Dans le premier groupe, les bactériophages T_1, T_3, T_5, T_7 provoquent la bactériolyse après un certain temps de latence qui dépend de divers facteurs (quantité de phages ensemencés, moment où cette addition a lieu, etc.). Cette lyse se traduit par une diminution rapide de la «consommation d'oxygène» pouvant aller jusqu'à la suppression presque totale de celle-ci; par suite, la courbe respiratoire fait une chute rapide qui aboutit presque à la valeur zéro. Cette courbe correspond également à celle d'*Esch. coli* 207 jusque dans ses moindres détails.

L'autre groupe qui comprend les bactériophages T_2, T_4, T_6 est caractérisé, après une phase de latence déterminée, par une bactériolyse qui s'effectue plus lentement. La «consommation d'oxygène» s'élève seulement jusqu'à une certaine valeur et y stationne longuement. Cette phase lytique régulière se traduit sur la courbe par un «plateau» qui peut être légèrement descendant; l'intensité respiratoire se maintient à un niveau déterminé ou diminue légèrement en dessous de celui-ci.

On peut en déduire que le dommage subi par les bactéries ou le nombre de celles qui sont atteintes par l'action phagique est différent dans les deux cas.

En outre, nous avons pu vérifier que la lyse produite avec le groupe $T_{2,4,6}$ quoique plus lente, est complète. Celle qui est déployée par $T_{1,3,5,7}$, d'abord beaucoup plus rapide, se révèle par la suite incomplète et montre l'apparition ultérieure d'individus résistants.

Conclusions. Le cours de la bactériolyse, suivi par la mesure du volume d'oxygène utilisé par les bactéries, est différent chez les bactériophages $T_{1,3,5,7}$ et $T_{2,4,6}$. Après un temps de latence, elle est rapide et incomplète avec $T_{1,3,5,7}$; elle est lente avec un «plateau» respiratoire et complète avec $T_{2,4,6}$. Le mécanisme biochimique de la bactériolyse semble donc différent dans les deux cas.

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Laboratoire de Recherche du Département pharmaceutique de CIBA Société Anonyme Bâle, le 2 mars 1959.

Summary

The course of bacteriolysis as determinated by the oxygen consumption of the bacteria is different during bacteriolysis produced by the phages $T_{1,3,5,7}$ as compared with that produced by phages $T_{2,4,6}$. With phages $T_{1,3,5,7}$, the bacteriolysis occurs, rapidly, but incompletely after a latent period. With the phages $T_{2,4,6}$ bacteriolysis occurs slowly and the course of the respiration shows a «plateau» but goes to completion. The biochemical mechanism of the bacteriolysis therefore seems to differ in both cases.

Increase of the Cathepsin Activity of the Liver and of the Skeletal Muscle of Rats treated either with 2,4-Dinitrophenol or with Bacterial Lipopolysaccharide

It is known that protein catabolism is strongly accelerated during fever, as is shown by the increased urinary elimination of nitrogen, especially in the form of ammonia, uric acid, and creatine (GRAFE[1], COLEMANN, and DUBOIS[2], SHAFTER and COLEMANN[3]). The reasons for this increased catabolism are not completely known.

The present paper deals with the behaviour of cathepsin activity of both liver and skeletal muscle of rats injected with two pyrogenic substances, i. e. either with 2,4-dinitrophenol (DNP) or with the lipopolysaccharide of *Salmonella abortus equi* (LPS)[4]. DNP, when injected intraperitoneally in doses of 4 mg/100 g body weight, produces a rise of body temperature of about 1°C within 1 h. LPS does not constantly increase the body temperature of rats, but in some instances it produces hypothermia. When the amount of LPS injected intraperitoneally in the rat was 0·25 µg/100 g body weight, an increase of 0·7–1°C of rectal temperature was recorded in about 70% of the cases studied within 1 h. In the present investigation, only the rats which developed fever were studied.

Albino rats weighing 160–180 g were used. They were fed on a standard diet and their body temperature was recorded for many days before the beginning of the treatment. The animals were killed by decapitation 2 h after the injection. Body temperature was measured before the injection and before the death. The liver or the muscle of the right leg were immediately dissected and transferred to the cold room at + 2°C.

