymes of Amine Oxidase in Human Plasma and Other Tissues

The enzyme plasma amine oxidase (PAO) uses benzylamine readily as a substrate[1], is apparently distinct from monamine oxidase of mitochondrial origin[1–3] and has been identified as existing in the plasma of many mammalian species, including humans[4–7]. PAO has been reported to be elevated in human serum in several diseases[8,9] including certain cirrhoses, massive neoplastic replacement of the liver and diabetes mellitus. Because of this, and our interest in amine oxidation, we have investigated the electrophoretic properties of this enzyme to determine if it exists in multiple molecular forms (isozymes) and if so what might be the relationship between these different forms. We examined 41 samples of human plasma by starch gel electrophoresis and have found the total plasma amine oxidase activity to consist of several different fractions. In addition, human tissue samples were studied and found to contain this enzyme activity but electrophoresis reveals somewhat different isozyme patterns from that found in the plasma.

Materials and methods. Plasma samples were obtained from apparently healthy employees of the Bronx Municipal Hospital Center who were referred to the Blood Bank for routine physical exams such as pre-employment exams. Human tissue samples were obtained from autopsy of victims who died of accidental causes. Three different cadavers were examined. Tissue homogenates were made in 0.2*M* phosphate buffer, pH 7.2 (1 g/ml). Starch gel electrophoresis was carried out with a Buchler vertical electrophoresis apparatus using primarily a Borate-NaOH buffer similar to SMITHIES'[10] (0.55*M* boric acid-0.22*M* NaOH for the gel and 0.3*M* boric acid-0.125*M* NaOH for the electrode chambers). Gels were run at 4 °C at 250 V for 16 h. Since the oxidative deamination of benzylamine to benzaldehyde is characteristic of PAO[1] and since such aldehydes can reduce tetrazolium salts to

[1] C. W. TABOR, H. TABOR and S. M. ROSENTHAL, J. biol. Chem. *208*, 645 (1954).
[2] D. S. ROBINSON, W. LOVENBERG, H. KEISER and A. SJOERDSMA, Biochem. Pharmac. *17*, 109 (1968).
[3] C. M. MCEVEN JR., G. SASAKI and W. R. LENZ JR., J. biol. Chem. *243*, 5217 (1968).
[4] J. G. HIRSCH, J. exp. Med. *97*, 345 (1953).
[5] F. BUFFONI and H. BLASCHKO, Proc. R. Soc. B *161*, 153 (1964).
[6] C. M. MCEWEN JR., K. T. CULLEN and A. J. SOBER, J. biol. Chem. *241*, 4544 (1966).
[7] C. M. MCEWEN JR. and J. D. COHEN, J. Lab. clin. Med. *62*, 766 (1963).
[8] C. M. MCEWEN JR. and D. O. CASTELL, J. Lab. clin. Med. *70*, 36 (1967).
[9] N. TRYDING, S. E. NILSSON, G. TUFVESSON, R. BERG, S. CARLSTROM, B. ELMFORS and J. E. NILSSON, Scand. J. clin. Lab. Inves. *23*, 79 (1969).
[10] O. SMITHIES, Biochem. J. *61*, 629 (1955).

