5,13, 27 bzw. 38% (DNA) oder 23, 19, 20 bzw. 39% (PVS) ($n = 5$) vermindert. Nicht dosisabhängig verhielt sich der gleichzeitig registrierte, maximal 200%ige Aktivitätszuwachs im Serum.

Diskussion. Nicht oder schwer abbaubare Makromoleküle, wie Dextran[15] und makromolekulare Schwefelsäureester[16–18], werden u.a. in der Niere, in grösserem Umfange allerdings in Leber und Milz gespeichert. RNA wird nach parenteraler Gabe grösstenteils abgebaut[19], der Rest verteilt sich unter anderem auf Milz, Leber und Niere[20]. Unter vergleichbaren Bedingungen bleibt ein beträchtlicher Teil der DNA (Rubachev et al.[21] veranschlagen ihn aufgrund von Versuchen an der Ratte auf 35–40%) makromolekular oder in Form grösserer Fragmente in Organen des Säugers (unter anderem in der Leber sowie in solchen mit hoher Proliferationstendenz) zurück. Die unter diesen Voraussetzungen nachträglich in den DNase I-Testansatz gelangenden Polyanionen-Mengen liegen vorwiegend um 1–2 Grössenordnungen unter den in vitro[10] wirksamen (ca. 25%ige Hemmung durch 6,5 µg PVS oder 420 µg SP_{54} pro 1,0 ml Ansatz) Polyanionenkonzentrationen.

Zeitlicher Verlauf der DNase I-Reaktion in Serum und Harn von Ratten[22, 23] und Gegenläufigkeit der Aktivitätsänderungen in den Verteilungsräumen Serum und Niere bei der Maus unter dem Einfluss von Polyanionen, GKB und TEM verweisen auf die ursächliche Beteiligung von Umverteilungsphänomenen. Als alleinige Ursache der geschilderten Polyanioneneffekte scheiden sie allerdings aufgrund der bisher vorgelegten Befunde[13, 14] aus. Davon abgesehen, stellen in vitro- und in vivo-Befunde zur Wirkung von makromolekularen Schwefelsäureestern[1–6] und Nukleinsäuren (vor allem der RNA)[19] einen unmittelbareren Eingriff der untersuchten Polyanionen in die Synthese der DNase I zur Diskussion. Die Entscheidung zwischen beiden Alternativen setzt die Markierung des Enzyms in Serum und Niere voraus.

Summary. Induction of DNase I by total-body X-irradiation and/or 2,4,6-triethyleneimino-1,3,5-triazine within kidneys of mice has been inhibited by heparin-like polyanions and enhanced by nucleic acids.

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Plasma Hippuryl-L-Lysine Hydrolase in Tourniquet Shock

It has been reported that the sera or plasma of many animals including human contain a carboxypeptidase which cleaves basic C-terminal amino acids and hydrolyses hippuryl-L-lysine (HLL), hippuryl-L-arginine and other peptides[1]. This enzyme was named carboxypeptidase N, which can be distinguished from the pancreatic carboxypeptidase. Lower levels of hippuryl-L-lysine (HLL) hydrolase in heparinized plasma have been noted in anaphylactic[1] and endotoxin shock[2]. The author observed that this enzyme also decreased in tourniquet shock and that adding aliquots of ultrafiltrated plasma obtained after release of the tourniquets to normal plasma led to a reduction of this enzyme activity. All of these results suggest that

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Table I. Hydrolysis of HLL in plasma before and after application of the tourniquets

Experiment	Enzyme solutions	Before	After release of the tourniquets (min)		
			10	30	60
A)	Plasma 0.1 ml	100	81	61	67
B)	Normal plasma 0.1 ml + Ultrafiltrate 0.01 ml	100	86	72	79

In the experiments A), HLL hydrolysis in plasma is expressed as percent activity and its activity in plasma obtained before application of the tourniquets is 100%. In the experiments B), HLL hydrolysis in normal plasma + ultrafiltrate obtained before application of the tourniquets is 100%.

Table II. Inhibition of plasma HLL hydrolase by corticosteroids and serotonine[a]

Concentration (M)	Corticosteroids				
	Hydrocortisone[b]	Predonisolone[c]	Dexamethasone[d]	Betamethasone[e]	Serotonin
10^{-8}	100	100	100	100	100
10^{-7}	95	94	89	100	95
10^{-6}	87	88	74	94	85
10^{-5}	68	78	51	71	74
10^{-4}	41	55	23	43	57

[a] HLL hydrolysis in samples is expressed as percent activity and HLL hydrolysis in plasma alone is 100%. [b] Solu-Cortef. [c] Predonine [d] DEXA-Sheroson. [e] Rinderon.

some substances, which are released during shock, may be responsible for the activity of HLL hydrolase in plasma. NAKAHARA recently isolated a low molecular weight inhibitor in lysosomes of the skeletal muscle[3]. The present paper was designed to investigate the effects of epinephrine, norepinephrine, histamine, serotonin and corticosteroids, which might be secreted as part of the metabolic response to ischemia, on plasma HLL hydrolase activity.

