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Relationship between Plasma Concentrations of Angiotensin I, Angiotensin II and Plasma Renin Activity during Cardio-Pulmonary Bypass in Man*

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Abstract. Several reports have demonstrated that the lungs are the most important site of conversion of angiotensin I to angiotensin II. The purpose of the present study was to assess the extent of extra-pulmonary conversion in man, during cardiopulmonary bypass. Plasma concentrations of angiotensin I and immunoreactive angiotensin II, and plasma renin activity were simultaneously determined, using specific radioimmunoassays, during extra-corporeal circulation in 13 patients undergoing major cardiac surgery. Generally the renin-angiotensin system was stimulated during cardio-pulmonary bypass with maximum values occurring at different time. A highly significant correlation was found between plasma renin activity and angiotensin I and II concentrations respectively, as well as between these two peptides. Positive correlations were also obtained between arterial and venous samples for plasma renin activity and angiotensin I and II. Thus the presence of angiotensin II in plasma in the absence of pulmonary circulation and its parallel variations with plasma renin activity indicate that converting activity by extra-pulmonary sources is not negligible.

Key words: Man, cardio-pulmonary bypass, angiotensin I, angiotensin II, renin, converting enzyme, radioimmunoassay, angiotensin fragments.

The circulating decapeptide angiotensin I, generated by renin activity upon its substrate angiotensinogen, is split by a converting enzyme into the biologically active octapeptide angiotensin II. The converting enzyme was first purified from plasma [1] where it was then thought to act mainly. However since Ng and Vane [2, 3] had shown in the dog that lungs were the major site of converting activity, many reports have confirmed this predominant localisation [4–7]. The relative importance of plasma and kidney converting enzyme has been variously evaluated [6, 8–12].

Cardio-pulmonary bypass with extra-corporeal circulation offers an opportunity to assess the role and extent of extrapulmonary conversion. In the dog, Stanley and Biron [13] observed during pulmonary bypass a definite plasma converting activity which was smaller than pulmonary converting activity. No similar investigation has yet been reported in man. Most experiments demonstrating pulmonary conversion have been performed by measuring with bioassays [2, 3, 5], or radioactive tracer techniques [6] the metabolism of supra-physiological doses of angiotensin I infused at either of the two sides of the pulmonary capillary bed.

The present study was designed to investigate the extrapulmonary conversion *in vivo* in man. The effect of pulmonary bypass upon the concentrations of endogenous plasma angiotensin I and II, determined by specific radioimmunoassays, and their relationship with the simultaneously measured plasma renin activity was examined during extra-corporeal circulation in 13 patients undergoing major cardiac surgery.

Subjects and Methods

Design of Clinical Studies

The investigation of the renin-angiotensin system during extra-corporeal circulation was carried out in 13 patients—8 males and 5 females—aged 14 to 63 years. In eight patients defective cardiac valves were being replaced by prosthetic ones while aorto-coronary anastomosis was performed in the other five because of coronary disease. The pulmonary bypass lasted at least one hour. A bubble oxygenator of the Travenol type 6 LF was used for the extra-corporeal circulation. The patients were perfused at a rate of 2.4 l/min/m^2 and a mean arterial pressure of 60 to 80 mm Hg. The temperature of the perfused blood was decreased to 25–30°C.

Venous blood samples were drawn from all 13 patients from the tube leading blood from the right atria to the oxygenator, at a distance of about one metre from the heart, and in 3 of these patients in addition arterial blood samples were simultaneously drawn from a catheter placed in the radial artery. The times of sampling were at the start of the perfusion when venous blood from the patient reached the oxygenator, and 10, 20, 30, 45 and 60 min later, except for the first two cases studied which included only 3 and 4 samples respectively.

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Treatment of Samples and Analytical Methods

Measurements of Plasma Levels of Angiotensin I and II and of Renin Activity. Blood was rapidly drawn into cooled Vacutainer® tubes, containing enough EDTA to block *in vitro* converting enzyme activity [15]. Tubes were immediately replaced in ice and soon afterwards centrifuged at 4°C. After addition of Diisopropylfluorophosphate (DFP) to inhibit angiotensinases, the separated chilled plasma was frozen and stored at −20°C.

