Effect of a thrombolytic agent (urokinase) on necrosis after acute myocardial infarction

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EFFECT OF A THROMBOLYTIC AGENT (UROKINASE) ON NECROSIS AFTER ACUTE MYOCARDIAL INFARCTION[1]

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SUMMARY

Using a two-compartment model with continuous input function, the rate and extent of myocardial damage were calculated from plasma enzyme levels measured in patients suffering from acute myocardial infarction (AMI). Results were compared for four different enzymes. As regards the course of necrosis, two different hypotheses were tested and it was shown that the necrotic process has to be considered as a multiplicative process rather than as a sudden disintegration of cells due to anoxia. Comparison of results obtained in a group of 5 patients treated with urokinase and in an untreated control group of 10 patients showed that the necrotic phase as defined by the enzyme release from the infarcted area was significantly shortened.

INTRODUCTION

The importance of determining plasma enzyme levels in patients suffering from acute myocardial infarction (AMI) has been well established. However, these data are mainly used in a qualitative way for diagnostic or prognostic reasons. As the routine laboratory determinations of these enzymes are sufficiently accurate to allow a thorough analysis of enzyme levels as a function of time, much information is lost that way. In the last few years, we have developed a method of obtaining a quantitative anlysis of myocardial damage after AMI (Hemker *et al.*, 1972; Witteveen, 1972). In the present paper, this method is used to investigate the effect of urokinase on the necrotic process after AMI.

MATERIALS AND METHODS

The experimental group consisted of 15 patients with AMI, of whom 5 received urokinase. Patients with cardiogenic shock or severe heart failure were not

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included in the investigation. For further clinical details see the publication by Witteveen (1972).

Blood was drawn from an indwelling catheter as soon as possible after admission to hospital and, subsequently, every 4 hours for 2 or 3 days. At the end of this period, progressively fewer samples were taken until plasma levels returned to normal.

Samples consisted of 10 ml of blood collected in tubes containing 0.2 ml of 20% sodium citrate to prevent clotting and were immediately centrifuged, initially for 10 min at 900 *g* to remove erythrocytes and leukocytes, and subsequently for 20 min at 40,000 *g* to remove thrombocytes. The plasma was then stored at −20°C until enzyme determinations were carried out.

The following enzymes were studied: glutamic oxalacetic transaminase (GOT; E.C. 2.6.1.1), creatine phosphokinase (CPK; E.C. 2.7.3.2), phosphohexose isomerase (PHI; E.C. 5.3.1.9), lactate dehydrogenase (LDH, E.C. 1.1.1.27), and α-hydroxybutyrate dehydrogenase (α-HBDH; E.C. 1.1.1.27). The last two enzymes are identical but are determined in a different assay, so that comparison of results permits an estimation of over-all experimental error.

Enzyme determinations were done spectrophotometrically. For GOT, LDH, and α-HBDH commercially available test kits were used (Boehringer). Recently, Boehringer introduced so-called optimized test kits. If these are used, the results presented below should be adjusted. CPK was determined according to Rosalki, (1967), and PHI according to Weber and Wegmann (1968). Enzyme activities were expressed as I.U./liter, in which 1 I.U. catalyzes the turnover of 1 μmole of substrate per minute.

Urokinase, a nonantigenic thrombolytic agent acting through activation of plasminogen, was made available by Hoffmann La Roche. The drug was administered as soon as possible after admission to hospital. An initial loading dose of 7,200 units/kg body weight was given in 10 min, after which an infusion of 3,600 units/hour/kg body weight was given for 18 hours.

Heart tissue was obtained at thoracic surgery. To determine the enzyme activities in the normal myocardium, the tissue was kept in physiological saline at 0°C and homogenized within 10–15 min (Turrax). After sedimentation of the remaining particles, the activities were measured in the supernatant (Table 2). To study the shed-out of enzymes *in vitro,* tissue was kept at 37°C for up to 100 hours in plasma under anoxic conditions and gentle shaking; the activity appearing in the plasma was measured. After this period, the tissue was homogenized to obtain the residual tissue activity (Table 1). The molecular weights of the human enzymes were determined by column chromatography with DEAE Sephadex G-200 of plasma containing high activities. To obtain a standard, albumin (M = 69,000) and ceruloplasmin (M = 160,000) were also determined by quantitative immunoelectrophoresis (Laurell).

