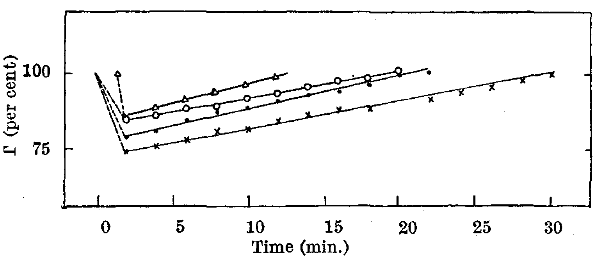
## Adenosine-triphosphate Initiating Contraction and Changing Bi-refringence in Isolated Cross Striated Muscle Fibres

SINCE previous investigations[1] have demonstrated close relationship between myosin and adenosine-triphosphate, it seemed of interest to investigate the action of adenosine-triphosphate and related phosphorus compounds on living isolated muscle fibres.

So far we have used adenosine-triphosphate (isolated from rabbit muscle and purified several times through the mercury and barium salts), inorganic triphosphate, pyrophosphate and orthophosphate. All substances were used in iso-osmotic solutions of *p*H 7·3, part of the sodium chloride in the Ringer solution being replaced by an equivalent amount of the sodium salts of the substances in question.

Micro-application with micropipette and micromanipulator[2] of adenosine-triphosphate in amounts of $2–3 \times 10^{-1}$ μgm. on non-curarized and curarized muscle fibres initiates repeated, twitch-like contractions. These are accompanied by action potentials. Repeated application (with washing out in between) has the same effect.




DECREASE AND RESTITUTION IN PHASE DIFFERENCE (Γ) (ORDINATE) AFTER ELECTRIC TETANIC STIMULATION, —Δ—Δ—.
—o—o—, after application of $2·85 \times 10^{-6}$ mol./ml. aden. triphos.
—.—.—., „ „ „ $4·25 \times 10^{-6}$ „ „ „
—×—×—, „ „ „ $5·7 \times 10^{-6}$. „ „ „

Bi-refringence, examined by means of the Babinet compensator[3], falls 20–30 per cent after application of adenosine-triphosphate ($1·2–7·3 \times 10^{-6}$ mol./ml.) and recovers spontaneously in the course of 15–20 min. The degree of decrease and the duration of recovery depend on the concentration applied, and the time course corresponds closely to that observed after tetanic electric stimulation (see graph).

Cozymase (60 per cent preparation) in concentrations of 0·05–0·5 mgm./ml. is without effect either on the mechanical response or bi-refringence.

Preliminary experiments with inorganic *triphosphate* ($6·1 \times 10^{-6}$ mol./ml.) likewise show release of contraction of a more contracture-like type, the decrease in bi-refringence being only a quarter to a third of that occurring after application of adenosine-triphosphate.

Pyrophosphate in concentrations of $6·7–9·8 \times 10^{-6}$ mol./ml. initiates contraction, and bi-refringence exhibits an irreversible fall of *c.* 40 per cent. Lower concentrations without releasing mechanical responses are accompanied by a decrease in bi-refringence the partial recovery of which takes more than 40 min.

Orthophosphate in concentrations of $2·5 \times 10^{-5}$ mol./ml. likewise initiates contracture-like contractions, but no changes in bi-refringence occur.

The experiments indicate that: (1) In spite of the fact that different investigators[4] find that muscle membranes are impermeable to adenosine-triphosphate, it may react directly or indirectly with contractile and doubly-refracting elements. (2) Adenosine-triphosphate normally could be an agent of contraction, while adenosine-triphosphate present in muscle cannot be in immediate contact with the contractile elements due either to differences in local distribution or to the presence of a non-reactive compound. Addition of adenosine-triphosphate thus would correspond to the establishment of contact between adenosine-triphosphate and contractile elements otherwise initiated by the nervous impulse. (3) In contrast to findings with pure myosin, phosphorus compounds other than adenosine-triphosphate may release contraction and cause changes in bi-refringence.

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## Colour of Small Objects

IF a circle, 2 cm. in diameter, is divided into quadrants and coloured as in Fig. 1, placed on either a black or white ground, and then viewed with one eye from a distance of about 3 m., it will be found that there is considerable difficulty in distinguishing the green from the light blue on one hand and the orange-brown from the light red-purple on the other, so long as the brightnesses of the fields are made as equal as possible. There will be no difficulty in distinguishing the green from the orange, provided that the observer is not red-green colour-blind in the sense that he habitually confuses such colours, and the circle will appear to be divided into two halves, right and left. Red-green colour-blind observers see the circle as uniform, or they may be able to separate the quadrants on subtle brightness differences.

Similar effects will be observed if the quadrants are coloured as in Fig. 2. The result in this case is even more striking since, if the circle is viewed at close range, it divides naturally into a yellow and green upper half and into a purple lower half. At a distance it divides vertically into a greenish-grey left half and a brownish-grey right half. It will be found that each particular green has its corresponding blue or mauve with which it is confused, and each yellow, orange or brown has its own particular reddish-purple.

In both cases, if the eye is focused not on the centre of the circle directly but on the point *A*, about 4 cm. from the centre of the circle, then all the quadrants stand out in their true colours. The point *A* may lie upon any radius of the circle. Similar effects are also obtained if the colours are arranged as in Fig. 3, so that they are probably not due to simultaneous contrast or to the overlapping of the images formed on the retina.