Ceruloplasmin Adrenaline Oxidase and Schizophrenia

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Akerfeldt's (1957) demonstration that ceruloplasmin levels (as measured by the oxidation of N.N. dimethyl para phenylenediamine) were elevated in some schizophrenic patients' blood serum initiated a series of studies and arguments which still remain unsettled. Leach and Heath's (1956) observation that adrenaline was oxidized more rapidly in schizophrenic plasma to adrenochrome and eventually into adrenolutin, gave some urgency to these problems for it was then believed that ceruloplasmin was the oxidase which oxidized both adrenaline and DPP. The critical comments published by Kety (1959) have had two consequences: (a) there was a marked drop in interest in pursuing these studies and few papers have appeared since 1959, while before they were coming along frequently, (b) most psychiatrists were convinced the matter was finally settled in the negative i.e. that oxidative rates in schizophrenic blood (plasma and serum) were in fact no different from those found in non schizophrenic but physically normal comparison groups. A review of this literature by Hoffer and Osmond (1963) suggested the contrary, that the great weight of evidence strongly favors Akerfeldt's early views and that, in fact, schizophrenic blood does oxidize adrenaline and DPP more rapidly than do comparison groups blood. Recently I read a paper by Osaki, McDermott and Frieden (1964) which completely alters the picture. Indeed, it now seems likely that the whole question must be reopened. In the light of our present knowledge most of the earlier studies were inadequate, not because they were poorly done, but because Osaki's findings were not then known.

Some workers have not distinguished clearly between serum and plasma—as if they were biochemically identical. Yet plasma contains substantial quantities of fibrinogen and serum does not. This may alter oxidation rates. Another major difference is that plasma contains anticoagulants. These are heparin, citrates, oxalates and EDTA. Osaki, et al, showed that citrates are powerful inhibitors of ceruloplasmin and that the quantities normally present in blood will inhibit ceruloplasmin 95%. They showed that this substance is the natural blood dialyzable inhibitor of ceruloplasmin.

These authors concluded that tests for ceruloplasmin activity in either serum or plasma are not valid unless the dialyzable inhibitors are removed. Obviously anticoagulants such as citrates oxalates or EDTA must be avoided. Since dialyzable inhibitors have not been removed in any of the studies previously published it seems that the entire question of ceruloplasmin oxidation and schizophrenia must be re-investigated. I have re-examined the published reports. In ten, serum was used, presumably with no anticoagulant. Six groups of investigators reported real (significant) differences between their schizophrenic populations and the comparison groups. Abood et al (1959) Akerfeldt (1957) Bonasera et al (1961) Friedhoff et al (1959) Saunders et al (1959) and Scheinberg et al (1957). Four groups found no significant differences i.e. Frohman et al (1958) Gussion et al (1958) Horwitt et al (1957) and Stennett et al (1960). The evidence on serum thus favors the view there is increased oxidation in schizophrenic serum.

There were only two reports on plasma.

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Both found significant differences i.e. Maas et al (1961) and Goldschmidt et al (1958). An additional report by Yuwiler et al (1961) cannot be accurately evaluated because it is not clear whether serum or plasma was used. Even then Yuwiler's data show there is a trend for their schizophrenics to have more ceruloplasmin activity.

Eight studies showed there were real differences; four failed to do so. The weight of evidence is clearly on the side of those who find increased levels in schizophrenics.

The whole matter should be re-opened using wherever possible plasma with heparin as the anti-coagulant. In our research group Payza and Zaleschuk (1959) found that heparin is not an inhibitor of ceruloplasmin. Citrate should be removed by dialyzing against aqueous solutions so that this complicating factor is removed.

It has been assumed that ceruloplasmin was also the enzyme which oxidizes adrenaline to adrenochrome and, in fact, it can do so, Walaas and Walaas (1961). But there is very strong evidence that there is another enzyme, an adrenaline oxidase, which is the more active enzyme in human blood. Payza and Hoffer (1959) Payza and Zaleschuk (1959) concluded that ceruloplasmin and adrenaline oxidase were not identical. The differences are shown in Table I.

There are other major differences. The distribution of ceruloplasmin and adrenaline oxidase in plasma from different mammalian species is different and using pig plasma only, ammonium sulfate fractionation shows different fractions contain different quantities. Finally in the pig the distribution among various tissues is different for both oxidases. Walaas and Walaas (1961) found that ceruloplasmin oxidizes adrenaline much less actively than it does P phenylene diamine. With prolonged incubation adrenochrome was formed.

Billewicz-Stankiewicz, Szczekala and Tyburczyk (1944) found serum contained adrenaline oxidase which catalyzed the combination of adrenaline with hydrogen peroxide to form adrenochrome. This was an adaptive enzyme and was not ceruloplasmin; an adaptive enzyme is one which increases in quantity as it has more work to do. Ceruloplasmin does not increase in concentration in the same way when adrenaline is infused into blood, Ostfeld, Abood and Marcus (1958). It is quite clear adrenaline oxidase and ceruloplasmin are not the same.