The mathematical analysis of the patient data is based upon the two-compart-

ment model presented in Figure 1, where V_1 is the vascular volume, V_2 the extravascular volume, $F(t)$ the enzyme release as a function of time, k the sum of first order clearance constants, and D the diffusion constant. This model is based upon the following considerations. The observed (double) exponential clearance from the plasma indicates that there is only first order elimination proportional to the enzyme concentration.

The assumption of the absence of extravascular elimination is based upon the *in vitro* experiments showing no appreciable denaturation. This suggests that the enzyme breakdown is circulation-dependent. Insofar as the enzymes from the necrotic cells do not diffuse directly into the blood stream, we assumed that, in view of the relatively direct transport of lymph from the myocardium to the vascular compartment by way of the thoracic duct, the input $F(t)$ is only into V_1. A more detailed discussion of the implicit assumptions of this model will be published elsewhere (Hermens *et al.*). It can be shown (Witteveen, 1972) that the plasma concentration of enzyme as a function of time $X(t)$ in this model is given by the double exponential function

$$X(t) = C_1(t)e^{-a_1 t} + C_2(t)e^{-a_2 t} + X_n \tag{1}$$

($a_1 > a_2 > 0$), where X_n is the normal steady state enzyme concentration, and the constants a_1 and a_2 depend on V_1, V_2, k, and D. The time-dependent coefficients in Eq. (1) are given by:

$$C_1(t) = [(a_1 - a_1 a_2/k)/(a_1 - a_2)] \int_0^t f(\tau)e^{a_1 \tau} d^\tau \tag{2}$$

$$C_2(t) = [(a_1 a_2/k - a_2)/(a_1 - a_2)] \int_0^t f(\tau)e^{a_2 \tau} d^\tau \tag{3}$$

where $f(t) = F(t)/V_1 - kX_n$ = extra enzyme release (i.e., due to infarction) per liter vascular volume.

As soon as the enzyme release from the infarcted area is completed, i.e., $f(t) = 0$, $C_1(t)$ and $C_2(t)$ become constants and a logarithmic plot gives a curve with slopes a_1 and a_2 as shown in Figure 1.

The vascular volume V_1 was calculated from body weight and hematocrit. The remaining parameters, k, V_2, and permeability constant P (which is related to the diffusion constant D by $P = DS/\delta$, where S and δ are the total interface area and the characteristic thickness of the membrane between both compartments), were obtained by curve-fitting techniques (Hermens *et al.*, to be published). However, an assumption has to be made regarding the release function $f(t)$.

Two different hypotheses concerning $f(t)$ were proposed as showin in Figure 2. In case A, we assume that necrosis is a multiplicative process; i.e., local degradation of cells has an amplifying effect on the necrosis of adjacent tissue, for instance, because of the release of cell contents, e.g., potassium. It can be shown

(Aitchison and Brown, 1963) that, under these circumstances, $f(t)$ will be a log normal function given by

$$f(t) = \frac{Q}{\sigma t\sqrt{2\pi}} \exp\left(-0.5(Int - \mu)^2/\sigma^2\right) \tag{4}$$

This is a three-parameter function in which Q is the total extra release of enzyme per liter vascular volume.

In case B, we assume that, after a period of ischemia, the majority of cells become permeable in a relatively short lapse of time (for instance, in a few minutes). This results in a sudden maximal release of diffusing enzymes with an exponential fall-off which is described by the following function:

$$f(t) = Qbexp(-b(t-t_p)) \qquad t \geqslant t_p \tag{5}$$

which is again a three-parameter function with total release Q.

