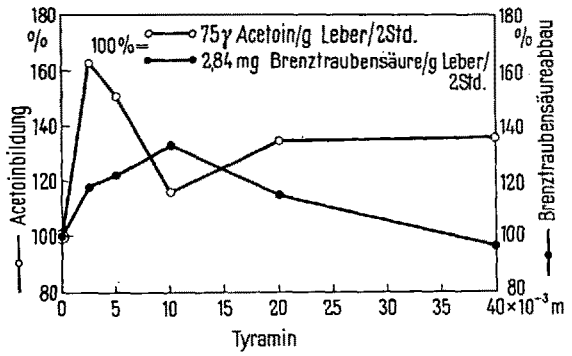

Fig. 1. Der Einfluss von Tyramin auf den Pyruvatabbau und die Acetoinsynthese in Rattenleberhomogenat. – Homogenate: Leber: 0,2 m KH_2PO_4-Na_2HPO_4-Puffer (pH 7,4) = 1:3 (G/V). Ansätze: 2,5 ml Leberhomogenat; Natriumpyruvat 10^{-2} m; Cocarboxylase $4{,}2 \times 10^{-5}$ m; Magnesiumsulfat $1{,}6 \times 10^{-3}$ m; Phosphat 0,11 m; Tyramin $0–4 \times 10^{-2}$ m. pH 7,4. Gesamtvolumen 5 ml. Gasphase: Luft. 2 h Inkubation bei 37°C unter Schütteln. Anschliessend Enteiweissung mit Trichloressigsäure und Bestimmung von Brenztraubensäure[4] und Acetoin[5] in den Filtraten. *Anmerkung:* Bei früheren Untersuchungen wurde nur die Wirkung von 10^{-2} m Amin (Tyramin, Dopamin, Tryptamin) auf die Acetoinsynthese aus Brenztraubensäure untersucht. Die Versuchsansätze waren sonst die gleichen.

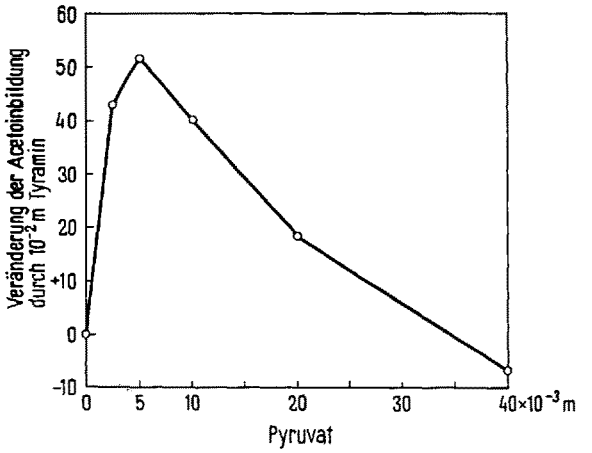

Fig. 2. Der Einfluss von Tyramin auf die Acetoinsynthese in Rattenleberhomogenat in Abhängigkeit der Pyruvatkonzentration. Ansätze: 2,5 ml Leberhomogenat (vgl. Fig. 1); Natriumpyruvat $0–4 \times 10^{-2}$ m; Cocarboxylase $4{,}2 \times 10^{-5}$ m; Magnesiumsulfat $1{,}6 \times 10^{-3}$ m; Phosphat 0,11 m; Tyramin 10^{-2} m. pH 7,4. Gesamtvolumen 5 ml. Gasphase: Luft. Nach 2 h Inkubation bei 37°C unter Schütteln Enteiweissung mit Trichloressigsäure und Bestimmung von Acetoin[5].

weitere Erhöhung der Tyraminkonzentration bewirkte einen Rückgang des Pyruvatabbaus. In einem Falle (Figur 1) wurde bei 4×10^{-2} m Tyramin eine geringe Hemmung des Pyruvatabbaus beobachtet.

Einfluss von Tyramin auf die Acetoinsynthese in Abhängigkeit der Pyruvatkonzentration: Die Wirkung von 10^{-2} m Tyramin auf die Acetoinsynthese in Leberhomogenat in Abhängigkeit der Pyruvatkonzentration zeigt Figur 2. Eine maximale Steigerung der Acetoinbildung wurde bei 5×10^{-3} m Pyruvat erreicht. Bei höheren Pyruvatkonzentrationen nahm die Förderung der Acetoinsynthese durch Tyramin ab. Bei Pyruvatkonzentrationen oberhalb $3{,}5 \times 10^{-2}$ m wirkte Tyramin hemmend. Der Übergang der Tyraminwirkung von Förderung in Hemmung der Acetoinbildung lag bei 4 verschiedenen Homogenaten im Bereich von $2{,}6 \times 10^{-2}$ m bis $3{,}5 \times 10^{-2}$ m Pyruvat.

