at any time, in five of the six pretreated dogs over the observed time, and a fifty percent reduction of the S–T amplitude was found in the dog that did develop these signs of injury and infarction, at the third hour. All control dogs showed S–T and Q wave changes by the third hour. The mean CAPB dropped significantly ($p < 0.01$) in the control but not in the treated group. There were significant elevations ($p < 0.05$) of CV concentrations of Lac and K in the control group at the third hour. There were no significant changes in concentrations for these biochemical variables in the pretreated group. The cardiac dynamics and biochemical integrity appeared to be sustained by these pharmaceutical agents in the early stages of acute coronary thrombosis.

K. Andrassy, E. Ritz, U. Bleyl and R. Egbring (Medizinische Universitäts-Klinik und Pathologisches Institut der Universität Heidelberg, Medizinische Universitäts-Klinik Marburg): **Purification of Human Urokinase and its Topographical Localisation in Renal Parenchyma. (73)**

Urokinase Leo was separated by agar zone electrophoresis into an anodic and cathodic fraction. The cathodic fraction, isolated from agar gel by ultracentrifugation, showed two precipitation bands with rabbit Urokinase antibodies. Band I displayed main Urokinase activity, in band II Urokinase was present in a high molecular weight complex with human serum proteins (albumin, a_2-macroglobulin, a_2HS glycoprotein); with affinity chromatography further separation of Urokinase isoenzymes from serum proteins was possible. The isoelectric point of these two Urokinase isoenzymes were pH 6.8 and pH 8.7 respectively in preliminary results with isoelectric focusing. Purification steps were controlled by disc gel electrophoresis and immunological techniques (Ouchterlony technique, immunoelectrophoresis, clot lysis test with Urokinase antibodies).

Topographic localisation of Urokinase in renal tissue, investigated with antibodies against Urokinase isoenzymes, revealed Urokinase activity both in the iuxtamedullary region (V. arcuatae, V. interlobulares, less V. recta) and in calyceal epithelia of the renal pelvis.

Th. Vukovich, B. Binder and W. Auerswald (Dept. of Physiology, School of Medicine, University of Vienna, 17 Schwarzspanierstr. A-1090 Vienna, Austria): **"Different" Forms of Urokinase. (74)**

The isoelectric inhomogeneity of human urokinase (UK) derived from urine and commercial preparations have been reported. The different isoelectric forms of UKs were in part related to different mol. wts.: UKs with pIs between 10.2 and 6.3 had a mol. wt. of 31.500 and UKs with pIs below 5.2 had mol. wts. greater than 100,000; a UK with a mol. wt. 54,700 was not f und after isoelectric focusing.

In the present experiments we used purified UK containing only the S_1-type UK – confirmed by us – as well as UK concentrated by ultrafiltration of native human urine containing all known UK-types and high mol. wt. aggregates. With short focusing times, e.g. 10 hours at 1200 V and a final 1 mA, UK activity was found only at a pI of 10.2; with extended focusing times e.g. up to 72 hours, such activity was additionally found at lower but defined pIs of 8.0, 7.5, 6.9, and 6.3. All active fractions with defined pIs lower than 10.2, when rerun immediately, focused at the initially determined pI values while the 10.2 fraction separated again into all the previously given lower pI fractions. The mol. wt. of all the UKs from pI 10.2 to 6.3 determined directly following isoelectric fractionation was ca. 31,500 (Sephadex G-150) while after storage for at least 1 month at 4° C at neutral pH, all the fractions contained active material of a mol. wt. less than 10,000. By focusing this low mol. wt. material UK activity could be detected only at a pI lower than 4.0.

Samples of our starting UK-containing materials were dialysed at 4° C against various buffer solutions (molarity 0.01–0.15, pH = 3.0–10.0). At pH values higher than 9.0 independent of molarity UK activities appeared in the dialysate. Isoelectric focusing of that material revealed its pI to be lower than 4.0.

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