Plasma renin activity (PRA), circulating angiotensin I (AI) and immunoreactive angiotensin II (AII) were determined in 1 to 2 ml plasma following extraction and using specific radioimmunoassays as previously described [14, 15]. Each assay was done in duplicate. The determination of AI was done after extraction of unincubated plasma as for AII but using labelled AI for the radioimmunoassay and a higher dilution of the same anti-AI antiserum as for PRA determination yielding a more sensitive standard curve (limit of detection 0.01 pmol per assay). The limit of detection for AII was 0.005 pmole per assay. PRA was determined by the radioimmunological measurement of AI generated in one ml plasma during one hour incubation at 37°C and pH 5.5. The detection level was 0.125 pmol/ml/h. In order to compare stoichiometrically the relationship of the components of the renin-angiotensin system, all the results have been expressed in molar form rather than in weight units (1 pmol Ile^5-AI = 1295 pg and 1 pmol Ile^5-AII = 1045 pg).

Chromatographic Studies of Angiotensin I and II Metabolites

Whereas the antiserum against AI was highly specific for the decapeptide with no cross-reaction with either renin substrate, AII, 2–8 heptapeptide or 3–8 hexapeptide, the antiserum against AII could not distinguish this octapeptide, the heptapeptide, and the hexapeptide [16, 17] which was also extracted by the procedure used. Neither antiserum bound fragments shorter than hexapeptide. Because of this cross-reaction and because of the possible presence of hexapeptide in plasma [18], the peptides were separated in a control experiment by thin-layer chromatography of plasma extract. The plasma was handled as for the assay of AI and AII, then the extract was dissolved in methanol containing glacial acetic acid to decrease the pH to 3. Chromatography was achieved on Silica Gel F 254 plates (Merck) in 8 hours with n-butanol-acetic acid-water (4:1:1, V/V), in which AI and AII migrated with similar Rf values, while 2–8 heptapeptide and 3–8 hexapeptide were clearly separated and had respectively higher Rf values. The Rf values were unaffected by the presence or absence of the iodine label in this system. When the extracts from incubated plasma samples were subjected to thin-layer chromatography and examined under ultraviolet light (285 nm) spots were observed migrating as AI or AII and as the hexapeptide, with 2 secondary spots of unidentified shorter fragments moving ahead of the hexapeptide but no spot migrated as the 2–8 heptapeptide. Since the hexapeptide has been shown to be a degradation fragment *in vivo* of AII but never of AI [6, 19], this was verified in the present system by examination of the products formed *in vitro* by hydrolysis of ^{125}I-AI and ^{125}I-AII. For this purpose an equal amount of either one of the labelled peptides was added to heparinized plasma without inhibition of converting enzyme and angiotensinases by EDTA or DFP. A control sample was not incubated, and 2 others were incubated at 37°C for 5 and 30 min respectively. They were then subjected to the usual extraction after incubation and the extracts deposited on thin-layer plates for chromatography as described above. The peptides and their metabolites containing labelled tyrosine were localized by autoradiography. With the plasmas containing labelled AI, autoradiography showed a spot at the level of either AI or AII, and several spots corresponding to unidentified fragments shorter than the 3–8 hexapeptide which appeared after 5 min of incubation, but none at the place where 2–8 heptapeptide and 3–8 hexapeptide migrated in this system. Plasma containing labelled AII revealed a spot with the Rf of the octapeptide which decreased in intensity with the incubation time while a spot with an Rf corresponding to the hexapeptide and other fainter spots moving faster and corresponding to fragments of hexapeptide appeared during the incubation. Thus a direct formation of 3–8 hexapeptide from AI could be ruled out.

Other Measurement. In each venous plasma sample sodium and potassium were measured by flame photometry and the haematocrit was determined.

Statistics

The data were analyzed statistically using both the Student paired *t* test and the Wilcoxon rank test which gave the same levels of significance. The regression lines of the correlations between the various parameters were calculated with the CDC 3800 Computer of the University of Geneva (Centre Cantonal d'Informatique) using the REGFIT programme conceived by Dr. R. Scherrer.

Results

Values of PRA, AI and AII during Cardio-Pulmonary Bypass (Fig. 1)

As expected the 13 patients studied showed different patterns of response of the renin-angiotensin system to the anaesthesia, the operative stress and the changing cardio-vascular dynamics induced by the extra-corporeal circulation. The fall of blood pressure under 50 mm Hg which occurred often at the onset of the perfusion may have been the most important.