Diskussion. Die vorliegenden Ergebnisse bestätigen frühere Befunde, wonach Tyramin unter aeroben Bedingungen die Bildung von Acetoin aus Brenztraubensäure in Rattenleberhomogenat beeinflusst. Tyramin bewirkte in der Regel eine Steigerung der Synthesegeschwindigkeit von Acetoin. Figur 1 lässt vermuten, dass diesem Vorgang ein komplexer Mechanismus zugrunde liegt. Eine signifikante Steigerung der Acetoinsynthese wurde nur bei gleichzeitiger oxydativer Desaminierung des zugesetzten Tyramins beobachtet. Es wurde daher vermutet, dass Abbauprodukte des Amins mit dem oxydativen Brenztraubensäureabbau interferieren und dadurch die Bildung von Acetoin begünstigen könnten. Während Ammoniak[3] und *p*-Hydroxyphenylessigsäure wirkungslos waren, führte Tyrosol (*p*-Hydroxyphenyläthanol) zu einer gesteigerten Bildung von Acetoin. Ebenso wurde die Acetoinsynthese durch *p*-Hydroxybenzylalkohol gesteigert, durch *p*-Hydroxybenzoesäure nicht verändert. *p*-Hydroxybenzaldehyd wirkte bei kleinen Konzentrationen fördernd, bei höheren Konzentrationen hemmend auf die Acetoinbildung.

Summary. Tyramine enhanced the production of acetoin from pyruvate in rat liver homogenates. A stimulation of acetoin synthesis was only observed, when tyramine was oxidized during the incubation. Tyrosol (*p*-hydroxyphenylethanol) stimulated acetoin synthesis whereas *p*-hydroxyphenylacetic acid and ammonia were ineffective.

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Medizinische Universitätsklinik, Basel (Schweiz), 16. Oktober 1962.

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STARLING'S Law of the Heart in Cardiac and Unstriated Muscle Killed by Heat

STARLING[1] showed that the energy of contraction of heart muscle is a function of the length of muscle fibres; up to a certain limit, the greater the initial length of the muscle fibres, the stronger the contraction. This holds true with non-cardiac striated muscle as well as unstriated muscle. This greater contraction of muscle with increase in initial length might be due to response of the excitatory system or the contractile system, or both.

That the contractile system may also contribute to the increase in contraction with increase in initial length is

[1] E. H. STARLING, *The Law of the Heart*, Linacre Lecture (London 1918).

suggested by the following experiments. If dog's stomach muscle or frog's stomach muscle is killed by heating to 70°C, it shows a reversible thermal contraction which is proportional to temperature (experimental range 20 to 70°C)[2]. The remarkable feature of this contraction is that it increases with increase in initial length up to a certain point like the contraction of living muscle by a physiological stimulus (Figure 1). The whole of frog's heart can be killed by heating, suspended in a bath, and it shows thermal contraction which obeys STARLING'S law (Figure 2). Dog's stomach muscle killed by heat shows staircase effect and fatigue with slowing of relaxation as shown by living muscle. The resemblance between the behaviour of dead and living muscle is so close that there must be a relationship between the thermal contraction of dead muscle and physiological contraction of living muscle.

It is difficult to explain these results unless one considers the intimate nature of muscular contraction. There is no analogous known physical or chemical system. Hair is known to contract when heated, and ASTBURY[3] has compared this thermal contraction of hair to contraction of muscle, and ascribed it to coiling of the

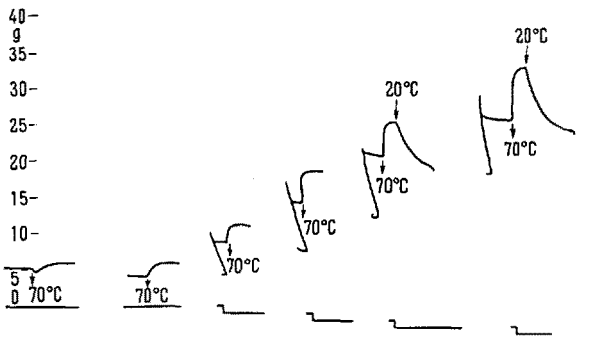

Fig. 1. Dog's stomach muscle killed by heating to 50°C. Shows the effect of initial length on tension produced by thermal contraction caused by raising the temperature from 20 to 70°C. Initial length of muscle 70 mm. Increased by 5 mm each time. As the length is increased, there is increase of resting tension as well as the tension on contraction. Note last contraction is about 4 times the first.

