Zusammenfassung. Werden Magenmuskeln von Hund und Frosch oder Froschherzmuskeln bei 70°C Wärme denaturiert und auf 20°C abgekühlt, so zeigen sie eine relative Wärmekontraktion proportional zur Temperatur. Die auffallendste Erscheinung dieser Kontraktion ist, dass sie mit der Zunahme der initialen Länge zunimmt, und zwar entsprechend dem Verhalten der Kontraktion eines lebenden Muskels bei physiologischer Reizung.

I. SINGH

Department of Physiology, Medical College, Agra (India), August 27, 1962.

Interaction Between 'Liquoid' (Sodium Polyanetholsulphonate) and Lysozyme in the Immune Haemolytic Reaction

A serum deprived of one of the components of the complement (C') constitutes the so-called reagent (R): R_1, R_2, R_3 and R_4 are sera respectively lacking in C'_1, C'_2, C'_3 and C'_4. The haemolytic activity of a reagent is restored only when the C' component, in which the reagent is deficient, is added. Several methods are available for obtaining reagents; all of them are more or less difficult because of the anticomplementary activity which develops in the serum during the treatment. Once a good reagent is obtained, it seems that only the addition of the appropriate C' component can reconstitute the lytic activity.

In the course of a study on the effect of lysozyme on the immune haemolytic reaction, we observed that this enzyme in the presence of 'Liquoid'—inactivated serum, in the form of R_3, following HEIDELBERGER and MULLER[1], induces lysis of sensitized erythrocytes (EA). The reagent was prepared by adding 0.30 ml of a 1:1000 solution of sodium polyanetholsulphonate ('Liquoid' Roche) in 0.9% NaCl to 1 ml of guinea pig serum. The mixture was incubated at 37°C for 15 min, cooled and diluted with 4 ml of veronal buffer (MAYER et al.[2]). For the preparation of EA and the methods of the immune haemolytic reaction see PLESCIA et al.[3]. The C' activity was determined by measuring the Hb liberated from the red cells at 5410 Å. The Table summarizes the results obtained when EA and R_3 were incubated in the presence of different amounts of lysozyme.

The mechanism of such an activity by lysozyme was therefore investigated in order to establish whether or not the enzyme could be identified with the C' component inactivated by sodium polyanetholsulphonate.

Red cells preincubated with guinea pig serum at 0°C, having fixed C'_1, C'_4 and C'_2 ($EAC'_{1,4,2}$) were suspended in veronal buffer containing 0.05 M EDTA. These cells undergo lysis only when in contact with C'_3 which is the only component not requiring Ca and Mg ions for fixation. The addition of lysozyme to $EAC'_{1,4,2}$ does not produce lysis of the cells. On the basis of these results, the possibility of identification of lysozyme with C'_3 was discarded.

The following experiments were carried out in order to verify a second hypothesis that the lysis-promoting power of the enzyme could be due to the formation of complexes of lysozyme with the 'Liquoid' previously added to the serum as C'_3 inactivating agent. It is well known that lysozyme forms complexes with substances of different chemical nature having in common the character of being electronegatively charged (CASELLI[4]). Inhibition of lysozyme by sulphonated macromolecules has also been known for a long time (BERGAMINI and FERRARI[5]).

In the present case, the phenomenon described above could be explained as a reactivity of sodium polyanetholsulphonate higher for the enzyme than for the plasma proteins of C'_3 activity furnished.

Reactivation by lysozyme of lytic activity of R_3 (0.2 ml of EA ($1 \cdot 10^9$ cells/ml) + 0.2 ml of R_3 + 0.2 ml of lysozyme solution)

µg of lysozyme added	Optical density at 5410 Å
0	0.020
50	0.080
100	0.100
200	0.115
300	0.350
500	0.620[a]

[a] At complete lysis of the cells the optical density was 0.625.