At the start of the perfusion, PRA varied between individuals from 0.3 to 6.4 pmol/ml/h with a mean of 2.4 ± 0.6 (SEM) pmol/ml/h. During the first 60 min of the pulmonary bypass, PRA in all but one case increased, the mean value of all the subjects doubling after 30 min. In only one case was a slight decrease amounting to 0.3 pmol/ml/h observed at 30 min. Maximal increments of PRA compared to initial values ranged from 0.3 pmol/ml/h to 11.3 pmol/ml/h with a mean of 3.2 ± 1.0 (SEM). The difference between peak values and control values was highly significant ($p < 0.005$). The maximum mean of 4.4 ± 1.2 (SEM) pmol/ml/h was noted at 30 and 45 min of pulmonary bypass. It was followed by a fall to 3.1 ± 0.8 (SEM) pmol/ml/h at 60 min.

The changes of the levels of plasma AI and AII followed closely the changes of PRA in most cases with an increase from the start up to 30 to 45 min of the pulmonary bypass. The mean AI plasma concentration at time zero was 0.039 ± 0.007 (SEM) pmol/ml and of AII 0.024 ± 0.004 (SEM) pmol/ml. The two peptides increased 1.5 times to a maximum mean of 0.062 ± 0.013 (SEM) pmol/ml for AI at 20 and 30 min and of 0.039 ± 0.010 (SEM) pmol/ml for AII at 45 min. The maximal increment of AI concentration ranged from 0.004 to 0.102 pmol/ml with a mean of 0.043 ± 0.009 (SEM) pmol/ml and of AII concentration from 0.002 to 0.075 pmol/ml with a mean of 0.034 ± 0.008 (SEM) pmol/ml. The difference between peak values and control values was again highly significant for the two peptides ($p < 0.001$). Between 45 and 60 min from the start of the pulmonary bypass, the levels of AI and AII, like PRA, decreased slightly to 0.049 ± 0.009 (SEM) pmol/ml and 0.031 ± 0.007 (SEM) pmol/ml respectively. The ratio AI/AII concentrations increased from 1.61 at time 0, to a maximum of 1.94 at 20 min then returned to starting value of 1.59 at 60 min, but these variations were not statistically significant.

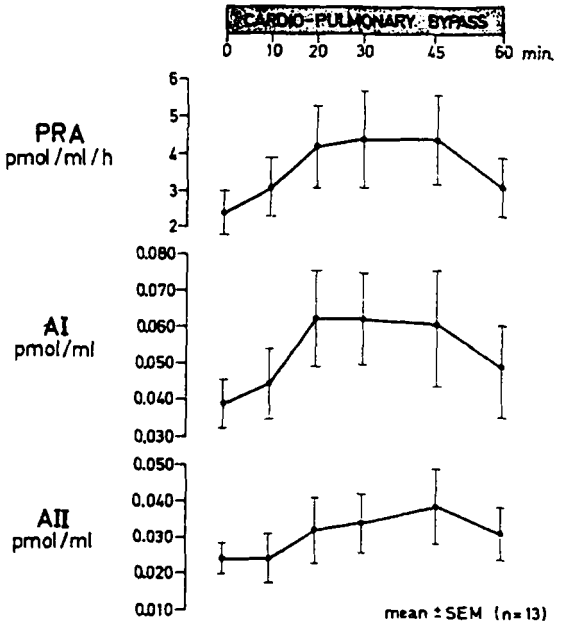

Fig. 1. Variations of venous plasma renin activity (*PRA*), angiotensin I (*AI*) and angiotensin II (*AII*) concentrations (mean ± SEM) in 13 patients during the first 60 min of cardio-pulmonary bypass