RESULTS

As reported earlier (Hemker *et al.* 1972), the biphasic disappearance shown in Figure 1 is only observed for PHI, meaning that the plasma clearance curves for CPK, GOT, and LDH (α-HBDH) are monoexponential. This phenomenon is caused by the fact that the enzyme release from the infarcted tissue continues for an appreciable period of time (about 34 hours for patients treated with urokinase and about 48 hours for untreated patients; see Figure 5). Owing to this tailing release, the fast phase of the clearance curve will not be observed in

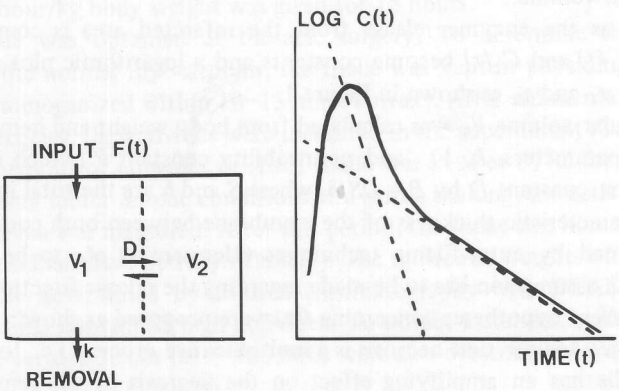

Figure 1. Two-compartment model.

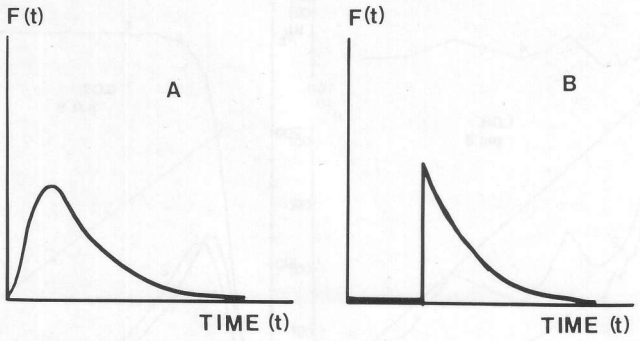

Figure 2. Two hypotheses regarding necrosis; A=multiplicative necrosis, B=sudden necrosis.

those cases where the exchange by diffusion (P) is fast compared to the clearance (k).

PHI is the only enzyme which is metabolized fast enough to show a biphasic disappearance of those patients with a relatively short period of enzyme release (mostly urokinase-treated patients).

The results of curve fitting for PHI showed that, in all cases, the assumption of multiplicative necrosis gave a better fit than the assumption of sudden necrosis; for more details, see the work of Hermens *et al.* (to be published). The best fit parameter values obtained for P and V_2/V_1 are (for PHI):

$$P = 0.15 \pm 0.1 \text{ (liter/hour)}$$

$$V_2/V_1 = 0.30 \pm 0.12 \tag{6}$$

For the molecular weights, the following values were found:

LDH (α–HBDH)	M = 145,000
PHI	M = 98,000
CPK	M = 82,000
GOT	M = 80,000

All values are ± 5,000.

As the diffusion constants of proteins are not very sensitive to molecular weight (Schultze and Heremans, 1966), it is improbable that the values for P and V_2/V_1 will differ much from those found for PHI. Values (6) were therefore used in a routine computer program for all enzymes. In this program, the slow phase of the clearance curve is used to calculate k. Subsequently, the extravascular concentrations, the release function $f(t)$, and the total release are calculated from the plasma concentrations. The results are shown in Figure 3.