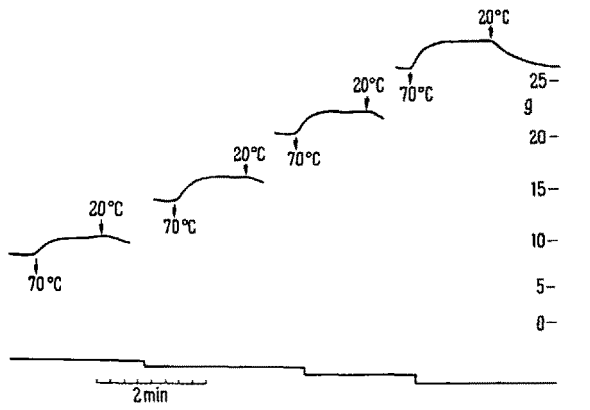

Fig. 2. Frog's heart killed by heating to 50°C (*Rana tigrina*). Suspended as an ordinary piece of unstriated muscle. Effect of initial length on tension produced by thermal contraction caused by raising the temperature from 20 to 70°C. Length increased by 3–4% each time. As the length is increased, there is increase of resting tension as well as the tension on contraction. Note last contraction is double the first.

polypeptide chains. If it is assumed that contraction of unstriated muscle is due to coiling or folding of the polypeptide chains, then increasing their length in some way makes them fold with greater force.

There is some evidence that contraction of unstriated muscle is due to coiling or folding of the polypeptide chains. The effect of various substances on the contractile mechanism can be tested by using the usual glycerol-extracted muscle[4]. In a second method the muscle is allowed to die in solutions of various substances[5,6]. As the muscle dies, its permeability and thus the excitability is destroyed and the substances can come in direct contact with the contractile mechanism. Using both these methods, it has been found that agencies, both physical and chemical, which denature proteins, cause unloaded pieces of the transverse muscle of the frog, *Rana tigrina*, to lengthen without the application of any external force. Denaturation of proteins is caused by acids, bases, amides, detergents, guanidine and urea. Some of these substances cause relaxation of unloaded muscle when treated as above; these are acids, bases, urea, formamide and acetamide. It is, therefore, presumed that the contractile mechanism relaxes actively in these experiments.

Heat is a physical agent that denatures proteins. Its effect on unstriated muscle is most interesting. When unloaded circular strips of frog's stomach muscle are heated to 50°C for 10 min, some of them contract by 2–5% and others lengthen by 10–30%; further heating up to 60°C causes all of them to lengthen by 50–100%. Muscles, which have been heated to 50°C and are thus killed, lengthen further if treated by the above substances which denature proteins. There is no doubt, therefore, that these substances act on the contractile mechanism. The action of heat and chemicals is thus complimentary, and therefore they may most likely have an identical action on the contractile mechanism in causing relaxation.

It is interesting to note that proteolytic enzymes, trypsin, papin and pepsin with hydrochloric acid cause lengthening of unloaded frog's stomach muscle whether living or killed by heating to 50°C[7]; these enzymes are presumed to denature proteins before hydrolysing them. The lengthening of unstriated muscle by heat and other denaturing agents may be considered as a visualizing of the unfolding of peptide chains during denaturation. These experiments therefore elucidate the nature of both denaturation of proteins and relaxation of muscle. But, as it is widely held that protein denaturation is accompanied by the unfolding of polypeptide chains, it is concluded that relaxation of muscle is also due to unfolding of polypeptide chains, and contraction therefore to an opposite process.

If the greater contraction of cardiac muscle with increase in initial length is due to response of the contractile mechanism, this increase in the strength of the contraction should take place without any concomitant increase of energy expenditure, though if the excitatory system also responds, there may be increase in energy expenditure, but the cardiac contraction should become more economical.

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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.

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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.

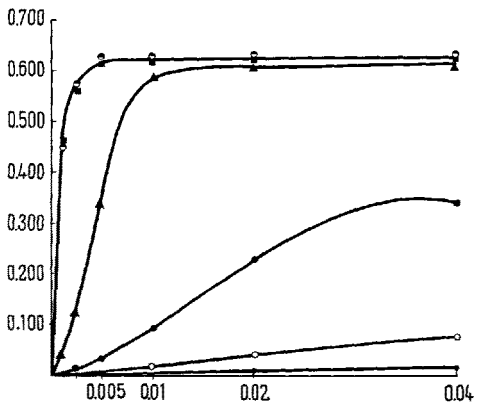

Fig. 1. C' activity of guinea pig serum alone and variously treated. Ordinates: values of the optical density at 5410 Å. Abscisses: ml of guinea pig serum. ·—·—· serum treated with 'Liquoid'; o—o—o serum treated with the supernatant of a mixture containing 500 µg of 'Liquoid' and 100 µg of lysozyme; •—•—• 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; ɵ—ɵ—ɵ 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.

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