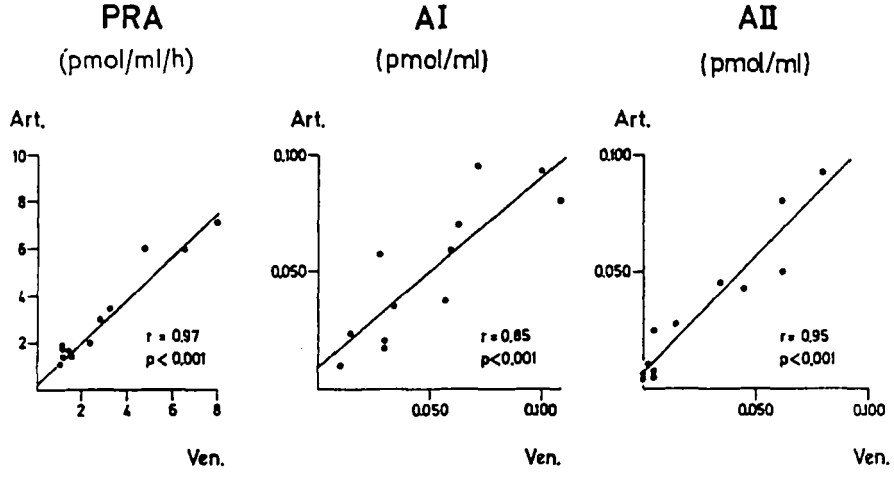

Fig. 2. Correlations between simultaneous venous (*Ven.*) and arterial (*Art.*) determinations of plasma renin activity (*PRA*), angiotensin I (*AI*) and angiotensin II (*AII*) concentrations in 3 patients (12 determinations) during cardio-pulmonary bypass

During extra-corporeal circulation the plasma potassium and sodium varied minimally around a mean of 3.6 mEq/l ± 0.2 (SEM) for K and of 130 mEq/l ± 1 (SEM) for Na. The rather low sodium values reflect the dilution of the plasma during the procedure. Also the low values of the haematocrit maintained between 30 and 35% reflect the blood dilution by the solution used to prime the pump.

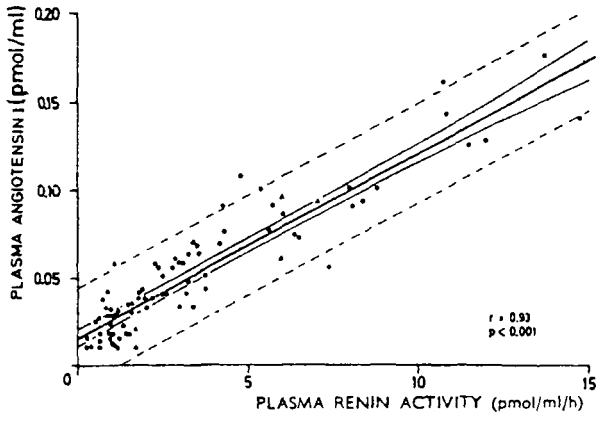

Fig. 3. Correlation between plasma renin activity and angiotensin I concentration in 13 patients during cardiopulmonary bypass, at times indicated on Fig. 1, with 72 determinations on the venous side (circles). Correlation coefficient $r = 0.93$ ($p < 0.001$). For comparison, 12 determinations were performed on the arterial side in 3 patients during bypass (triangles). The regression line with its confidence limits (continuous lines) and the intervals (dashed lines) of 2 SD on each side of the regression line are indicated

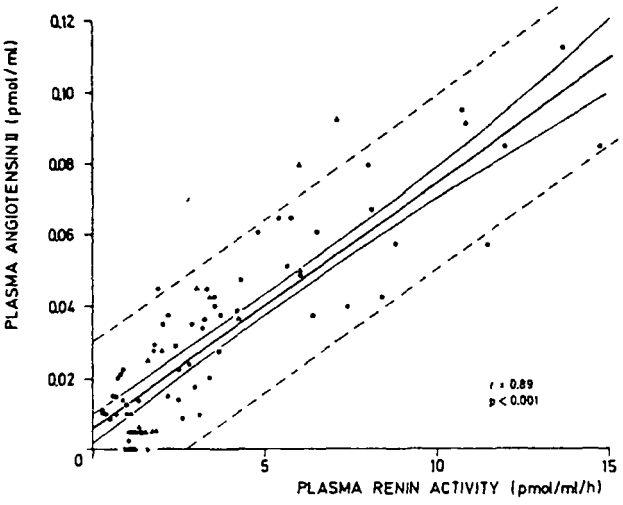

Fig. 5. Correlation between plasma renin activity and angiotensin II concentration in 13 patients during cardiopulmonary bypass with 72 determinations on the venous side (circles). Correlation coefficient $r = 0.89$ ($p < 0.001$). For comparison, 12 determinations were also performed on the arterial side in 3 patients during bypass (triangles). Regression line and confidence limits as in Fig. 3