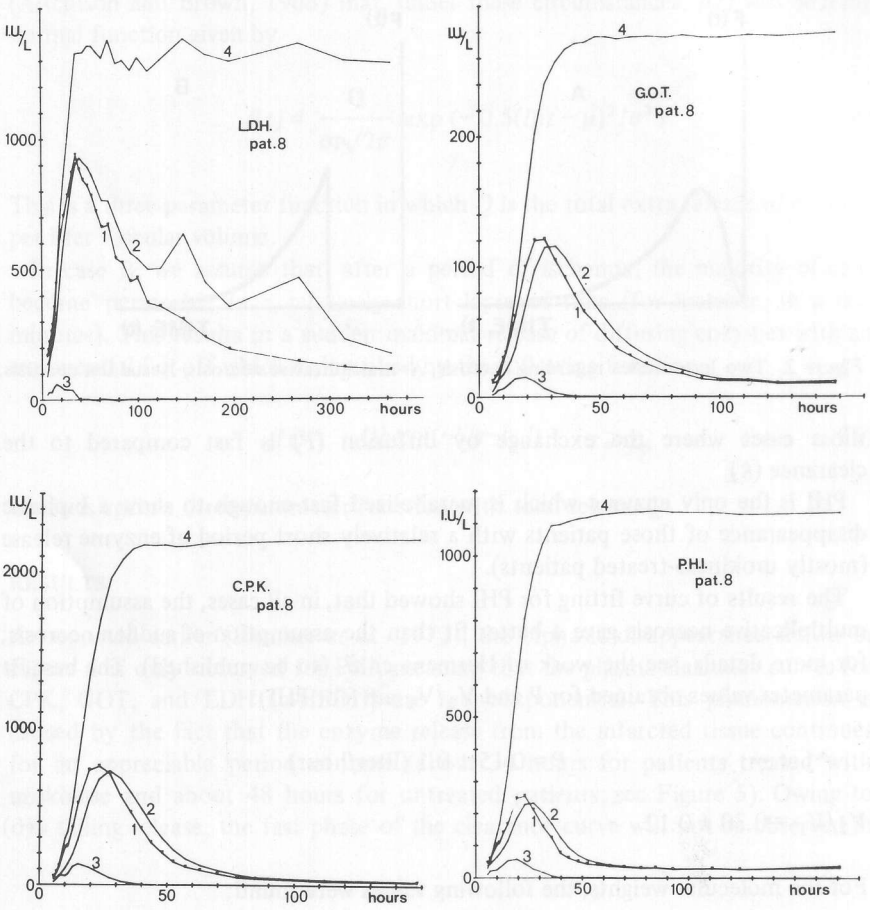

Figure 3. Patient 8. *1* = measured plasma concentrations. *2* = calculated extravascular concentrations. *3* = calculated enzyme release/hour. *4* = calculated total enzyme release.

Figure 4 shows the correlations between the calculated total release Q for the different enzymes compared to LDH. As the clearance for LDH (α-HBDH) is slow (cf. Table 3), this enzyme is the least sensitive to the irregularities in the release and was therefore chosen as reference. The systematic error in Q_{LDH} corresponding to the uncertainty in P and V_2/V_1 is estimated at 10–15%.

The correlation for CPK is not satisfactory, possibly attributable to the fact that this is the only enzyme that showed an unpredictable denaturation during in vitro studies (Witteveen, 1972). This phenomenon could invalidate our assumption of absence of extravascular elimination for this enzyme.

