Intestines from pretreated rabbits responded in an apparently normal way to sympathetic nerve stimulation *in vitro* in the presence of ethanol (< 1.0% w/v) or acetaldehyde (< 0.01% w/v).

Conclusions. The results suggest that a decrease in peripheral vascular resistance is a major contributing cause of the hypotension during the Antabuse-alcohol reaction. No evidence of any impaired function in the sympathetic nervous system was obtained. This indicates that the hypotension is not primarily due to a blockade of the vasoconstrictor system. However, the homeostatic regulation of the blood pressure is notoriously poor in the rabbit, and this probably explains why the hypotension during the Antabuse-alcohol reaction is so pronounced in this animal.

The present findings seem to provide some experimental support for the clinical observation[3] that exogenous administration of the sympathetic transmitter (= noradrenaline i.v.) may be an adequate supportive therapy for the serious hypotension which can occur during Antabuse-alcohol reactions in man.

A more detailed report[4] of these studies will appear in the near future[5].

Résumé. Nos études sur le système circulatoire des lapins anesthésiés à l'uréthane montrent que la baisse de la résistance vasculaire périphérique, provoquée par un facteur transporté par le sang, est une cause majeure contribuant à l'hypotension pendant la réaction Antabuse-Alcool. Aucune évidence de la présence d'un bloc primaire au niveau du système vasoconstricteur n'a été constatée.

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[3] E. JACOBSEN, *Journées Thérapeutiques de Paris* (G. Doin et Cie, Paris 1958), p. 89.

[4] E. S. PERMAN, Acta physiol. scand. *55*, Suppl. 190 (1962).

[5] Research grants from the Alcohol Research Committee of the Swedish Medical Research Council (to E.S.P.) and from Magnus Bergvalls Stiftelse (to S.B.) are gratefully acknowledged.

In vitro Guanidino-Resistance and Guanidino-Dependence of Poliovirus

The discovery of the *in vitro* antipolio activity of guanidine[1,2] has been quickly followed by the observation that the poliovirus becomes easily resistant to this chemical[3,4]. It seemed to us useful, from the view-point of viral biology and from that of chemotherapy, to study in detail some features of this resistance.

The technical procedure employed in these *in vitro* experiments has been described in detail elsewhere[5]. In brief, we have been able to confirm that a guanidine-resistance easily develops in poliovirus 1 and 2. As shown in Table I, it is possible to obtain an appreciable degree of resistance after only 4–5 transfers in HeLa cell cultures containing increasing amounts of guanidine HCl (from 1/16000 to 1/4000 which is the maximum dose tolerated by the cultured cells). However, when the initial amount of guanidine in the medium is very high (1/4000) it is never possible to isolate resistant viruses.

The stability of the guanidine-resistance was then investigated. A strain of guanidine-resistant poliovirus was subjected to passages in guanidine-free HeLa cell cultures. After 10 transfers (Table II), a clear diminution of its guanidine-resistance was observed and after 30 transfers the resistant virus had a guanidine-sensitivity very similar to that of the original sensitive virus.

These results suggest that the guanidine-resistance evoked *in vitro* should be considered as an adaptative character of the virus-cell system rather than a genetic one. Other experiments have demonstrated that the virus which has become guanidine-resistant in HeLa cell cultures shows the same degree of resistance even when propagated in other cell-lines. It seems, therefore, that the resistance we induced is a property acquired by the virus or by a virus-cell system not limited to a single cellular type – for instance the virus plus the 'receptor' described by HOLLAND and MCCLAREN[6].

Finally, we have observed that guanidine not only interferes with, but even enhances the viral growth. In fact, if the transfers of a resistant virus in cell cultures

Tab. I. Guanidine resistance induced in poliovirus strains (CPU present in culture media after 4 or 5 serial passages[a])

	Virus	CPU[a] without guanidine	with guanidine γ/ml	
1 S:	Polio 1 Strain (Brunhenders)	10^6	250	<10
			125	10
			83	10^2
			62	10^3
			50	10^5
			33	10^6
1 R:	1 S propagated in HeLa cells once with 62 and 3 times with 250 γ/ml of guanidine HCl	10^6	250	10^6
1 Rx:	1 S propagated in HeLa cells 4 times with 250 γ/ml of guanidine HCl	10^3	250	<10
			125	<10
			83	<10
			62	<10
			50	10
			33	10
2 S:	Polio 2, MEF 1; mouse adapted	10^6	250	<10
2 R:	2 S propagated in HeLa cells once with 15, twice with 62 and twice with 250 γ/ml of guanidine HCl	10^6	250	10^6

[a] End-point method, performed in HeLa stationary cultures

[1] B. LODDO, Boll. Soc. Ital. Biol. sper. *37*, 395 (1961). – B. LODDO, W. FERRARI, G. BROTZU, and A. SPANEDDA, Nature *193*, 97 (1962).

[2] W. A. RIGHTSEL, R. J. DICE, R. J. MCALPINE, E. A. TIMM, I. W. MCLEAN, J. G. DIXON, and F. M. SCHABEL Jr., Science *134*, 558 (1961). – D. CROWTHER and J. L. MELNICK, Virology *15*, 65 (1961).

[3] J. L. MELNICK, D. CROWTHER, and J. G. BARRERA ORO, Science *134*, 556 (1961).

