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	10^3	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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et al.[11]). The isolation procedure of the sarcosomes differed from the method[10,12] for liver mitochondria as follows: 10% homogenates were prepared with special coarse-surfaced glass homogenizers[13] in 0.44 M sucrose *without* ethylenediaminetetraacetate (EDTA); after a first centrifugation at 580 × g, for total recovery of the sarcosomes the nuclei and cell debris sediment was extracted twice by re-homogenization in sucrose and re-centrifugation, and the three supernatants pooled for the high-speed sedimentation as described[12]. The addition of EDTA to the isolation medium had to be abandoned because, unlike with liver mitochondria, very low heart sarcosome yields were obtained in the presence of this agent. Systematic study indicated that there is an increased 'trapping' of the sarcosomes in the homogenate at concentrations of EDTA above $3.5 \times 10^{-4} M$, which may well be related to its chelating (or cross-linking) ability. Microscopic examination with phase contrast indicated that the sarcosomal particles isolated by the present procedure were not swollen and had a highly dynamic and mobile appearance; addition of a small drop of $1 \times 10^{-2} M$ ATP to the preparation on the slide caused clustering and immediate cessation of all sarcosomal movement. The swelling assays (photometrically, at 525 mμ) were carried out as previously[10,12] except that the test system used here is the one given in the legend to the Figure. In all swelling assays the initial absorbance was adjusted to 0.300, and the percentage swelling at 40 min was used. Each value given represents the average from five individual rats.

Since thiamine is a component of the coenzyme of pyruvate decarboxylase, its deficiency should constitute, at the main entry of the aerobic segment of carbohydrate metabolism, a biochemical block. Although this is possibly a major facet of the decreased energy production in the failing beri-beri heart, no evidence appears to have been furnished for the decrease of ATP synthesis, originating from glucose as energy source, in thiamine deficient sarcosomes. The data presented in the Figure show that as a result of progressive thiamine deficiency there is, after an initial compensatory response, a successive decrease in the availability of pyruvate for mitochondrial ATP synthesis as evidenced by decrease of the inhibition of sarcosomal swelling produced by the pyruvate plus fumarate substrate combination. During these experiments the unexpected observation was made that the decrease of energy production in thiamine deficiency due to this block is further aggravated by an actual decrease of the number of sarcosomes per unit weight of tissue (Table). The maintenance of rats on the 'thiamine devoid' and 'thiamine low' diets does not appear to produce, however, alterations in the sarcosomal membrane at the level of receptor sites for swelling-inducer compounds: diphosphopyridine nucleotide (DPN) $8 \times 10^{-3} M$; pentachlorophenol (PCP) $1 \times 10^{-3} M$; p-chloromercuribenzoate (PCMB) $1 \times 10^{-4} M$; thyroxine $3 \times 10^{-5} M$; $HgCl_2$ $1 \times 10^{-5} M$; $CaCl_2$ $5 \times 10^{-3} M$ and Na_2HPO_4 $1 \times 10^{-3} M$. *In fact*, unlike the drastic changes in the swelling ratio measuring substrate-produced inhibition (Figure), there is no noticeable change, relatively to the normal, in the swelling induced by these agents after respectively 5 and 10 weeks feeding of the thiamine deficient diets.

Compared to the swelling of liver mitochondria, sarcosomal swelling is much less affected by a wide range of osmolarities, which is in agreement with the relative stability of oxidative phosphorylation in sarcosomes[15] under hypotonic conditions. Sarcosomal swelling showed a series of statistically significant minima and

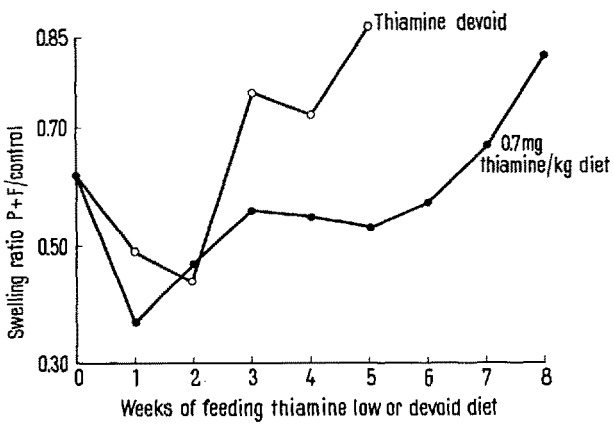

Fig. Effect of thiamine deficiency on the inhibition of rat heart sarcosomal swelling by pyruvate plus fumarate. The test system consisted of 1 ml 0.04 M sucrose buffered with 0.03 M trishydroxymethylaminomethane, at pH 7.4. Both substrates were at the concentration of $1 \times 10^{-2} M$, and the use of this combination corresponds to maximal loading of the oxidative system[14]. Each point on the curves represents the ratio of percentage swelling in the presence of substrates over the percentage swelling in the absence of substrates (control), both at the same time interval of feeding. Since ATP both inhibits and reverses the swelling of mitochondrial particles, this ratio gives a measure of the utilizability of pyruvate for ATP synthesis at different stages of thiamine withdrawal. The inhibition of swelling in the *simultaneous* presence of the two substrates is statistically significant ($0.02 < p < 0.05$), while there is no significant inhibition with pyruvate alone ($0.60 < p < 0.70$) and with fumarate alone ($0.40 < p < 0.50$). Furthermore, the observed decrease of swelling by the substrate combination is not due to possibly increased lability of the sarcosomal structures and thus leakage of DPN from the particles, since the inhibition is not influenced by addition of 0.07 μM of this coenzyme per test system.

