because even a cyclopropylmethyl group was removed. In no case did both dealkylation and reduction take place on the same substrate. Among the alkaloids studied, IV (R = H) was the only substrate that was unattacked by any of the organisms used and it is probably significant that it is the only substrate which did not carry any alkyl function on the piperidino-nitrogen.

Previous work on the microbiological transformation of morphine alkaloids has led to a variety of interesting results[11–16], but our findings represent reactions which are apparently novel in this class. Virtually all the positions involved in previous studies are blocked by the ethenobridge in our substrates and the shape of the molecule is rigidly prescribed. It is, therefore, not surprising that our transformations followed another path.

Zusammenfassung. In der vorliegenden Arbeit werden mikrobiologische Umwandlungen von mehreren 6,14-*endo*-Äthenotetrahydrothebain Alkaloiden beschrieben. Mit Hilfe von verschiedenen Mikroorganismen konnten stereospezifische Reduktionen und *N*-Desalkylierungen ausgeführt werden.

L. A. MITSCHER, W. W. ANDRES,
G. O. MORTON and E. L. PATTERSON

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The Variation in Active Tension with Sarcomere Length in Vertebrate Skeletal Muscle and its Relation to Fibre Width

According to the sliding-filament model of muscular contraction[1,2], the tension output is related to the number of active sites formed, and hence to the area of overlap between the 2 sets of interdigitating filaments of the myofibril. As has recently been demonstrated[3,4], the length-tension relation exhibited by the vertebrate skeletal muscle fibre is accordant with this idea. Contraction does not, however, merely involve a longitudinal movement of the myofilaments. Due to the fact that the fibre maintains a virtually constant volume throughout changes in length[5,6], the myofilament packing density, i.e. the centre-to-centre distance between the filaments, may be assumed to vary as an inverse square root function of the sarcomere length. Data obtained in X-ray diffraction studies[7–9] support this view. On this basis it may be assumed that as the number of interacting sites is increased when the fibre shortens, the distance over which the active links have to operate in order to propel the A and I rods relative to each other is steadily increased. For example, by shortening of the sarcomere spacing from 3.6 μ (zero overlap) to 1.4 μ the centre-to-centre distance between the A and I filaments is increased by a factor of 1.6. For an adequate evaluation of the kinetics of the sliding-filament process, it is essential to find out whether the variation in active tension with sarcomere spacing exclusively refers to the length dimension of the contractile system, i.e. the area of overlap between the A and I filaments, or whether the tension output is also dependent on the width of the myofilament lattice. In the present study we have approached this problem by defining the length-tension curve in isolated skeletal muscle fibres that were subjected to various degrees of hydration. Evidence will be presented that changes in width of the fibre do not affect the relation between tetanic output and sarcomere spacing to any substantial degree.

Methods. The recording technique used was similar to that described previously[4,10]. The isolated fibre (dissected from the ventral head of the semitendinosus muscle of *Rana temporaria*) was mounted horizontally in a thermostated Perspex trough (1–2 °C) between an RCA 5734 tension transducer and a light isotonic lever. The resting length of the fibre and the amount of active shortening could be adjusted by means of microscrews in front of and behind the lever. The sarcomere spacing in the middle segment of the fibre was measured at rest for various degrees of stretch of the fibre in the beginning and at the end of the experiment[4]. The sarcomere spacing during activity was derived by cinephotographic recording (64 f/sec, 2.5 msec exposure time) of thin nylon filaments placed on the fibre surface across the fibre axis. Tetanic contractions were produced at 3 min intervals by passing a square pulse train of 1.0–1.5 sec duration (40 c/sec, pulse width 1 msec) through a pair of platinum electrodes mounted in the floor of the Perspex trough. Changes in fibre width produced by altering the osmotic strenght of the Ringer's solution were measured from the cine film (see above) for a given region of the fibre (close to a nylon filament) at 3 different sarcomere spacings (2.5–3.0 μ). The photographic records after enlargement were read to

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1% of the size. The fibre width has been expressed in units of the thickness recorded in the ordinary Ringer's solution. The data given are mean values of measurements from 10 frames at each of 3 different sarcomere spacings. The following solutions were used (mM): (1) ordinary Ringer's solution: NaCl 115.5, KCl 2.0, $CaCl_2$ 1.8, Na-phosphate buffer 2.0, pH 7.0. (2) 'Hypertonic' Ringer: same as (I) except for addition of 50 mM sucrose. (3) 'Hypotonic' Ringer: same as (1) except for reduction of NaCl to 80% of the normal concentration.