[4] J. G. BARRERA ORO and J. L. MELNICK, Texas Repts. Biol. Med. *19*, 528 (1961).

[5] B. LODDO and C. E. ZANDA, Arch. int. Pharmacodyn. Therap. *133* 1 (1961).

[6] J. J. HOLLAND, L. C. MCLAREN, J. exp. Med. *114*, 161 (1961).

containing the maximum dose of guanidine are repeated beyond a certain number, the CPE and the virus replication take place only, or at least more efficiently, in the presence of guanidine (Table III) than in its absence. That is, the virus-cell system is capable, perhaps by means of enzymatic adjustments, not only of coping with the toxic action of guanidine but also of utilizing this substance for its own replication. While the resistance of some viruses to some chemotherapeutic agents has been observed several times, we are not aware of any previous demonstration of the transformation of a viral inhibitor into a viral growth-factor.

Tab. II. Reversibility of the guanidino-resistance induced in polio 1 (see Table I)

Virus	CPU	
	without guanidine	with guanidine HCl 250 γ/ml
1 S	10^6	<10
1 R	10^6	10^6
1 R 15: 1 R after 15 transfers in presence of 250 γ/ml of guanidine HCl	10^6	10^7
1 R 15 S: 1 R 15 after 30 passages without guanidine HCl	10^8	10^5

Tab. III. Guanidine-dependence induced in polio 1 (see Tables I and II)

Virus	CPU	
	without guanidine	with guanidine HCl 250 γ/ml
1 R	10^6	10^6
1 R 15	10^6	10^7
1 D: 1 R 15 after other 30 passages in presence of 250γ/ml of guanidine HCl	10^8	10^8
1 D diluted 1/10000	<10	10^4

The most obvious conclusion is to consider the resistance of a virus as a selective phenomenon acting at the genetic level. However, our results indicate that the resistance that we can induce in polio virus against guanidine must be considered as an acquired and unstable characteristic.

Considering these facts under a broader aspect, they strongly support the idea of the virus as a living organism which is more complicated than generally supposed and perhaps more autonomous with respect to the host cell. From the view-point of chemotherapy, it is important to know that a viral agent does not seem to be, in its fundamental aspects, very different from the schyzomycetes.

Riassunto. Il virus poliomielitico può essere reso, *in vitro*, guanidino-resistente se propagato serialmente in culture cellulari contenenti dosi crescenti di guanidina. Proseguendo nei passaggi seriali in presenza delle dosi massime di guanidina tollerate dalle cellule, si ottiene un ceppo di polio virus che si sviluppa assai meglio in terreni contenenti la sostanza che non in terreni che ne siano privi.

Tale caratteristica, che richiama quella della antibiotico-dipendenza di alcuni schizomiceti, sembra deporre per una autonomia del virus rispetto alla cellula ospite maggiore di quanto sinora ritenuto.

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An Evidence for Decrease of Energy Production in Thiamine Deficient Failing Rat Heart[1]

Investigations on alterations of the mechanisms of energy production is one avenue of gaining insight into the conditions at the cellular level leading to heart failure. Gertler[2], and Schwartz and Lee[3] found marked decrease in the efficiency of oxidative phosphorylation in heart mitochondria (sarcosomes) isolated from guinea pigs subjected to experimental aortic constriction. The relationship between efficiency of oxidative phosphorylation and shape changes of muscle mitochondria has been strikingly illustrated by Harman and Feigelson[4]. Another change of mitochondrial morphology, reversible swelling, which also influences the efficiency of oxidative phosphorylation, may be induced by hypotonicity and different chemical agents[5–7] and reversed by the addition of adenosinetriphosphate[8] (ATP). Mitochondrial swelling may be inhibited also by citric cycle substrates leading to the *de novo* synthesis of ATP[9]. In the present report such substrate inhibition of swelling has been used to detect alteration of the sarcosomal oxidative system in the thiamine deficient failing heart.

Sprague-Dawley male rats (range 300–400 g) received Purina Chow or a dextrose–casein base semi-synthetic diet[10] containing 3 mg/kg (normals), 0.7 mg/kg (thiamine low) and 0 mg/kg (thiamine devoid) levels of thiamine. The hearts obtained from the animals on the two deficient diets were flabby and had heart-weight per body-weight ratios significantly higher than the normals (Yoshitoshi

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[2] M. M. Gertler, Proc. Soc. exp. Biol. Med. *106*, 109 (1961).
[3] A. Schwartz and K. S. Lee, Circulation Res. *10*, 321 (1962).
[4] J. W. Harman and M. Feigelson, Exp. Cell Res. *3*, 509 (1952).
[5] J. Raaflaub, Helv. physiol. pharmacol. Acta *11*, 142, 157 (1953).
[6] D. F. Tapley, J. biol. Chem. *222*, 325, 341 (1956).
[7] D. F. Tapley, C. Cooper, and A. L. Lehninger, Biochim. biophys. Acta *18*, 597 (1955).
[8] A. L. Lehninger, J. biol. Chem. *234*, 2465 (1959).
[9] G. DiSabato and A. Fonnesu, Biochim. biophys. Acta *35*, 358 (1959).
[10] J. C. Arcos, H. H. Gosch, and D. Zickafoose, J. biophys. biochem. Cytol. *10*, 23 (1961).