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Angiotensin-1-Converting Enzyme (ACE) Activity in Human Serum following Plasma Exchange[1] (41635)

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Abstract. Although angiotensin-1-converting enzyme (ACE), which catalyzes both the conversion of angiotensin 1 to angiotensin 2 and the hydrolysis of bradykinin, is known to be present in serum, little information is available about its source or turnover characteristics. We determined the removal and recovery characteristics of serum ACE for two normal subjects and 16 patients, with various diseases, who were undergoing partial plasma exchange. Cholesterol was removed as predicted, but the average actual removal of ACE, 70% of that originally present, was 28% greater than predicted. Recovery of serum levels of ACE after plasma exchange was 34% on Day 1, 62% on Day 3, 94% on Day 5, and 104% on Day 8, and the level remained relatively constant thereafter up to 28 days of study. The pattern of recovery of serum ACE is more compatible with a system of synthesis and release of ACE than with a simple exchange mechanism from sites on the plasma membrane of the endothelial cell or a shift of the enzyme from one space to another.

Angiotensin-1-converting enzyme is a dipeptidylhydrolase with a molecular weight of approximately 140,000. This enzyme catalyzes the conversion of angiotensin 1 to vasoactive angiotensin 2 and inactivates bradykinin, thus promoting a vasoconstrictive effect. The enzyme is known to be present in plasma (1) and also exists in association with a variety of cells (2, 3). The most prevalently studied cellular association of the enzyme is that with the vascular endothelium, where the enzyme is attached to the luminal surface of endothelial cells (4). Serum levels of angiotensin-1-converting enzyme appear to be relatively constant and, with the exception of thyroid function (5), are not under any known hormonal or humoral regulatory control. Serum levels of this enzyme are recognized to be elevated in some diseases such as sarcoidosis (6, 7) and Gaucher's disease (8), and may be elevated less frequently in a variety of other diseases. The elevated serum enzymatic activity may be derived from epithelioid or macrophage-like cells (9). Experimental lung injury also produces a transient elevation of angiotensin-1-converting enzyme in serum (10, 11) and in lung lymph (12).

Little information is available about the source of angiotensin-1-converting enzyme that occurs normally in serum or about the turnover characteristics of the enzyme in serum. Precise kinetic data of the enzyme in serum will not be rapidly forthcoming; it is very difficult to purify sufficient quantities of serum enzyme from animals to radioactively label it for turnover studies. Furthermore, enzyme purified from lung tissue, where it may be recovered in large quantities, may have quite different turnover characteristics from that of serum, because of its different carbohydrate content (13). We designed our study to look at the removal characteristics of angiotensin-1-converting enzyme by plasma exchange and to obtain an approximation of the rate of synthesis and/or release of this enzyme by longitudinal measurements of recovery of angiotensin-1-converting enzyme in the serum from 16 patients and two normal subjects who had undergone partial plasma exchanges. Our results show that calculated predicted removal of serum angiotensin-1-converting enzyme moderately underestimated its actual removal and recovery to preexchange serum levels took approximately 5 to 7 days. Al-

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though reduction in the serum enzyme level occurred, no change in blood pressure following plasma exchange was observed for these normotensive subjects.

Methods. Sixteen patients with a variety of neuromuscular and collagen-vascular diseases, who were being treated by plasma exchange, and two normal subjects were studied. All subjects were normotensive and no alteration in blood volume or electrolyte balance would have been expected in this patient population. Plasma exchange was performed on a Haemonetics Model 30 or a CS-3000 cell separator using a double-venipuncture technique. Anticoagulation was achieved with acid citrate dextrose (ACD) Formula A. Removed plasma was replaced with 5% normal serum albumin in nearly equivalent volumes in a manner so that intravascular volume fluctuations were minimized. The replacement did not alter the serum level of albumin. Equimolar sodium loss was also replaced. Serum samples were obtained immediately prior to and following the plasma exchange in all cases. In one normal subject, samples were obtained during the exchange at specified intervals (see data points of Fig. 1). The recovery data were obtained by measuring the serum enzyme level at varying later time intervals after plasma exchange. Six patients underwent repeated plasma exchanges (two to four procedures) and data of those repeated exchanges where preexchange levels of angiotensin-1-converting enzyme had returned to normal were included in Fig. 3. Cholesterol determinations were performed in the clinical chemistry laboratory. Angiotensin-1-converting enzyme activity was determined with the use of hippurylhistidyl leucine as previously described (7). The predicted removal of this enzyme was calculated according to a previously reported formula used for anticipated removal of substances in the blood by plasma exchange (14) and assumes that no synthesis or catabolism takes place and that no equilibration occurs between intravascular and extravascular compartments during the course of the procedure. The method for comparing actual to predicted removal and determining the percentage recovery has been previously published (15).