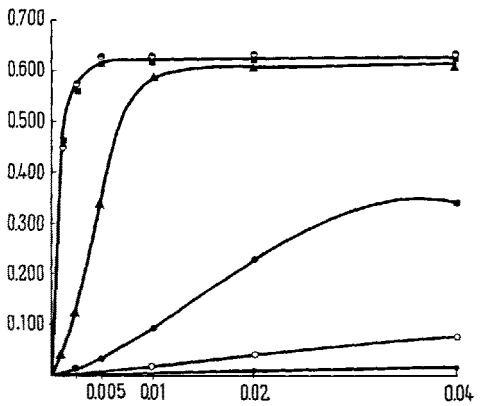

Fig. 1. C' activity of guinea pig serum alone and variously treated. Ordinates: values of the optical density at 5410 Å. Abscisses: ml of guinea pig serum. ·—·—· serum treated with 'Liquoid'; o—o—o serum treated with the supernatant of a mixture containing 500 µg of 'Liquoid' and 100 µg of lysozyme; •—•—• serum treated with the supernatant of a mixture containing 500 µg of 'Liquoid' and 300 µg of lysozyme; Δ—Δ—Δ serum treated with the supernatant of a mixture containing 500 µg of 'Liquoid' and 400 µg of lysozyme; □—□—□ serum treated with the supernatant of a mixture containing 500 µg of 'Liquoid' and 500 µg of lysozyme; ⊖—⊖—⊖ serum untreated. – The mixtures of the reactants were composed by 0.2 ml of EA and 0.2 ml of diluted serum after treatment with «Liquoid» or «Liquoid» + lisozyme.

[1] M. HEIDELBERGER and R. H. MULLER, J. clin. Invest. *28*, 282 (1949).
[2] M. M. MAYER, C. C. CROFT, and M. M. GRAY, J. exp. Med. *88*, 427 (1948).
[3] O. J. PLESCIA, G. CAVALLO, K. AMIRAIAN, and M. HEIDELBERGER, J. Immunol. *80*, 374 (1958).
[4] P. CASELLI, Atti 1° Symp. intern. sul Lisozima di Fleming, Milano (1959), p. 53.
[5] L. BERGAMINI and W. FERRARI, Boll. Ist Sier. Mil. *27*, 98 (1948).

It has been possible to give an indirect demonstration of such an hypothesis.

If a 1:500 solution of 'Liquoid' is mixed with equal volume (1 ml) of a solution containing lysozyme and incubated for 30 min at 37 °C a precipitate is formed in the tube. The clear supernatant gives only partial inactivation of complement when added to the guinea pig serum. The degree of inactivation of C′ is lower the higher the amount of lysozyme added to the solution of 'Liquoid'. Figure 1 shows these results.

It has also been demonstrated that methyl-lysozyme (enzymatically inactive) and protamine sulphate act as lysozyme in reactivating the haemolytic activity of the 'Liquoid' treated serum. These substances, moreover, have a combining power higher than lysozyme.

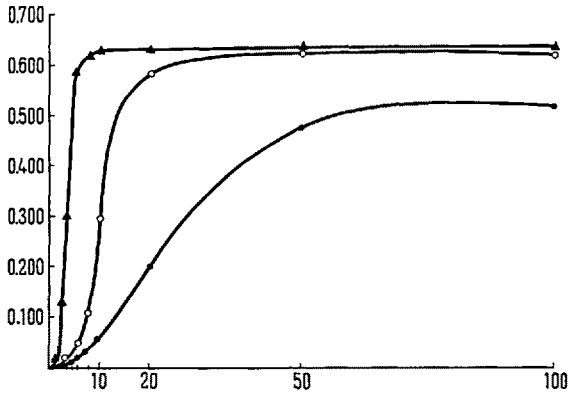

Fig. 2. Action of basic proteins to systems containing 0.2 ml EA + 0.2 ml of 'Liquoid' prepared R_3. Ordinates: values of the optical density at 5410 Å. Abscisses: μg of added proteins. ·—·—· Lysozyme; o—o—o Methyl-lysozyme; Δ—Δ—Δ Protamine sulphate.