Decrease in the sarcosomal content of rat myocardial tissue during feeding thiamine deficient diets. This relative decrease was followed by measuring the volume of sarcosomal 'stock suspension' (containing particles from 1 g of tissue per ml) necessary to obtain a standard 0.300 initial optical density in the test system. Alteration of sarcosome content per unit weight of tissue is expressed as percentage of inverse change of 'stock suspension' volume (average normal, 0.065 ml = 100%)

Weeks of feeding	Relative sarcosomal yield at 0 mg thiamine	at 0.7 mg thiamine
Control	100%	100%
1	106	116
2	106	102
3	95	100
4	81	98
5	65	106
6	—	104
7	—	93
8	—	97
10	—	80

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maxima between 0.01 and 0.30 *M* sucrose concentrations, the greatest swelling occurring at 0.04 *M*, which was the molarity used in the thiamine deficiency studies. Liver mitochondria, on the other hand, showed one unique maximum at 0.15 *M*. The variety of agents that induce or enhance the swelling of sarcosomes was found by us to be much more restricted [16] than for liver mitochondria [6, 8]. The sarcosomal receptors for swelling-inducer compounds appear to be sites in the pharmacological and enzyme kinetic sense. It was now found that the sarcosomal swelling reaction velocities *versus* concentration of inducer (DPN, PCP, or PCMB) follow the Michaelis-Menten equation (liver mitochondria, see [17]).

Résumé. La combinaison des substrats – acides pyruvique et fumarique – inhibent le gonflement de mitochondries isolées du coeur de rat, en accord avec le maintien de l'intégrité de la structure mitochondriale par l'adénosine triphosphate produite. On constate la diminution successive de cette inhibition dans des rats déficients en thiamine, par suite du blocage au niveau de la décarboxylation pyruvique. De plus cette carence vitaminique provoque une décroissance graduelle du nombre des mitochondries dans le tissu cardiaque.

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On the Action of β-Melanocyte Stimulating Hormone (β-MSH) on Spontaneous Electric Discharge of the Transparent Knife Fish, *G. eigenmannia*

In previous communications from these laboratories [1–3], it was reported that β-MSH facilitated submaximally induced spinal reflexes in the cat, although behavioral changes were not observed unless the animal had been depressed previously. Failure to have observed behavioral changes might be attributed to two factors: either β-MSH does not modify behavior except under unusual conditions; or β-MSH modifies behavior, but the change could not be detected.

The second supposition stimulated our interest in quantitation of behavioral changes in a species for which special experience with psychological techniques would not be required.

On the basis of EULER's observation that the nervous system of various fishes contain substance P [4], KRIVOY and LANE (in preparation) found that LSD modified the spontaneous electric discharge of the transparent knife fish. It was further observed that LSD and substance P combined produced a greater effect than LSD alone. The nature of the effect was a decrease in the number of alterations in amplitude of the spontaneous 300 cycle per second discharge which is continuously emitted by this fish. Since we had been able to demonstrate the actions of substance P on this species, it became of interest to determine the effects of β-MSH.

Methods and Materials. Fishes were placed in individual aquaria which had been divided into two unequal compartments by means of a plastic screen. One fish was kept in the smaller compartment and a pair of silver electrodes was placed in the larger. The electrodes were placed close together and at some distance from the fish. The placement of these electrodes was such that they recorded changes in the amplitude of the discharge from the fish-generator, as opposed to changes due to simple geometric manipulation associated with free movements of the fish. The potential was amplified, recorded on tape, and simultaneously monitored on a cathode ray oscilloscope. For analysis of this activity, the tape was played back through an analysis system designed to count the number of changes in amplitude of the 300 cycle per second (cps) signal. A band-pass filter was used to reject artefacts (60 cps etc.) that were recorded with the signal. The signal was then amplified, rectified and integrated, yielding the contour of the 300 cps signal. This signal was then capacitance coupled to a squaring amplifier in a manner which would provide a square wave corresponding to each change in amplitude of the 300 cps signal. These square waves were then counted by means of a decade counter to give the number of amplitude changes. Analysis of the record for changes in the fundamental 300 cps signal showed this not to be influenced by drug administration.

Prior to each experiment, the aquarium was enclosed to maintain the fish in relative darkness for a period of 2 h before any drug was administered. At the end of this time, the electrical activity was recorded on tape for a period of 1 h, and the drug to be tested was then pipetted into the aquarium.

The β-MSH was prepared by the method of SCHALLY et al. [5], by Dr. J. Fischer, of the Armour Pharmaceutical Co. It had a potency of 5×10^6 U/mg.

Results. Disturbances, such as modification of the conductivity of the environment of the fish, or the introduction of drugs (KRIVOY and LANE, in preparation), resulted in the production of a potential of constant strength relative to the control period. The frequency of discharge of each fish was found not to change, even during strong stimulation, or from day to day.

With β-MSH concentrations of 0.1–0.2 μg/ml the frequency of change of amplitude was reduced to from 19–65% of control values ($P = 0.1$ or less). This effect became maximum between 40 and 90 min after introduction of the β-MSH. The changes observed with concentrations below 0.05 μg/ml were not consistently significant ($P \geqq 0.1$).

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