The results of four *in vitro* experiments of anoxic incubation of tissue are

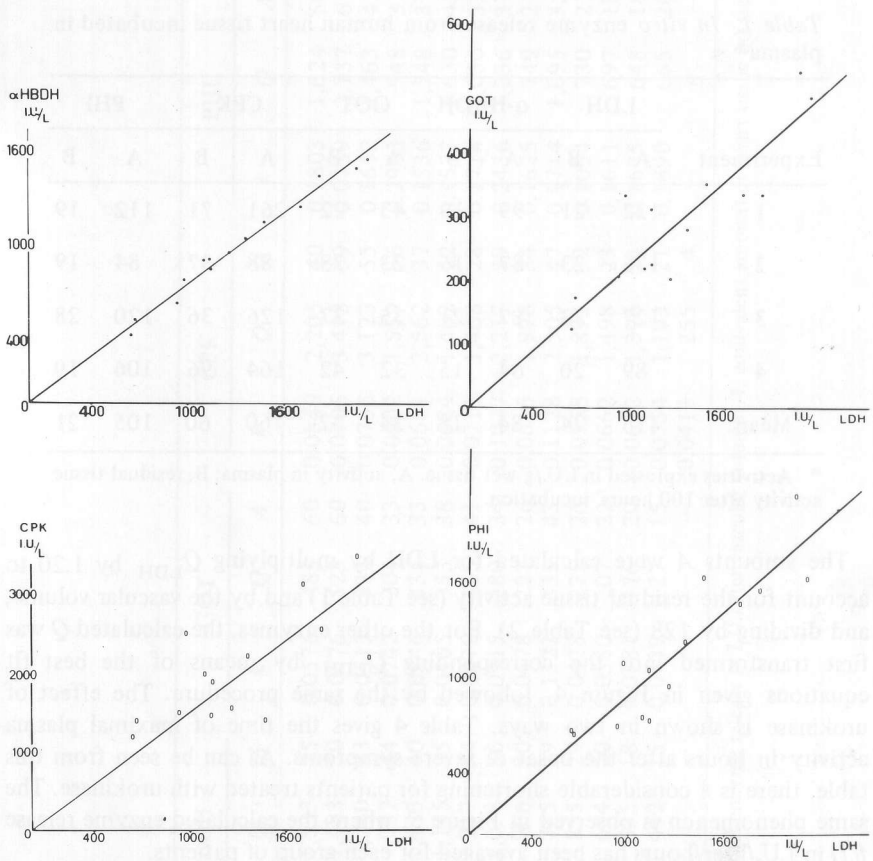

Figure 4. Correlations between calculated total enzyme release. Best fit straight line through the origin for α-HBDH: $y = 0.74x$; GOT: $y=0.22x$; CPK: $y=1.50x$; PHI: $y=0.89x$. 0.89 x.

shown in Table 1. As seen from Figure 4 and Table 1, the calculated total amounts of enzyme released from the infarcted area in patients exhibit approximately the same ratio as the amounts released *in vitro*. However, if we compare these *in vitro* values with the activities measured in fresh tissue (Table 2), we find that, for LDH (α-HBDH) and PHI, the original activities are recovered but, for GOT and CPK, roughly one-third of the activity has disappeared by a local process which apparently (also) proceeds *in vitro*.

Table 3 shows the calculated clearance constants k, total enzyme release Q, and equivalent amount of heart tissue A in grams for all patients except patient 15; in this patient, the levels were too low to permit calculation of LDH, GOT, and PHI.

Table 1. In vitro enzyme release from human heart tissue incubated in plasma*

	LDH		α-HBDH		GOT		CPK		PHI	
Experiment	A	B	A	B	A	B	A	B	A	B
1	122	21	99	19	43	22	261	71	112	19
2	136	23	87	16	23	28	88	37	84	19
3	127	33	87	21	35	37	126	36	120	28
4	89	20	64	15	32	42	164	96	106	19
Mean:	118	24	84	18	34	32	160	60	105	21

* Activities expressed in I.U./g wet tissue. A, activity in plasma; B, residual tissue activity after 100 hours' incubation.

The amounts A were calculated for LDH by multiplying Q_{LDH} by 1.20 to account for the residual tissue activity (see Table 1) and by the vascular volume, and dividing by 128 (see Table 2). For the other enzymes, the calculated Q was first transformed into the corresponding Q_{LDH} by means of the best fit equations given in Figure 4, followed by the same procedure. The effect of urokinase is shown in two ways. Table 4 gives the time of maximal plasma activity in hours after the onset of severe symptoms. As can be seen from this table, there is a considerable shortening for patients treated with urokinase. The same phenomenon is observed in Figure 5, where the calculated enzyme release $f(t)$ in I.U./liter/hours has been averaged for each group of patients.

DISCUSSION

The effect of urokinase in patients presenting electrocardiographic evidence of necrosis, such as elevation of the ST-segment, has been revealed in the multicentered European Urokinase Trial in myocardial infarction. It could be demon-

Table 2. Human heart tissue enzyme activity*

	LDH	α-HBDH	GOT	CPK	PHI
Mean	128	101	96	370	143
S.D.	15.4	11.1	18.2	37.0	14.3

*Activities expressed in I.U./g wet tissue (results of 15 determinations).