This study presented an opportunity to observe that lysozyme plays an aspecific action in its reconstituting haemolytic activity when mixed with a 'Liquoid' prepared reagent. The effect could be due to the formation of complexes with the 'Liquoid' previously fixed to the globulins of C'_3 activity furnished. Such an activity seems to be related to the basic property of lysozyme since other basic proteins act in the same way as lysozyme does.

The possibility should also be considered that a 'Liquoid' prepared R_3 fails to indicate the real content of C'_3 when used for the titration of this component in a serum containing lysozyme.

Riassunto. È stato osservato che il lisozima provoca lisi di emazie sensibilizzate in presenza di un reagente per la titolazione di C'_3, preparato con siero di cavia trattato con 'Liquoid'. È stato studiato il meccanismo di tale azione ed è stato dimostrato che essa è dovuta alla formazione di complessi tra il lisozima ed il 'Liquoid' fissato dalle globuline del siero fornite di attività di C'_3. Tale azione del lisozima è indipendente dall'attività enzimatica in quanto anche il metil-lisozima ed il solfato di protamina provocano lo stesso fenomeno.

G. Cavallo, G. Pontieri, and S. Imperato

Istituto di Patologia Generale, Università di Napoli, and Istituto di Microbiologia, Università di Pisa (Italy), July 24, 1962.

Inter-Relations of the Effects of Psilocybin on Subjective Sensation, Photopic Critical Frequency of Fusion, and Circulating Non-Esterified Fatty Acids

Psilocybin (O-Phosphoryl-4-Hydroxydimethyltryptamine), a hallucinogenic and psychotomimetic agent found in certain fungi of the genus Psilocybe has been synthesized. Its administration significantly alters biochemical, physiological, and psychological behavior. The study to be reported was designed to investigate interrelations among some of these effects.

The subjective elements of the Psilocybin syndrome include awareness of autonomic, perceptual, and general psychological changes. The autonomic effects are predominantly adrenergic; the perceptual effects commonly include distortions of color and detail and hallucination of color and form. The general psychological response includes mild intoxication and changes in psychic function that resemble those of clinical functional psychopathology. The syndrome is not that of a toxic delirium; memory and orientation are preserved.

As Psilocybin so profoundly effects vision it seemed indicated to seek a neurophysiological correlate of this effect. Takashina[1] has reported that lysergic acid diethyl amide, a drug similar in effect to Psilocybin, significantly increases photopic CFF. CFF (critical fusion of frequency) may be defined as the minimal rate at which intermittent photic stimuli are perceived as continuous illuminations. A pilot study as well as the study to be described demonstrate that Psilocybin also has such action. The existence of subjective visual changes and changes in CFF does not in itself demonstrate that these changes are related. They might reflect effects of a common process at psychological and physiological levels or they might reflect the effects of different basic processes. One method of ascertaining relations between two effects is to study their times of onset, height, and diminution. Effects that are temporally coincident are more likely to depend on a common underlying process than are changes that occur at different intervals after drug administration.

In view of the predominantly adrenergic nature of the autonomic symptomatology it seemed of interest to study, directly or indirectly, changes in circulating epinephrine and nor-epinephrine after Psilocybin administration. The direct assay of circulating epinephrine and nor-epinephrine is technically difficult and requires relatively large amounts of blood (a problem if serial determinations are necessary). It has been demonstrated, however, that circulating non-esterified or free fatty acids (FFA) vary with circulating catechol amines in a wide range of circumstances. Changes in circulating FFA are not specific to catechol amines. They depend on an intact and responsive adrenal cortex, are elevated by

[1] K. Takashina, Psychiat. Neurol. Japan *62*, 1745 (1960).