Mongrel dogs were subjected to tourniquet shock by application of tourniquets to both thighs following the method of the previous paper[4]. Heparinized plasma was obtained before and after release of the tourniquets. 1 ml of plasma cleaves at the rate of 0.95 μM/min[3]. The activity of HLL hydrolase decreased after release of the tourniquets, and reached a minimum 30 min after release. Aliquots of each sample were ultrafiltered using an Amicon Ultrafiltration Chamber with UM-10 membrane. When each ultrafiltrate was added to normal plasma, HLL hydrolase activities decreased suggesting that HLL inhibitors appear in plasma after release of the tourniquets (Table I).

Increase in blood glucose and reduction in the tolerance for glucose after clamp release have been shown in shocked rats[5-7]. The glycogen level in uninjured muscles and the liver continuously decreases after limb ischemia, but a medullectomy can prevent hyperglycemia and loss of glycogen from uninjured muscles[5]. Plasma concentration of epinephrine and norepinephrine in hemorrhagic and anaphylactic shock increases[8,9]. In the present experiment, adrenaline and noradrenaline did not affect HLL hydrolysis of the plasma over a concentration range of 10^{-3} to $10^{-8}M$. Glucose also had no effect on its activity up to 500 mg/100 ml. However, insulin enhanced HLL hydrolysis of plasma; 4×10^{-3} insulin-units showed 42% enhancement of its activity compared with the control. The high initial glycogenolytic rate is generally attributed, in part at least, to secretion of epinephrine and norepinephrine[10].

Relatively large amounts of histamine were released in the peritoneal cavity of rats after release of the tourniquets, but only small amounts of serotonin were present[11]. Serotonin reduced HLL hydrolysis of the plasma; HLL hydrolase was significantly inhibited at a concentration of $10^{-4}M$, whereas histamine had no effect on its activity (Table II).

Hydrocortisone was effective in lowering the level of the lysosomal enzyme after tourniquet release, presumably by stabilizing lysosomal membranes[12]. An increased secretion of adrenal cortical steroids follows operative or traumatic injury[13,14]. It is also known that the adrenal corticosteroids of the 17-hydrocorticoid-type contribute to continued hyperlgycemia[10]. Corticosteroids used in this experiment effectively reduced the levels of the HLL hydrolase with increasing concentrations up to $10^{-4}M$ (Table II). It is thought from the above findings that corticosteroids and serotonin may be responsbile for the reduction of plasma HLL hydrolase levels after ischemia. But, there may be no relationship between the reduction of plasma HLL hydrolase activity by corticosteroids and survival rate following administration of corticosteroids in tourniquet shock. Hydrocortisones were ineffective for increasing the survival rate in tourniquet shock[7,12]. The significance of the reduction of this enzyme level in plasma is under investigation.

Résumé. L'activité de l'hydrolase de la hippuryl-L-lysine dans le plasma du chien est diminuée par l'épreuve du tourniquet. L'hydrocortisone, la prédnisolone, la dexaméthasone, la β-méthasone et la sérotonine réduisent l'activité de cet enzyme in vitro, tandis que l'histamine, l'adrénaline et la noradrénaline sont sans effet.

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Sources of Metabolic Energy used by Isolated Strips Ventricle of Frog Heart for the Uptake and Retention of H^3-Norepinephrine

The uptake and retention of H^3-norepinephrine (H^3NE) by isolated strips ventricle of frog heart is an active process requiring metabolic energy. In a recent paper[1] we obtained evidence that under anoxia and glucose deprivation, the uptake and retention of H^3NE by isolated strips ventricle of frog heart was 42% compared with controls under standard conditions (O_2 plus glucose absent in the incubation medium) and that the energy for this uptake and retention was produced by the glycolysis of endogenous carbohydrates since the pretreatment with iodoacetate (IAA) reduced the uptake and retention to 8%. These results differ greatly from those obtained on isolated atrium of the guinea-pig[2].

However, further experiments carried out in our laboratory under similar circumstances to those described above (isolated strips ventricle of the frog heart, under anoxia plus glucose deprivation) showed significant differences in the H^3NE uptake and retention compared with the results previously described[1].

The present paper reports some of the results lately obtained, and an attempt to connect these results with those previously published. A theory is also proposed, elaborated with the data obtained in this work and in several previous publications[1,3,4], that would explain the sources of metabolic energy utilized for the uptake and retention of H^3NE by isolated strips ventricle of frog heart.

Methods. Ventricles of frog (*Rana pipiens*) were prepared and mounted as previously described by FURCHGOTT et al.[5], for isolated atrium of guinea-pig, Ventricles were suspended in an organ bath containing 20 ml of regular Ringer solution[6], containing 10^{-5} g/ml of ethylene diamine tetraacetic acid (EDTA). A mixture of 95% N_2