Different groups of animals were used to study the cathepsin activity of the liver and of the skeletal muscle, in order to decrease the time necessary for the preparation of the homogenates. 10% homogenates were prepared in the Potter-Elvehjem homogenizer. Skeletal muscle was previously finely minced with scissors.

In each case two types of suspension fluids were used: the first one was 0·25 *M* sucrose, the second one was 0·25 *M* sucrose containing 0·1% Triton X-100. This was used to produce maximal activation of cathepsin as a result of the disintegration of the particles (lysosomes) in which it is contained (WATTIAUX and DEDUVE[5]).

Cathepsin activity was determined at 37°C by the method of GIANETTO and DEDUVE[6], with 0·17 *M* acetate buffer, pH 5·0, and 0·00026 *M* hemoglobin as substrate. In the case of the homogenates prepared with Triton X-100, also the reaction fluid contained this substance in 0·1% concentration. The reaction was stopped by addition of ice cold 0·3 *M* trichloroacetic acid. Three different amounts of enzyme material were used for each determination: 20, 50, and 100 mg. In each case, two samples were used: in the first one the reaction was stopped 2 min after the beginning of the incubation; in the second one it was stopped after 10 min. The aromatic degradation products of hemoglobin were then determined on the deproteinized filtrate, with tyrosine as standard. The differences between the readings at 10 and at 2 min were retained as a measure of cathepsin activity. The values obtained were submitted to statistical analysis, the standard deviation being calculated for each average and the '*t*' value of Fisher for each difference between two averages. Only the data with a '*t*' value corresponding to a probability $P < 0{\cdot}05$ were accepted as significant.

Table I shows that in the rats treated either with DNP or with LPS, the cathepsin activity of liver homogenates

[1] E. GRAFE, *Die Wärmeregulation und ihre Störungen*, Oppenheimers Handbuch der Biochemie, Ergänzungswerk, vol. 1.

[2] E. DU BOIS, J. Amer. med. Ass. *77*, 35 (1920).

[3] SHAFTER and COLEMAN, Arch. int. Med. *4*, 538 (1909).

[4] The author is indebted to the Firma Wander S.A., Berne, for generous supply of LPS (Pyrexal).

[5] R. WATTIAUX and C. DEDUVE, Biochem. J. *63*, 606 (1956).

[6] R. GIANETTO and C. DEDUVE, Biochem. J. *59*, 433 (1955).

Table I. Behaviour of cathepsin activity of the rat liver after treatment either with 2,4-dinitrophenol (DNP) or with the lipopolysaccharide of *Salmonella abortus equi* (LPS)

The standard deviation is given after each average

	Amount of the enzyme used mg	Normal rats (15 experiments)		Rats treated with DNP (9 experiments)		Rats treated with LPS (8 experiments)	
		µg tyrosine set free in 8 min	µg tyrosine mg N	µg tyrosine set free in 8 min	µg tyrosine mg N	µg tyrosine set free in 8 min	µg tyrosine mg N
Without Triton	20	0	0	0	0	14·4 ± 5·3	26·3 ± 7·8
With Triton	20	32·7 ± 9·6	57·5 ± 32·3	38·5 ± 13.3	68·8 ± 23.7	54·7 ± 14·7	99·3 ± 28·6
Stimulation by Triton %		∞				279·8 +	
Without Triton	50	7·3 ± 7·0	5·1 ± 5·0	17·7 ± 12·0	12·5 ± 8·8	26·1 ± 8·0	19·5 ± 6·2
With Triton	50	62·4 ± 18.3	44·1 ± 16.4	47·3 ± 19·3	33·7 ± 14·9	68·6 ± 24.8	51·0 ± 19·4
Stimulation % by Triton		+754·8		+167·7		+162·8	
Without Triton	100	14·3 ± 9.6	5·1 ± 3.1	46·0 ± 20·7	16·6 ± 8·4	32·0 ± 12·9	11·9 ± 5·1
With Triton	100	93·7 ± 18·8	34·0 ± 7·8	76·1 ± 28·5	24·9 ± 9·8	87·8 ± 24.0	32·7 ± 9·3
Stimulation % by Triton		+583·2		+65·2		+174·3	

