EMRICH and ULLRICH[9] showed that diamox inhibited human sweat flow rate. They suggested that it was the transport process responsible for the production of the 'primary sweat' that was inhibited. SCHULTZ et al.[10] and MARTINEZ et al.[11] found from micropuncture studies of human sweat glands and rat submandibular glands that the 'primary secretion' formed in the glomerulum and the acini respectively had a plasma-like composition with chloride as the dominating anion.

The marked inhibition of the secretory rate found in the present work can thus not be explained by a mere inhibition of bicarbonate formation in the acini, but could be explained by an inhibition of LUNDBERG's[4] 'chloride pump'.

Previous unsuccessful attempts by others[12] to inhibit salivary secretion with diamox have probably been due to the employment of too small a dose, and to the fact that the maximal inhibition does not occur until about 5 h after intravenous administration of diamox.

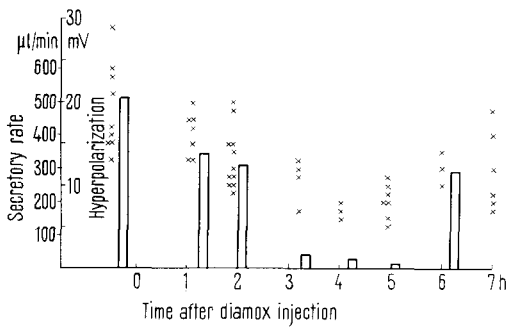

Fig. 3. The time course of inhibition in experiment No. 42 of both secretory potentials and secretory rate. Each cross represents one secretory potential. × = secretory potential; ⌐¬ = salivary flow rate.

Zusammenfassung. Es wird eine Hemmung der Speichelflussrate in der Submandibularisdrüse der Katze durch den Kohlensäureanhydratasehemmstoff Acetazolamid hervorgerufen und gleichzeitig eine Hemmung der sekretorischen Potentialunterschiede über Azinuszellmembrane gemessen. Die Sekretionshemmung liesse sich durch die Hemmung der sekretorischen Potentiale, die ein Mass für einen aktiven Anionentransport ist, erklären.

O. H. PETERSEN and J. H. POULSEN[13,14]

Institute of Medical Physiology A, University of Copenhagen (Denmark), June 24, 1966.

[9] H. M. EMRICH and K. J. ULLRICH, Pflügers Arch. ges. Physiol., in press.
[10] I. SCHULTZ, K. J. ULLRICH, E. FRÖMTER, H. HOLZGREVE, A. FRICK, and U. HEGEL, Pflügers Arch. ges. Physiol. *284*, 360 (1965).
[11] J. R. MARTINEZ, H. HOLZGREVE, and A. VOGEL, Pflügers Arch. ges. Physiol. *283*, R1 (1965).
[12] A. S. V. BURGEN and N. G. EMMELIN, *Physiology of the Salivary Glands* (E. Arnold Ltd., London 1961).
[13] We are greatly indebted to Dr. N. A. THORN for his valuable help and criticism throughout this study.
[14] The work was supported by a grant from the Danish State Research Foundation. – We thank Dr. C. CRONE for loan of various equipment supplied by the F. L. Smidth Foundation and the Danish State Research Foundation.

Effect of Actinomycin D on Mice Infected with the Lactate Dehydrogenase Virus[1]

Following infection of mice with the lactate dehydrogenase (LDH) virus[2], there is a viremia and an elevation in plasma LDH activity which persists for weeks or months until death[3,4]. Although studies have been reported to indicate that the increase in enzyme activity is due to an impaired rate of plasma LDH clearance, related perhaps to an effect of the virus on the host's reticuloendothelial system[5–7], there is recent evidence to suggest that the mechanism of enzyme elevation may also involve an increase in the influx of endogenous LDH into the plasma[8,9]. In an attempt to obtain additional information relevant to these possibilities, experiments were initiated to determine the effect(s) of certain metabolic inhibitors on mice infected with the LDH virus. This paper will describe results obtained in studies with actinomycin D.