Results. *Removal.* The removal characteristics of angiotensin-1-converting enzyme in a normal subject is shown in Fig. 1. The total predicted removal was 81%. The actual removal was 94%. The logarithmic removal could be described by the relationship:

$$\log y = -0.93\,x + 1.94\ (r = -0.94).$$

Removal characteristics of angiotensin-1-converting enzyme for 16 patients and two normal subjects were analyzed and compared to removal of cholesterol. The average percentage exchange of plasma was 55% and the average percentage removal of serum ACE activity was 70%. The actual removal of cholesterol and angiotensin-1-converting enzyme by plasma exchange were compared to the predicted removal. The means ± one standard deviation (SD) of the differences of actual from predicted values are shown in Fig. 2. Cholesterol was removed with a mean of 7 ± 14% more than was predicted and the removal of angiotensin-1-converting enzyme was 28 ± 16% more than predicted. The percentage of removal of cholesterol was significantly different from that of angiotensin-1-converting enzyme ($P < 0.001$). No differences were noted in removal characteristics of patients that underwent plasma exchange on different machines.

Recovery. The percentage of total recovery of angiotensin-1-converting enzyme in serum measured at various times following plasma exchange is shown in Fig. 3. The figure is presented as a composite of data, and includes

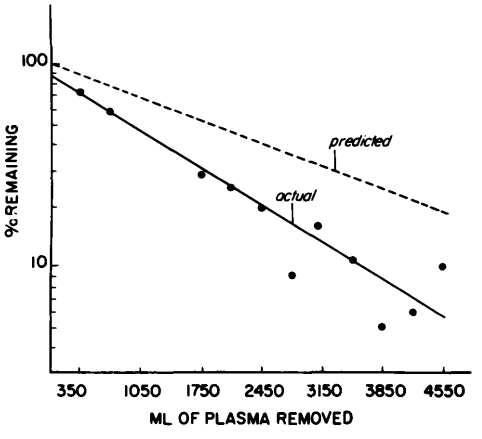

FIG. 1. Change in the concentration of angiotensin-1-converting enzyme during a single partial plasma exchange with albumin replacement. The actual removal (solid line) is greater than predicted.

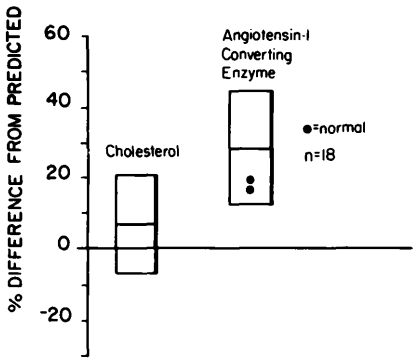

FIG. 2. Removal characteristics of cholesterol and angiotensin-1-converting enzyme in the 16 patients and two normal subjects studied. The mean ± SD are shown. Measurements for the two normal subjects are shown by black dots.

multiple studies on 6 of the 18 patients as noted in Methods. One-hundred percent recovery was considered to represent the preexchange value. Mean percentage recovery increased gradually from 34% on Day 1 to 94% on Day 7 and 104% on Day 8. At 2 weeks post-plasma exchange the mean percentage recovery was 111%, and at 22 and 28 days, the mean percentage recoveries were 101 and 104%, respectively. Values on Days 2, 3, and 5 were statistically different from that for Day 1 ($P < 0.01$), and the value for Day 5 was statistically different from that for Day 3 ($P < 0.1$). The pattern of return of values for normal subjects was similar to that for the patients studied.

Discussion. Plasma exchange is used to treat a wide variety of diseases. Replacement of removed plasma with normal serum albumin results in the removal of many normal plasma constituents. Depending on the extent and frequency of the therapeutic exchange and the removal and recovery kinetics of the removed normal plasma constituents, there may be a prolonged depletion of these constituents. The physiological effects of the depletion are not entirely known, since many constituents have not yet been studied.

The results of our study indicate that, during the course of plasma exchange, reduction in the concentration of angiotensin-1-converting enzyme occurs logarithmically. The actual removal of angiotensin-1-converting enzyme was nearly 30% greater than predicted for both normal subjects and patients.