Results. The length-tension relation of an isolated fibre immersed in ordinary Ringer's solution is illustrated in Figure 1 (middle curve). The curve has the characteristic shape described previously[3,4], exhibiting a plateau within the sarcomere range 2.2–2.0 μ and a distinct bend at 1.7 μ. Figure 1 also illustrates the length-tension relation of the fibre after increasing (upper curve) and decreasing (lower curve) the fibre diameter by altering the osmolarity of the extracellular medium. The relative measures of the fibre thickness, defined at a given sarcomere length (see above), was 1.08, 1.00 and 0.92 in the hypotonic Ringer, ordinary Ringer and hypertonic Ringer, respectively.

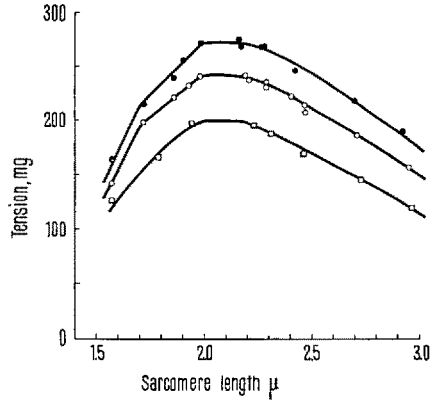

Fig. 1. Relation between tetanic tension and sarcomere length of an isolated frog semitendinosus fibre in ordinary Ringer (o), hypotonic Ringer (•) and hypertonic Ringer (□), respectively. Smallest and largest fibre diameters measured in the ordinary Ringer at 2.50 μ sarcomere spacing: 60 and 140 μ. For further information, see text. From experiment 6. 7. 1967.

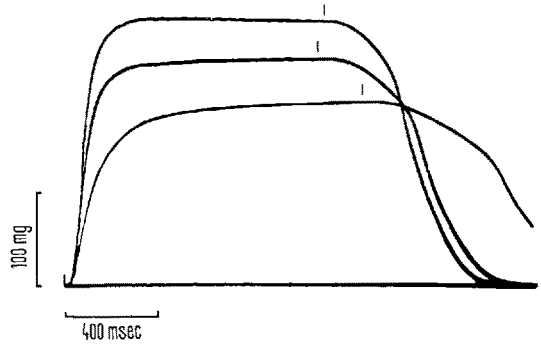

Fig. 2. Photographically superimposed oscilloscope records of tetanic contractions in an isolated semitendinosus fibre of the frog at 2.20 μ sarcomere spacing. Upper: hypotonic Ringer. Middle: ordinary Ringer. Lower: hypertonic Ringer. Smallest and largest fibre diameters measured in the ordinary Ringer at 2.35 μ sarcomere spacing: 70 and 170 μ. Onset and end of stimulation (40 c/sec) indicated by vertical bars. From experiment 20. 6. 1967.

These values agree with measurements of osmotic volume changes of single muscle fibres reported previously by SATO[11] and BLINKS[12]. Under all conditions employed, the fibre was able to produce a fused tetanic contraction (Figure 2). As is evident from Figure 1 and Figure 2, there was a substantial increase (about 15%) of the maximal tetanic output, when the fibre was immersed in the hypotonic solution. Conversely, the maximal tension was lowered (about 20%), when the normal Ringer was replaced by the hypertonic solution. The effects could be fully reversed by re-immersion of the fibre in the ordinary Ringer solution. It should be noted that the shape of the length-tension curve did not undergo any fundamental change by altering the state of hydration of the fibre, i.e. the active tension was affected to virtually the same degree at all sarcomere lengths considered. This is demonstrated in Figure 3, illustrating the data of Figure 1 after recalculation of the tension values as percentage of the maximum output produced in the respective solution. As can be seen there was a very similar shape of the length-tension curves defined in the 3 different osmotic media. The findings described in Figure 1 and Figure 3 have been confirmed in 3 other experiments.

By changing the fibre volume, it was possible to produce a substantial shift of the relation between the length and thickness dimensions of the sarcomere. For instance, the fibre width existing at 2.0 μ sarcomere spacing in the ordinary Ringer solution was made to correspond to 1.70 μ and 2.35 μ sarcomere spacings, respectively, by immersion of the fibre in the hypertonic and hypotonic media. The fact that the relation between mechanical output (taken as percentage of maximum) and sarcomere spacing remained unchanged under these different conditions (Figure 3) would seem to make clear that the length-tension diagram is referable to the length dimension of the contractile system, i.e. to the area of overlap between the 2 sets of interdigitating filaments. The shape of the curve evidently bears very little relation to the actual width of the myofilament lattice.