Table 3. Calculated parameters*

Patient	LDH			α-HBDH			GOT			CPK			PHI		
	k	*Q*	*A*	*k*	*Q*	*A*	*k*	*Q*	*A*	*k*	*Q*	*A*	*k*	*Q*	*A*
1 ϕ	0.0126	2,113	58	0.0125	1,533	55	0.0576	485	60	0.0849	2,205	40	0.1803	1,624	50
2 ϕ	0.0134	2,042	52	0.0113	1,483	50	0.0576	523	60	0.0705	3,473	59	0.0759	2,137	61
3	0.0157	1,699	45	0.0164	1,240	43	0.0513	334	40	0.0736	3,123	55	0.1652	1,463	43
4	0.0194	1,253	44	0.0202	957	44	0.0742	204	33	0.0602	1,559	36	0.2903	948	37
5	0.0131	1,819	39	0.0133	1,423	40	0.0546	334	33	0.0661	2,562	37	0.1536	1,548	37
6	0.0139	1,469	34	0.0167	1,145	35	0.0405	353	38	0.0684	1,402	22	0.1577	1,630	43
7	0.0182	1,131	32	0.0181	917	34	0.0670	315	41	0.0711	1,468	28	0.1714	1,050	33
8	0.0157	1,355	36	0.0189	1,038	36	0.0715	280	34	0.1037	2,217	39	0.2436	1,236	37
9	0.0129	1,090	38	0.0134	906	30	0.0478	221	26	0.0615	1,998	34	0.1625	749	21
10 ϕ	0.0102	969	27	0.0130	785	29	0.1321	335	43	0.1128	2,505	47	0.1714	1,095	35
11 ϕ	0.0082	1,134	29	0.0086	855	29	0.0428	225	26	0.0558	1,895	32	0.1054	730	21
12 ϕ	0.0135	928	22	0.0122	634	20	0.0580	207	22	0.0662	1,498	23	0.1611	697	18
13	0.0274	664	17	0.0243	535	18	0.0781	176	20	0.0692	1,396	24	0.1675	648	18
14	0.0124	639	17	0.0121	442	15	0.0439	127	15	0.0754	1,193	21	0.1890	665	20
15	–	–	–	–	–	–		–	–	0.0413	255	4	–	–	–

*k, first order clearance constant $(\text{hours})^{-1}$; Q, total enzyme release in I.U./liter vascular volume; A, equivalent amount of heart tissue (g) (see text); and ϕ, patients treated with urokinase.

Table 4. Time of maximal plasma enzyme activity*

	LDH	α-HBDH	GOT	CPK	PHI
A	37.7 (6.3)	39.1 (7.0)	27.6 (4.9)	22.9 (5.4)	22.2 (5.3)
B	25.7 (6.3)	25.7 (6.3)	19.6 (4.2)	17.3 (3.6)	16.8 (2.8)

*Values are expressed as means, with S.D. shown in parentheses. A, mean value for control patients (N = 10); B, mean value for patients treated with urokinase (N = 5). Difference between A and B is significant at a 0.02 level for LDH, α-HBDH, and GOT, and at a 0.05 level for CPK and PHI (Wilcoxon).

strated that the electrocardiogram of patients treated with urokinase showed a more rapid normalization of the ST-segment as compared to the control group (Burkart *et al.*, 1973).

As yet, no important therapeutic advantages of thrombolytic agents have been shown for AMI. Clinical trials on urokinase and another thrombolytic agent, streptokinase, revealed no appreciable effect on mortality in patients treated in coronary care units. In some earlier studies (Verstraete, 1973) in which patients were not treated in coronary care units, a favorable effect was found in trials with streptokinase. Whether this effect implies that there have been fewer fatal arrhythmias in the treated group, a finding which could be explained, for example, by a shorter period of tissue necrosis and, thus, a shorter period of electrical instability, remains to be demonstrated.