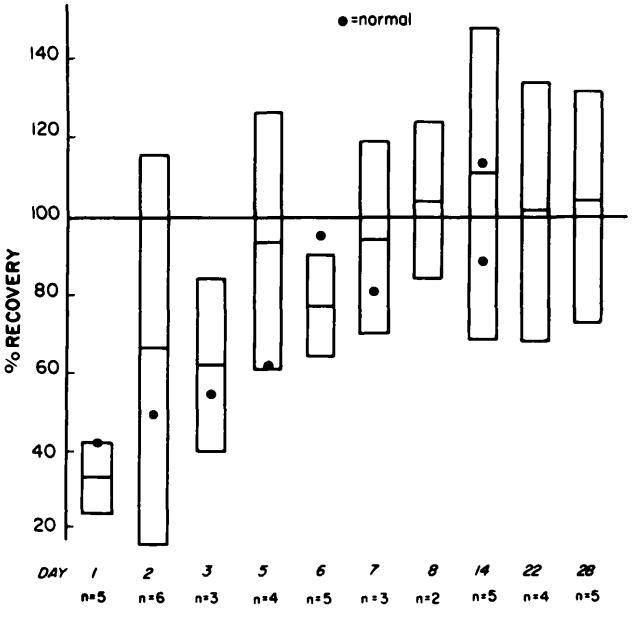

FIG. 3. Measurements of angiotensin-1-converting enzyme made at varied time intervals after plasma exchange. Percentage of total recovery is shown for each day as the mean ± SD. The measurements for the two normal subjects are shown by black dots. Six of the 16 patients had multiple recovery studies at different intervals after plasma exchange as noted in Methods.

Cholesterol was removed as predicted, confirming a previous report (15). Plasma exchange with 5% albumin has been reported to cause intravascular volume to increase by approximately 5% (14), but this increased volume cannot explain the deviation of removed angiotensin-1-converting enzyme from the predicted value. Utilization of angiotensin-1-converting enzyme or rapid metabolism without reequilibration during the procedure might explain the observed results. The assay for angiotensin-1-converting enzyme was not affected by a 50% dilution of test samples with 5% normal serum albumin, which excludes a possible artifact of measurement resulting from the exchange with 5% albumin. We also tested the possibility that albumin or its breakdown fragments might account for the low serum angiotensin-1-converting enzyme activity immediately following plasma exchange (16). *In vitro* mixing experiments of pre- and postexchange plasma from five patients showed only an average 4% reduction of calculated enzymatic activity at a time when postexchange plasma levels had been reduced by 70%. Thus, there was no evidence that components of postexchange plasma accounted for the reduction of the enzymatic activity.

Measurements of the recovery of angiotensin-1-converting enzyme after plasma exchange indicate that wide variations exist from patient to patient in the recovery and return to baseline, or preexchange, levels of this enzyme. Similar observations have been reported for measurements of recovery of other molecules following plasma exchange (15). Nevertheless, there was a general pattern of return to a baseline level of this enzyme in the serum between 5 and 7 days after removal, and this level subsequently remained relatively constant throughout the 28-day period after plasma exchange. The studies with the two normal subjects paralleled the data obtained with patients. Recovery of cholesterol after plasma exchange is also prolonged and requires 7 to 14 days to reach pre-apheresis levels (15).

Previous studies done following plasma exchange have shown that there is a rapid reequilibration (24 hr) of some molecules such as ions and glucose. Other molecules, such as fibrinogen, complement, cholesterol, IgG, and IgM, take variably longer periods of time to return to pre-apheresis levels (15). Since angiotensin-1-converting enzyme required 5 to 7 days to achieve pre-apheresis levels, our studies suggest that recovery of angiotensin-1-converting enzyme in serum is not due to a simple exchange mechanism from sites on the plasma membrane of the endothelial cell, since it is likely that this exchange would have occurred more rapidly. Similarly, a shift of the enzyme from one space to another is unlikely, as this would be expected to occur rapidly as well. Although the mechanism of recovery of the enzyme still remains to be determined, we can conclude from our observations that reestablishment of the serum enzyme level after removal of approximately one plasma volume requires 5 to 7 days; likely this is due to some combination of synthesis and release of the enzyme. Once reestablished at pre-plasma-exchange levels, the enzyme concentrations are relatively constant.

Although an average of 70% of the plasma enzyme activity was removed in studies carried out on these patients, we did not observe any alteration in blood pressure measurements for these subjects. This observation may not be unexpected, since the lung contains large quantities of angiotensin-1-converting enzyme relevant to that in serum (17); however, our observations do point out that removal of serum angiotensin-1-converting enzyme is not likely to cause the previous reports of other investigators of an antihypertensive effect of plasma exchange (18).

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