Materials and methods. Adult C57BL/Fg mice, weighing 18–20 g, received an intraperitoneal injection (0.1 ml/mouse) of $10^{7.0}$ ID_{50}/ml of virus. 1 h after infection, the animals were injected i.p. with actinomycin D (10 µg/mouse) dissolved in equal parts of ethyl alcohol and phosphate-buffered saline (PBS), pH 7.2. For controls, 28 infected mice received an i.p. injection of the solvent (0.1 ml/mouse). Blood was collected by tail bleeding from experimental and control animals at intervals of from 24 h to 3 weeks after treatment; the plasma LDH activity of each sample was determined as described previously[10]. Plasma samples were then pooled (4 specimens/pool), according to treatment and time after infection, and frozen at – 30 °C. After thawing, serial tenfold dilutions of the plasma pools were prepared in cold PBS and inoculated i.p. (0.1 ml/mouse) into recipient animals. Test mice were bled 1 week after inoculation and

[1] Supported by Public Health Service research grant No. 5 R01 CA05742-05 from the National Cancer Institute.
[2] V. RILEY, F. LILLY, E. HUERTO, and D. BARDELL, Science *132*, 545 (1960).
[3] A. L. NOTKINS and S. J. SHOCHAT, J. exp. Med. *117*, 735 (1963).
[4] P. G. W. PLAGEMANN, K. F. GREGORY, H. E. SWIM, and K. K. W. CHAN, Canad. J. Microbiol. *9*, 75 (1963).
[5] A. L. NOTKINS and C. SCHEELE, J. natn. Cancer Inst. *33*, 741 (1964).
[6] J. M. BAILEY, J. CLOUGH, and M. STEARMAN, Proc. Soc. exp. Biol. Med. *115*, 642 (1964).
[7] B. W. J. MAHY, K. E. K. ROWSON, C. W. PARR, and M. H. SALAMAN, J. exp. Med. *122*, 967 (1965).
[8] V. RILEY, J. D. LOVELESS, M. A. FITZMAURICE, and W. M. SILER, Life Sci. *4*, 487 (1965).
[9] C. G. CRISPENS JR., J. natn. Cancer Inst. *36*, 81 (1966).
[10] C. G. CRISPENS JR., J. natn. Cancer Inst. *34*, 331 (1965).

their plasma LDH levels determined[10]. Infective titers were calculated by the method of REED and MUENCH[11].

4 groups of mice were: (a) injected with actinomycin D (10 μg/mouse) at 1 h after infection; (b) infected only; (c) injected with actinomycin D (10 μg/mouse) only; and (d) not treated. 24 h later, 50% of the animals in each group received i.p. 6500 U of homologous murine LDH obtained from C57BL/Fg mouse erythrocytes as described previously[9]. The mice were bled at intervals thereafter and the LDH activity in each plasma sample was determined. Mean plasma enzyme activities in animals not injected with mouse LDH were subtracted from the mean levels observed in injected mice. The figure obtained was divided by the number of units of homologous LDH administered; this value was then subtracted from 100 to give an estimate of the % LDH cleared in each group at each of the time intervals tested.

Table I. Plasma virus titers in mice treated with actinomycin D 1 h after infection

Time after infection	ID_{50}/ml (log 10)			
	Experimental		Control	
	No. 1	No. 2	No. 1	No. 2
24 h	10.0	10.0	10.0	10.3
48 h	9.5	10.0	8.0	8.5
72 h	8.5	8.5	7.5	8.0
96 h	9.0	8.0	7.5	7.5
120 h	8.5	8.0	7.5	6.5
1 week	8.0	7.5	7.3	6.5
2 weeks	7.0	7.0	6.8	6.5
3 weeks	7.0	6.5	7.0	6.5