prepared without Triton is strongly increased. No significant differences were noted with homogenates prepared in the presence of Triton X-100. Table II shows that also cathepsin activity of skeletal muscle is strongly increased in treated rats. In this case, however, some differences existed between the animals treated with DNP and those treated with LPS. In fact, in the latter group the cathepsin activity was found to be increased not only in the experiments made in the absence of Triton, but also in those made in the presence of this substance. The extent of the increase was higher in animals treated with LPS than in those treated with DNP.

Since the cathepsin which acts on hemoglobin is located within lysosomes, the fact that the enzyme activity of the liver of treated animals increases in the absence of Triton but remains unmodified in the presence of this substance, suggests that the change is not due to a real increase of the amount of the enzyme contained in the tissue, but to a damage of the particles in which it is contained. In the case of the skeletal muscle of rats treated with LPS, in which the increase was observed also in the presence of Triton X-100, it seems probable that an increase of the amount of the enzyme, or also a different type of activation, play an important role.

The increase of cathepsin activity in the organs of animals treated with pyrogenic substances may have some importance in understanding the mechanism of the accelerated protein catabolism found by many authors during fever.

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Institute of General Pathology of the University of Genoa (Italy), December 9, 1958.

Riassunto

L'attività cateptica dell'omogenato aumenta notevolmente nel fegato e nel muscolo scheletrico di ratti trattati con 2,4-dinitrofenolo, oppure col lipopolisaccaride della *Salmonella abortus equi*, due sostanze piretogene. L'aumento è evidente in animali uccisi 2 h dopo l'iniezione, in fase febbrile. Esso si osserva solo con gli omogenati preparati senza Triton X-100, una sostanza tensioattiva che disintegra i lisosomi, organelli nei quali la catepsina è contenuta. Nessun aumento si osserva, nel caso del fegato, in presenza di Triton. Nel caso del muscolo scheletrico, invece, l'attività cateptica è aumentata anche in presenza di Triton, specialmente negli animali trattati col lipopolisaccaride.

Table II. Behaviour of cathepsin activity of the rat skeletal muscle after treatment either with 2,4-dinitrophenol (DNP) or with the lipopolysaccharide of *S. abortus equi* (LPS)

The standard deviation is given after each average

	Amount of the enzyme used mg	Normal rats (9 experiments)		Rats treated with DNP (8 experiments)		Rats treated with LPS (15 experiments)	
		µg tyrosine set free in 8 min	µg tyrosine mg N	µg tyrosine set free in 8 min	µg tyrosine mg µg	µg tyrosine set free in 8 min	µg tyrosine mg N
Without Triton	20	0	0	7·9 ± 7·7	18·7 ± 18·3	22·6 ± 7·5	50·2 ± 19·1
With Triton	20	22·7 ± 7·1	47·9 ± 16·2	18·3 ± 7·4	43·6 ± 18·2	55·6 ± 12·7	118·1 ± 30·2
Stimulation by Triton %		∞		+131·6		+146·0	
Without Triton	50	13·9 ± 12·8	11·1 ± 8·7	32·7 ± 21·2	29·2 ± 18·1	69·4 ± 18·9	53·0 ± 15·4
With Triton	50	31·2 ± 10·5	24·8 ± 9·8	41·5 ± 18.8	37·8 ± 16·9	115·7 ± 33·3	100·2 ± 35·4
Stimulation % by Triton		+124·4		+ 26·9		+ 65·1	
Without Triton	100	20·7 ± 7·5	8·3 ± 3·5	91·2 ± 30·9	38·8 ± 11·9	79·3 ± 28·4	33·7 ± 12.8
With Triton	100	85·9 ± 13·1	34·9 ± 6·3	96·7 ± 16·7	41·5 ± 6·5	148·9 ± 40·9	63·8 ± 17·1
Stimulation % by Triton		+314·9		+6·0		+87·7	