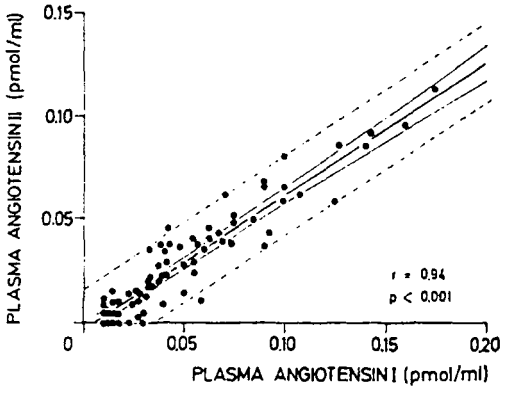

Fig. 4. Correlation between angiotensin I and angiotensin II concentrations in 13 patients during cardio-pulmonary bypass with 72 determinations on the venous side. Correlation coefficient $r = 0.94$ ($p < 0.001$). Regression line and confidence limits as in Fig. 3

Correlation between Venous and Arterial Values (Fig. 2)

In the 3 cases in whom venous and arterial blood samples were simultaneously drawn during pulmonary bypass, a positive correlation between venous and arterial values was obtained for PRA ($r = 0.97$; $p < 0.001$), circulating AI ($r = 0.85$; $p < 0.001$) and circulating AII concentrations ($r = 0.95$; $p < 0.001$). The difference between arterial and venous values was not significant for PRA ($p > 0.6$), AI ($p > 0.6$) and AII ($p > 0.05$). As a control, the suspension of erythrocytes, used for priming the oxygenator, was also examined for its content of the components of the renin-angiotensin system: PRA, AI as well as AII were all undetectable.

Correlation between PRA, AI and AII Venous Levels

A highly significant correlation was obtained between the venous values of all three parameters of the renin-angiotensin system at each time, $r = 0.93$ ($p < 0.001$) for the correlation between PRA and AI (Fig. 3), $r = 0.94$ ($p < 0.001$) for the correlation between AI and AII (Fig. 4), and $r = 0.89$ ($p < 0.001$) for the correlation between PRA and AII (Fig. 5). The venous or arterial values of circulating AI were statistically significantly higher than the values of circulating immunoreactive AII ($p < 0.001$).

Discussion

Since the original observation of Ng and Vane [2, 3], the lung has been considered as the principal site of AI conversion into AII. A high level of converting activity has also been demonstrated *in vitro* in extract of lung tissue [7]. *In vivo*, all investigations of the role of pulmonary conversion have involved infusions of supraphysiological doses of AI into the circulation either before or after passage through the pulmonary capillary bed. The extent of the conversion into AII was assessed by estimation of the pressor activity of the plasma by bioassay [2, 3, 5], or with chemical or radioimmunological techniques [6, 7, 20, 21]. These experiments have revealed the great capacity of conversion in the pulmonary capillary bed. Nevertheless the role of extra-pulmonary conversion was demonstrated by some authors in plasma, or in kidneys or in other vascular beds [6, 8–12]. Only two investigations of AI conversion *in vivo* have been reported in man. Biron *et al.* [5] applied the pressor response technique to study the fate of AI and AII during

passage through the lungs. In two patients AI injected in the pulmonary artery was twice as potent as when injected in the aorta. Friedli [22] demonstrated in children that AI injected in the pulmonary artery induced a slightly greater pressor response than when injected into the aorta. The latter observation, and further arguments drawn from the lag-time of the pressor response, suggested that pulmonary conversion though evident, was not the only effective one, but that further conversion occurred also in the systemic circulation.