The question of adrenaline oxidases must be considered separately from that of ceruloplasmin. Only three groups have investigated rates of adrenaline oxidation using optimum conditions for measuring rates of change. Leach and Heath (1956) established a method for measur-

TABLE I

Differences Between Ceruloplasim and Adrenaline Oxidase

Property	Adrenaline Oxidase	Ceruloplasmin
Optimum pH	6.8	5.0
Optimum Molarity	0.05	0.04
Effect of dialysis into EDTA	Removes activity	No effect
Activators	Cupric ion Semicarbarbazide Iproniazide Hemoglobin	Cupric ion Sulfanilamide Hemoglobin
Inhibitors	NaCN Tris buffer EDTA Cysteine Ascorbic acid	Adrenaline Adrenolutin Cysteine Gluththione EDTA Iproniazide Semicarbazide
No effect	Adrenochrome semicarbazide Heparin Nicotinic Acid Mescaline	Adrenochrome LSD-25 GABA Mescaline

ing oxidation of adrenaline. Fortunately they used heparinized plasma. Schizophrenic plasma oxidized adrenaline more rapidly than did non schizophrenic non-physically ill comparison groups. Physically ill subjects also had higher rates of oxidation. This is proof in a way that schizophrenics are also physically ill, and that other factors play a role. These factors could be changes in protein levels such as increased erythrocyte sedimentation rates, increased hemolysis of blood red cells, increased consumption of ascorbic acid due to infectious, fevers and wastage of tissue, etc.

In 1957 I reported that ascorbic acid added to plasma produced a marked bleaching effect at 420°A. Leach and Heath's (1956) method was used and, after the reaction had run the eighty minutes recommended ascorbic acid was added. If ascorbic acid is not added there is a peak at 395°A which is a measure of the amount of adrenolutin formed and of the amount of adrenochrome formed. Both added together provide an estimate of the amount of adrenaline converted into both adrenochrome and into adrenolutin. Since ascorbic acid decolorizes adrenochrome the amount of bleaching at 420°A is another measure of the amount of adrenochrome formed and present in plasma before any adrenaline was added to it. Thus if there is any adrenochrome in plasma at the beginning the amount of bleaching will be greater than the amount of adrenochrome plus adrenolutin formed. My results are shown in Table II.

Since optical densities are not absolutely quantitative measures of the quantities of adrenochrome and adrenolutin formed, one should not expect precise relationships. It is shown in the table that the equivalence between optical density increases at 460°A, and decreases in optical density at 420°A are roughly comparable except for schizophrenics i.e. for the non schizophrenic groups the two measures of adrenochrome formation are practically identical. But for untreated schizophrenics the amount of adrenochrome present (using bleaching by ascorbic acid at 420°A as the measure) was greater than the amount of adrenochrome or adrenochrome plus adrenolutin i.e. O.D. decreased (0.21 - 0.095) or while the increase in adrenochrome plus adrenolutin was 0.065. Blood drawn during operation produced much more indoles as measured by increase in OD than was measured by decrease in OD at 420°A. This is probably due to slight quantities of freshly liberated hemoglobin in plasma due to the tissue damage as a result of the operation. Hemoglobin is also bleach-

TABLE II

Oxidation Rates of Adrenaline Using the Leach and Heath (1956) *Method, for Some Group of Human Subjects*

Group	Number	Optical Density (395°A)	Optical Density (460°A)	Optical Density (395° & 460°)	Optical Density Increases (420°)
Normal	18	0.30	0.10	0.40	0.10
Neurotic	16	0.31	0.10	0.41	0.09
Surgical pre op.	28	0.36	0.12	0.48	0.13
Surgical - op.	12	0.43	0.19	0.62	0.22
Schiz. - acute	13	0.35	0.12	0.47	0.21
Schiz. - after treatment	11	0.35	0.13	0.48	0.06

ed by ascorbic acid but not to the same degree as adrenochrome. This data suggests that acute schizophrenic plasma has more adrenochrome or similar substances before the adrenaline is added.

Schizophrenics (N = 24) did oxidize adrenaline more rapidly than normal or neurotic comparison groups but not to the same degree as surgically stressed normal subjects. This must be due to the increased secretions of adrenaline known to increase the quantities of the adaptive adrenaline oxidase. Schizophrenics could have increased levels of adrenaline oxidase for similar reasons.

Our work, therefore, fully corroborated Leach and Heath's work on plasma. A further report by Angel et al (1957) on serum suggested these differences held but were less striking. The report by Yuwiler et al (1961) is of no value since they used only 0.1 ml. of serum and other changes in the basic Leach and Heath method. The Leach and Heath method used 1.0 ml. — ten times as much. Yuwiler et al used so little serum they found only non specific auto oxidation.

Conclusion

Because dialyzable inhibitors of ceruloplasmin in serum were not removed none of the published studies are adequate. The two studies with plasma where heparin was the anti-coagulant show that schizophrenics contain greater quantities of adrenaline oxidase than do comparison groups not physically ill.

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