The two largest infarctions were found in urokinase-treated patients, with the result that this group showed a somewhat larger average enzyme release than did the control group. This effect is not significant in a rank test (see Table 4) and

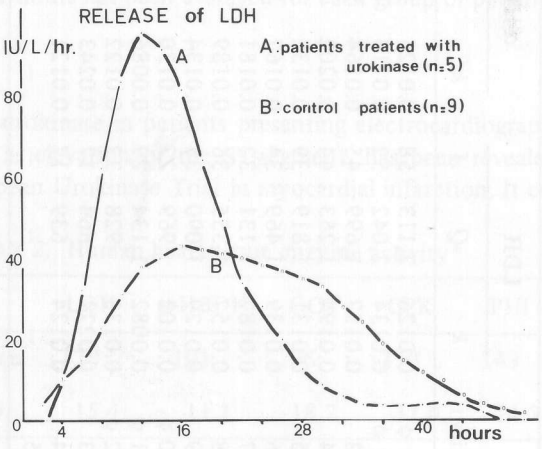

Figure 5. Averaged release functions $f(t)$ for LDH.

might have been caused by the fact that the urokinase treatment was given to early recognized and hospitalized patients, the group that tends to present the larger infarctions. However, it remains to be answered whether urokinase, apart from accelerating the enzyme release, could also enhance it.

As regards the quantitation of the amount of necrotic tissue, it should be noted that, although we account for the amount of enzyme that is not released *in vitro* (20% for LDH) and although the release *in vitro* shows the same ratio for the amounts of different enzymes as the release *in vivo,* there might be an over-all underestimation due to local *in vivo* breakdown of tissue, e.g., by inflammatory processes.

Experiments in which CPK release was studied after experimental AMI in dogs showed that less than 30% of total tissue CPK appeared in the circulation (Shell, Kjekshus, and Sobel, 1971). However, the time course of plasma enzyme activity in dogs differs considerably from that in man; therefore, caution should be used before applying these data in studies of infarction in man.

REFERENCES

AITCHISON, J., and J. A. C. BROWN. 1963. *In* The Lognormal Distribution. , Cambridge.

BURKART, F., F. DUCKERT, P. W. STRAUB, P. G. FRICK, W. SCHWEIZER, und F. KOLLER. 1973. Die fibrinolytische Therapie beim akuten Myokardinfarkt. Schweiz Med. Wschr. 103: 1814.

HEMKER, H. C., S. A. G. J. WITTEVEEN, W. Th. HERMENS, and L. HOLLAAR. 1972. Quantitation of the size of a myocardial infarction by determination of plasma enzymes. *In* H. A. Snellen, H. C. Hemker, P. G. Hugenholtz, and J. H. van BEMMEL (eds.), Quantitation in Cardiology. Leiden University Press, Leiden.

HERMENS, W. Th., S. A. G. J. WITTEVEEN, L. HOLLAAR, and H. C. HEMKER. Study of myocardial necrosis after acute myocardial infarction in man by means of plasma enzyme levels. (To be published).

ROSALKI, S. B. 1967. An improved procedure for serum creatine phosphokinase determination. J. Lab. Clin. Med. 69: 696.

SCHULTZE, H. E., and J. F. HEREMANS. 1966. *In* Molecular Biology of Human Proteins, Vol. 1, p. 176. Elsevier Publishing Company, Amsterdam.

SHELL, W. E., J. K. KJEKSHUS, and B. E. SOBEL. 1971. Quantitative assessment of the extent of myocardial infarction in the conscious dog by means of analysis of serial changes in serum creatine phosphokinase activity. J. Clin. Invest. 50: 2614.

VERSTRAETE, M. 1973. The present status of thrombolytic agents (Review article). Drugs 5: 353.

WEBER, H., und T. WEGMANN. 1968. *In* Atlas der klinischen Enzymologie (Methoden). Georg Thieme Verlag, Stuttgart.

WITTEVEEN, S. A. G. J. 1972. Assessment of the extent of a myocardial infarction on the basis of plasma enzyme levels. Thesis, Leiden.

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