The purpose of the present study was to evaluate *in vivo* the extent of conversion during cardio-pulmonary bypass by measuring the levels of circulating AI and AII and to relate them to the values of PRA measured simultaneously. The demonstration that AI circulates in the plasma and can be measured by specific radioimmunoassay of unincubated plasma should be critically evaluated since renin may continue to act and generate AI after the blood sample has been drawn. The addition of EDTA and DFP blocks *in vitro* the converting enzyme and angiotensinases but not renin [15], whose activity under the present conditions was stopped only by the low temperature of 4°C. Thus special care was taken to draw and refrigerate the blood rapidly. The great closeness of the AI values found in different samples drawn at the same time and the significant positive correlation between AI levels and either PRA or AII concentrations permit one to attach a real significance to circulating AI. Morton *et al.* [23] have also found a good correlation between these values in peripheral venous blood in normal subjects and in patients with various forms of hypertension. It remains possible however that the higher values of circulating AI compared to AII could be due to generation of AI *in vitro* or to incomplete conversion *in vivo* because of the pulmonary bypass or alternatively to AII disposal being more rapid than formation from AI. The first possibility appears the least likely since in kinetic studies we observed that lowering the temperature effectively inhibits renin activity. The half-life of AI has been calculated by Oparil *et al.* [6] to be much shorter in whole dog blood with no anticoagulant (3 min) than in dog plasma anticoagulated with heparin (15 min). Since the blood from the patients in the present study was heparinized the possibility thus exists that the half-life of AI was prolonged explaining the relatively higher values of this decapeptide.

During cardio-pulmonary bypass, PRA levels rose in most of the 13 patients studied, with the maximum levels occuring however at different times. They then started to decline, the difference between maximum values reached and control values being highly significant. The cause of these changes was not elucidated because of the complex situation induced by the surgical stress, the anaesthesia and the extra-corporeal circulation. Whatever the pattern of PRA response and its cause were, the present investigation was, first of all, concerned with the correlation between PRA and the levels of circulating AI and AII. The PRA and the two peptides concentrations increased together from the start of perfusion to a maximum 30–45 min after. Consequently the plots of all values of plasma AI and AII versus PRA and of AI versus AII showed a linear regression with highly significant correlations. The present data are in complete agreement with the correlation of PRA and AII in peripheral venous blood reported by Gocke *et al.* [24] in normal subjects, and by Cain *et al.* [25] in women during oral contraceptive therapy.

Such a correlation in our study implies the presence of extra-pulmonary converting enzyme in sufficient amount to act upon AI at physiological levels in plasma and to form AII in quantities proportional to PRA. If the extra-pulmonary converting activity had been incomplete, one would have expected that with high values of PRA an accumulation of AI would have occurred with low levels of AII. This may have actually occurred however in the earlier phase of the pulmonary bypass since a delayed maximum of AII values was observed compared to AI and PRA (Fig. 1), though the variation of the AI/AII ratio was not significant.

Significant correlations were also determined between venous and arterial values of PRA, AI and AII obtained from blood sampling at two different distances from the point of renin secretion. The AII present in venous samples could have originated in the plasma, in the kidneys which were relatively close to the sampling site, or from the peripheral tissues. It could probably not be generated in the oxygenator itself, since in a U-shaped bubble oxygenator such as the Travenol model used in this study, the passage of blood is very rapid (about 1 min).

Concerning the radioimmunoassay of AII, a further specification of the peptide effectively measured in this study was required, since fragments from degradation of angiotensin II, such as the 3–8 hexapeptide which cross-reacted with AII [16, 17], have been reported to occur in plasma [18]. Therefore chromatographic separation for the metabolites of ^{125}I-labelled AI and AII was performed after incubation of the plasma without inhibition of converting enzyme or angiotensinases. Although the 3–8 hexapeptide appeared as one of the metabolites of AII in the system used for chromatography, no similar product was observed after incubation of AI. Thus if a certain amount of hexapeptide contributed to values of immunoreactive AII concentrations in plasma, this fragment was not a direct degradation product of AI and could not have led to a false evaluation of converting activity. The 2–8 heptapeptide fragment was not detected among the degradation product of AII in plasma. On the other hand neither the 2–8 heptapeptide nor the 3–8 hexapeptide cross-reacted with the antiserum directed against AI and thus could not

account for the higher values of AI found. Studying the metabolism of radioactive AI, Oparil *et al.* [6] did not detect any products other than leucine or histidyl-leucine *in vitro* as well as *in vivo*, and Ryan *et al.* [19] reported the presence of di-, tri-, and tetrapeptides, but did not find hexapeptide. We thus feel confident that what was measured as immunoreactive angiotensin II was actually the product of the action of converting enzyme upon angiotensin I and conclude from this study that effective extra-pulmonary conversion takes place during cardiopulmonary bypass in man.

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