It is of interest to note that the capacity of the contractile system to produce tension can be raised beyond the level attained in the ordinary Ringer's solution by increasing the degree of hydration of the fibre (cf. BLINKS[12]). The results have shown that the tension is

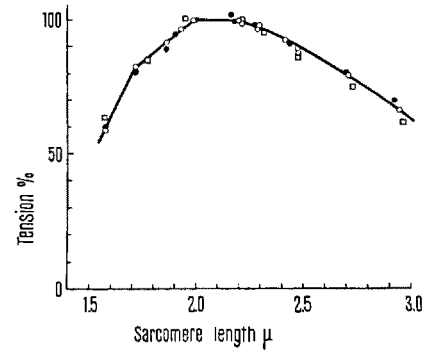

Fig. 3. Tension values of Figure 1 expressed as percentage of maximum tension recorded in the 3 osmotic media used. Meaning of symbols the same as in Figure 1.

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affected to the same proportion at all sarcomere lengths concerned between 1.5 and 3.0 μ. This means, if the length-tension curve is considered an index of the state of overlap between the A and I filaments (see above), that the tension delivered per unit overlap area may be varied over a substantial range by altering the degree of hydration of the fibre. This is an interesting finding because it suggests, in terms of the sliding-filament model, that the mechanical output produced by the individual active link is affected by changing the state of hydration of the cell. The nature of this effect is unclear. It may reflect a change of the interaction between the actin and myosin components due to alterations of the ionic composition[13,14] and the total ionic strength of the intracellular medium[15].

Zusammenfassung. Die Relation zwischen tetanischer Spannung und Sarkomerabstand wurde an der isolierten Semitendinosus-Faser des Frosches bei verschiedenen Graden von Hydratisierung der Faser untersucht. Es trat keine Veränderung des Länge-Spannungs-Verhältnisses auf, obwohl die Relation zwischen Länge-und Querschnittsdimensionen der Muskelfaser durch Immersion in den verschiedenen osmotischen Medien beträchtlich verschoben werden konnte. Die Ergebnisse stützen die Annahme, dass das Länge-Spannungs-Diagramm durch den Grad des Überlappens zwischen den A- und I-Filamenten und nicht durch den Querabstand zwischen den Filamenten bestimmt wird.

K. A. P. EDMAN and K.-E. ANDERSSON

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Distribution of 4-Iodine-Antipyrine (I^{131}) in the Rat

Since SOBERMAN et al.[1] proposed in 1948 the utilization of antipyrine for measurement of body water in mammals, wide experience has been accumulated in several species[1–8]. In some of them it has been shown that the indicator follows closely the distribution of water in the body[1,8].

In 1955 TALSO et al.[9] proposed the utilization of a derivative of antipyrine, the 4-iodine-antipyrine labelled with I^{131} for the measurement of body water in men. In this situation, the antipyrine played the role of a carrier, facilitating the penetration of the indicator (I^{131}) in the body water compartment. The volumes of distribution obtained in men[9] and sheep[10] were consistent and similar to those obtained with other chemical or radioactive indicators.

SULLIVAN and ROSE[11] suggested that an early metabolization of the carrier indicator complex may liberate the indicator (I^{131}), which will join the iodine body pool. Their suggestion was based on the finding of a lower concentration of indicator in brain than in skeletal muscle of rats.

Material and methods. In the course of studies on body composition, determination of body water content was attempted with 4-iodine-antipyrine (I^{131}) in 108 male rats from the strain bred at the Institute of Physiology.

Animals anaesthetized with sodium pentobarbital (40 mg/kg b.w.) were injected i.v. with 4-iodine-antipyrine (I^{131}), the amount injected being determined as in previous experiments in cpm[12].

Measurements were made in 9 groups of 12 animals of similar weight (mean 194.1: S.D. 15.2 g) at different time intervals, after injection: 90, 105, 120, 135, 150, 240, 300, 360 and 420 min. From the results obtained in all groups, the volume of distribution of the indicator was calculated by extrapolation to 'zero time', with the aid of the regression line logarithmic equation. In each group a sample of arterial blood was obtained at the predetermined time and immediately testis, kidney, myocardium, spleen, liver, lung and skin were extracted.

An aliquot of plasma and each organ was placed in plastic containers and their radioactivity in cpm measured in a well scintillation counter, ensuring a similar geometric efficiency for each reading. Volume of distribution in ml/100 g was calculated from the following general equations:

$$\frac{\text{cpm injected} \times 100}{\text{cpm in 1 ml of plasma} \times \text{body weight}} = \text{volume of distribution/100 g body weight}$$

$$\frac{\text{cpm in 1 g of tissue} \times 100}{\text{cpm in 1 ml of plasma}} = \text{volume of distribution/100 g tissue weight}$$

Corrections were made for plasma density to refer the results to water content in ml/100 g.

Results. From the regression line, the slope of disappearance of the indicator from plasma and tissues was calculated and expressed as % of decay of radioactivity in 60 min. The Table shows the results obtained, where

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