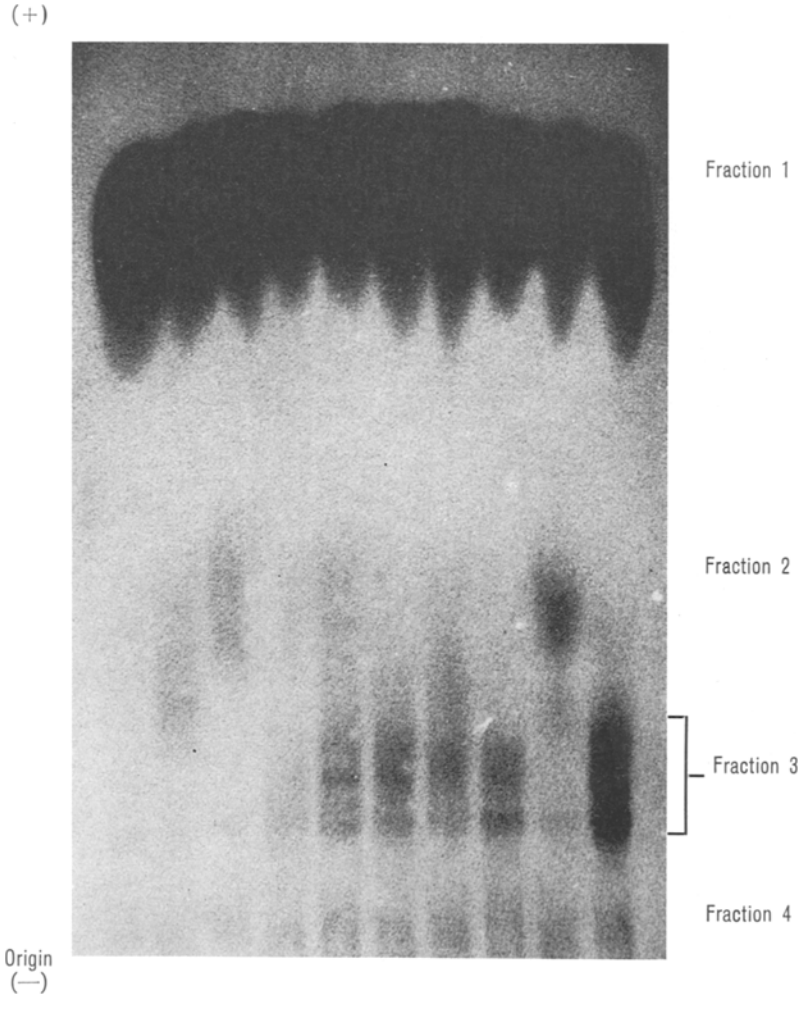

Fig. 1. Starch gel electrophoresis of 10 different samples of human plasma stained for plasma amine oxidase (PAO) activity. 4 areas of enzyme activity can be seen.

formazan dyes[11], PAO activity could be detected on the starch gel by incubating the sliced gel at 37 °C in a solution of the following composition: benzylamine, 8 g; nitro blue tetrazolium 1.0 mg/ml, 18 ml; phosphate buffer 0.1 *M* pH 7, 150 ml.

Molecular weight studies were performed with a Sephadex column (2.5 cm diameter) containing Sephadex G-200 with the following proteins as molecular weight standards: cytochrome c, myoglobin, ovalbumin and human gamma globulin. Their elution patterns were determined by their absorption peaks[12]. PAO activity was indicated by staining on a starch gel in order to detect the individual isozyme components. All proteins were dialyzed against, and eluted with, 0.1 *M* NaCl in 0.01 *M* phosphate buffer, pH 7.

Results and discussion. Electrophoresis of human plasma reveals four areas of enzyme activity (Figure 1). The most rapidly migrating anodal band (fraction 1) is the most uniform, showing up with similar mobility and intensity in every sample examined. It is also the most active enzyme fraction. Following that band is a region that variably exhibited enzyme activity (fraction 2). It was present in about 40% of these samples. A set of 3 bands follows (fraction 3) which vary widely in the relative intensities of the bands in different samples. Closest to the origin is a lighter staining, more difuse area of enzyme activity (fraction 4). This general pattern of enzyme activity was seen with many different gel buffers (*Tris*-citrate, phosphate, phosphate-citrate, glycine-NaOH, barbital, EDTA-borate-*tris*) but none gave as clear a resolution as the borate-NaOH used in this study.

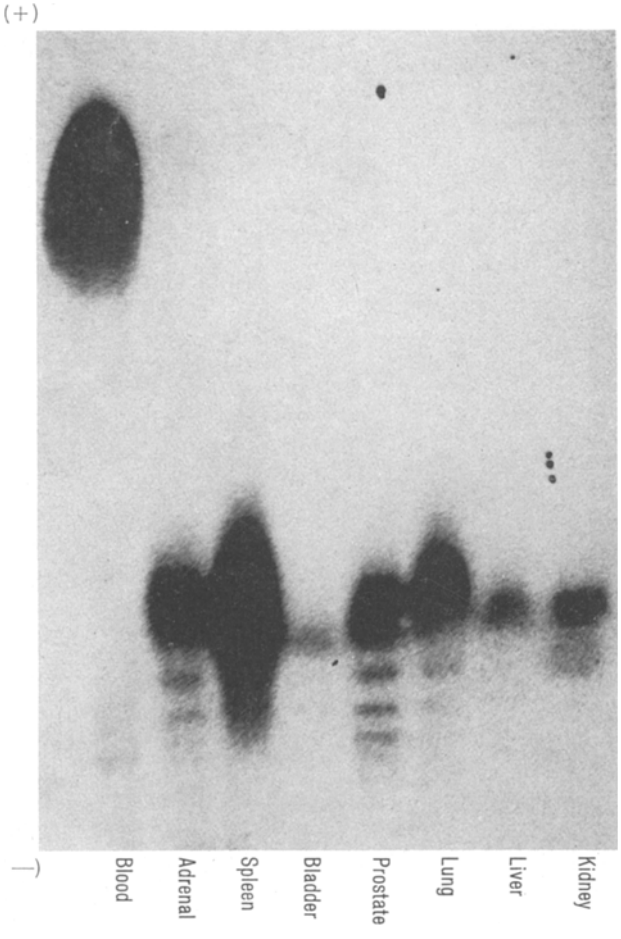

Fig. 2. Electrophoresis of human tissue extracts stained for PAO activity. The first sample, blood, was placed on the gel as a reference for the tissue isozymes. Tissues were obtained from autopsy and all were from the same individual.