Table II. Plasma lactate dehydrogenase (LDH) levels in mice treated with actinomycin D 1 h after infection

Time after infection	Plasma LDH (U/ml)			
	Experimental[a]		Control[b]	
	Range	Mean	Range	Mean
24 h	1400– 3300	1950	700–1700	1450
48 h	2700– 4400	3550	2000–2800	2400
72 h	4600– 8400	6200	2900–3700	3400
96 h	4700– 9800	6150	2400–4300	3400
120 h	5400–10,800	7400	3800–5600	4550
1 week	5200– 9900	7050	2500–4900	3700
2 weeks	3300– 4700	4000	3200–4300	3750
3 weeks	2900– 5200	4250	3100–4900	3850

[a] Values shown for each time interval represent a compilation of data from 16–30 animals. [b] Values shown for each time interval represent a compilation of data from 8–12 mice.

Table III. Clearance of lactate dehydrogenase (LDH) from the plasma of normal and infected mice treated with actinomycin D

Time after injection (h)	% homologous LDH cleared[a]			
	Infected actinomycin-treated	Actinomycin-treated only	Infected only	Not treated
24	23	65	68	80
48	40	93	91	97
72	52	100	94	100
96	73	100	98	100
120	94	–	100	–
144	100	–	100	–
168	100	–	–	–

[a] Each % represents a compilation of data from 8–12 mice in 2 separate experiments.

Results. Table I shows that virus titers were 1–1.5 logarithms higher during the period of 48 h to 1 week in mice injected with actinomycin D 1 h after infection than in infected animals not treated with this antibiotic. Since high interferon titers have been reported in the plasma of mice 24 h after infection[12], an inhibition of interferon production by actinomycin D[13] appears to be a likely explanation for this finding. An alternative possibility, that the increase in virus titers was due to a delay in antibody production caused by actinomycin D[14], is inconsistent with recent evidence to indicate that neutralizing antibodies are not demonstrable in mouse plasma until 21 days after LDH virus infection[15].

In addition to the increase in virus titers, mice injected with actinomycin D 1 h after infection had plasma LDH levels which were approximately 2 times higher than those observed in control animals 24 h to 1 week post-inoculation (Table II). It would appear that the higher enzyme levels can be accounted for on the basis of an impaired rate of plasma LDH clearance, since homologous mouse LDH, introduced by intraperitoneal injection, was cleared more slowly from the plasma of infected actinomycin-treated mice than from the plasma of infected animals not treated with this drug (Table III). The possibility remains, however, that the elevation in enzyme activity also involved, at least in part, an increase in the influx of endogenous LDH into the plasma related to the increase in virus production. At present, the evidence is not sufficient to permit a conclusion.

Résumé. Chez la souris traitée avec de l'actinomycine D, 1 h après injection de virus LDH, la quantité de l'enzyme LDH dans le plasma sanguin et le taux de virus sont élevés. Ces résultats sont discutés au point de vue d'une inhibition possible d'interferone et du mechanisme responsable de l'augmentation de la quantité de l'enzyme LDH.

CH. G. CRISPENS JR.[16]

Department of Anatomy, University of Maryland School of Medicine, Baltimore (Maryland, USA), June 20, 1966.

[11] L. J. REED and H. MUENCH, Am. J. Hyg. *27*, 493 (1938).
[12] S. BARON, H. G. DU BUY, C. E. BUCKLER, and M. L. JOHNSON, Proc. Soc. exp. Biol. Med. *117*, 338 (1964).
[13] H. B. LEVY, D. AXELROD, and S. BARON, Proc. Soc. exp. Biol. Med. *118*, 384 (1965).
[14] C. J. WUST, C. L. GALL, and G. D. NOVELLI, Science *143*, 1041 (1964).
[15] K. E. K. ROWSON, B. W. J. MAHY, and M. BENDINELLI, Virology *28*, 775 (1966).
[16] I am indebted to Miss IRIS CARTER for her technical assistance.