Chromatography on the Sephadex column indicated that the different isozymes were of different molecular weights, since they eluted in different fractions. The most anodal isozyme was the smallest, eluting near ovalbumin, thus having a molecular weight of about 4.5×10^4. Enzyme activity corresponding to fraction 2 eluted earlier than fraction 1 and was therefore of a bigger molecular size, in the area of 2×10^5. The set of three bands (fraction 3) eluted at or near the peak of blue dextran which was used to determine the void volume of the column. Sephadex G-200 has an exclusion limit of around 10^6 (Ref. 12). Consequently these bands appear to be much bigger molecules than the more rapidly migrating isozymes. They could represent aggregates of the smaller isozymes, as bovine PAO has been shown to undergo reversible aggregation at pH 7[13], or may represent completely different proteins.

The origin of this amine oxidase in the plasma is not known[7,14]. We thus looked at the isozyme patterns of different human tissues to see if any might be similar to that of the blood and therefore suggest that the blood isozymes were being synthesized there. Results of one such electrophoresis are seen in Figure 2. The first gel channel contains a plasma sample for comparison and the following organs are illustrated: adrenal, spleen, bladder, prostate, lung, liver, kidney. Although not illustrated, we have in addition looked at these samples: pancreas, brain, heart (ventricle), and aorta. No tissues contained the fast migrating anodal band present in blood (fraction 1) except that pancreas did electrophorese as a smeared band with a small concentration of activity which had a similar mobility to this fraction. Brain had very low activity, and in fact was difficult to detect on the gel. Heart had a similar pattern to kidney with one additional slower moving band. Aorta had low activity on the gel and contained only one band, which migrated a little slower than the major band seen in heart, kidney and liver. Thus, with the possible exception of pancreas, the major component of the plasma enzyme appears to be unique to the plasma. On the basis of electrophoretic mobility, fraction 3 in the plasma may be similar to the set of more slowly migrating isozymes which are seen in the organs illustrated in Figure 2. It has been suggested that connective tissue might be the source of the plsma enzyme due to similar properties of the enzyme in the plasma and the aorta[14]. But the isozyme pattern of the aorta was not any closer to that of the plasma than the other organs studied and on this basis seems no more likely than these tissues to be the source of this enzyme.

It is clear from the diverse mobilities of the enzymes in the tissues studied that different enzymes may exist in different tissues which are capable of converting benzylamine to benzaldehyde, are readily soluble unlike monoamine oxidase present in mitochondria[15,16], do not utilize

[11] T. BARKA and P. J. ANDERSON, *Histochemistry* (Harper and Row, N.Y. 1963), p. 324.
[12] P. ANDREWS, Biochem. J. *96*, 595 (1965).
[13] F. M. ACHEE, C. H. CHERVENKA, R. A. SMITH and K. T. YASUNOBU, Biochemistry, N.Y. *7*, 4329 (1968).
[14] R. B. RUCKER and B. L. O'DELL, Biochim. biophys. Acta *235*, 32 (1971).
[15] P. BAUDHUIN, H. BEAUFAY, Y. RAHMAN-LI, O. Z. SELLINGER, R. WATTIAUX, P. JACQUES and C. DE DUVE, Biochem. J. *92*, 179 (1964).
[16] M. B. H. YOUDIM and T. L. SOURKES, Can. J. Biochem. Physiol. *44*, 1397 (1966).

tryptamine well, (as gels did not stain when tryptamine was substituted for benzylamine) and would thus appear to function more like the amine oxidase previously described for plasma. The relationship between these different tissue enzymes and the blood enzyme, as well as the role of the enzymes in the overall celular economy is an intringuing question[17].

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Zusammenfassung. Menschliche Plasmaaminooxydase, bestehend aus verschiedenen Isoenzymen, deren Hauptfraktion in zahl reichen untersuchten parenchymatösen Organen vorhanden ist, wurde mit der Stärkegelektrophorese und Sephadex Säulenchromatographie untersucht. Isoenzyme aus Gewebehomogenaten und Plasma unterscheiden sich.

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Phagocytosis of Latex Particles in vitro: Effects of Antilymphocyte and Antithymocyte Serum

Several investigators have considered the possibility that impairment of phagocytosis contributes to the immunosuppressive capacity of antilymphocyte serum[1-3]. The primary site of action and the mechanism of phagocytic depression is not yet clear, however. Preferential impairment of splenic dendritic macrophages was found in ALS-treated mice by Barth et al.[4], while selective impairment of hepatic phagocytosis was described by Pisano et al.[5]. The finding that ALS-sensitized lymphocytes localized predominantly in the liver[6], raised the possibility that an immunoglobulin component is present in ALS which promotes phagocytosis by the Kupffer cells[7].

The present communication reports the results obtained when monolayers of mouse macrophages were incubated with ALS or ATS in vitro and their phagocytic activity was measured at different time intervals.

Materials and Methods. Horse antimouse thymocyte serum (ATS) was obtained from the Institute of Microbiology and Hygiene of the University of Montreal, through the courtesy of Dr. H. E. Taylor, Medical Research Council of Canada. Rabbit anti-mouse lymphocyte serum (ALS) was purchased from Microbiological Associates, Bethesda, Md. Peritoneal cells were obtained

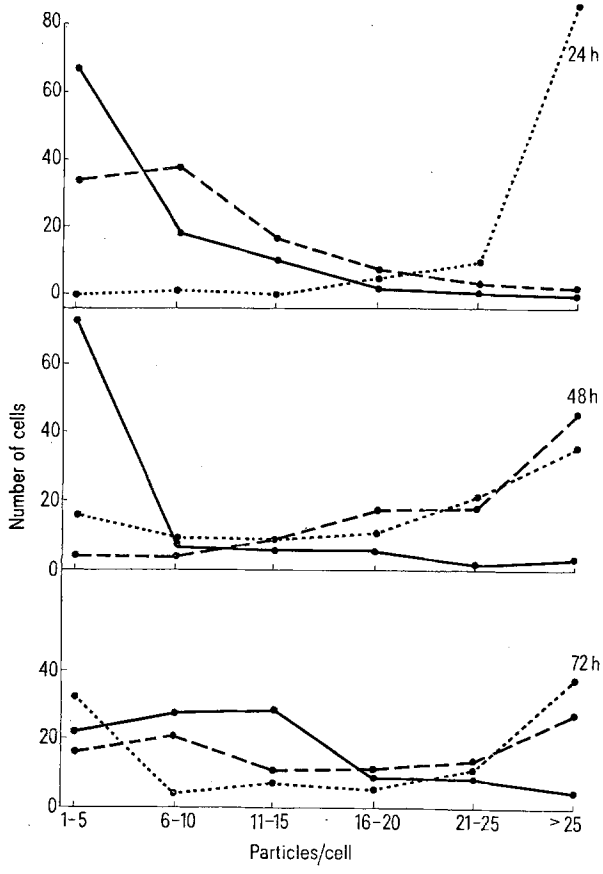

Fig. 1. Particle distribution within peritoneal macrophages after 1, 2 and 3 days in culture. Solid line: 40% ATS in both the culture medium and test medium; broken line: 40% ATS followed by Latex in 40% horse serum; dotted line: control.

Table I. Effect of ALS on the phagocytic activity of macrophages in vitro

Incubation time	Peritoneal macrophages Percent ALS			Spleen macrophages Percent ALS		
	4	20	40	4	20	40
30 min	97	92	70	92	94	69
2 h	79	65	—	76	79	—
48 h	84	99	75	84	88	69
72 h	—	—	95	—	—	98

Results are expressed as percent control values. Control cultures were maintained in medium supplemented with 40% inactivated newborn calf serum.

[1] J. N. Sheagren, R. F. Barth, J. B. Edelin and R. A. Malmgren, J. Immun. *105*, 634 (1970).
[2] N. S. Harris, G. E. Merino and J. S. Najarian, Transpl. Proc. *3*, 797 (1971).
[3] J. C. Pisano, J. T. Patterson and N. R. Diluzio, in *The RES and Immune Phenomena* (Ed. N. R. Diluzio and K. Fleming; Plenum Press, New York 1971), p. 237.
[4] R. F. Barth, R. L. Hunter, J. Southworth and A. S. Rabson, J. Immun. *102*, 932 (1969).
[5] J. C. Pisano, J. T. Patterson and N. R. Diluzio, Proc. Soc. exp. Biol. Med. *132*, 517 (1969).
[6] W. J. Martin and J. F. A. P. Miller, Int. Arch. Allergy *35*, 163 (1969).
[7] M. Rabinovitch, in *Mononuclear Phagocytes* (Ed. R. van Furth; Blackwell Scientific Publications, Oxford 1970), p. 299.