concerning P suggest that both these compounds are produced at the same time from ATP, according to the following reaction:

$$ATP \rightleftharpoons ADP + P \quad (1)$$

The rapid fall of both these compounds after the first 45 minutes of incubation can easily be explained assuming a development of the reaction in the opposite direction.

This fact is also supported by the curve representing ATP, which indicates a resynthesis of this compound.

The curves of AMP and PP, on the other hand, although comparable in their shape, show a considerable discrepancy when values for PP, experimentally determined, are compared with the amount of PP to be expected stoichiometrically, assuming that all AMP is produced from ATP according to reaction (3).

This difference suggests that AMP is formed from ATP by the simultaneous occurrence of the two reactions:

$$(ATP) \rightleftharpoons ADP \longrightarrow AMP + 2P \quad (2)$$

$$ATP \longrightarrow AMP + PP \quad (3)$$

It appears most probable that the partial resynthesis of ATP, occurring when reaction (1) is reversed, accelerates reactions (2) and (3). This fact explains the remarkable increase in the rate of formation of AMP that can be observed after 90 minutes of incubation.

A reaction of the myokinase type can not be excluded: it is, however, inadequate in itself to account for all features of the phenomena quoted above.

A full report of these findings and of other results concerning the features of the enzymic breakdown of ATP under wider experimental conditions will be given together with a kinetic treatment of the data in a forthcoming paper.

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A SIMPLE METHOD OF ANALYSING PAPER-STRIPS IN ELECTROPHORESIS ON FILTER-PAPER

by

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In paper-electrophoresis, which is becoming more and more valuable for routine analysis of serum proteins, the analysing of the paperstrip may take place in two ways. By the first method[1,2,3,4] the paper is cut into a great number of narrow segments, the dye absorbed by the protein is eluted from each segment and the optical density (O.D.) of the elution fluid is measured. Instead of this laborious method GRASSMAN[5,6] uses a faster one. The paper, made transparent by dipping into a suitable liquid, is laid between two glass plates and passed along a slit illuminated by monochromatic parallel light. The O.D. can then be measured point by point by means of a selenium cell and a galvanometer.

For more than a year we have been using a paper-photometer as shown by Fig. 1. The dry paper-strip lies directly, *i.e.* without glass-plates, between the two slits S_1 and S_2. This avoids the necessity of parallel light and therefore the use of lenses. The strip being stained with bromphenol-blue, a sodium vapour lamp is particularly suitable as a monochromatic light source, with, moreover, the advantage that the light-output is very little influenced by mains variations. If a suitable kind

of paper is used (Whatman No. 1) the error caused by irregularities in the paper is negligible in practice. This error can, however, also be corrected, as the O.D. caused by the stain E_s is the difference between the total O.D. E and that caused by the paper E_p. By measuring the E_p curve before the test and subtracting it from the E curve found after the test we can obtain the E_s curve. The sensitivity of the galvanometer is best regulated in such a way that on the point of the paper with the smallest O.D. we read an O.D. zero. An example is given in Fig. 2. When checked it appeared that blank paper without protein after electrophoresis, staining and washing showed exactly the same curve as before and had not altered appreciably in length (1 mm in 270 mm).

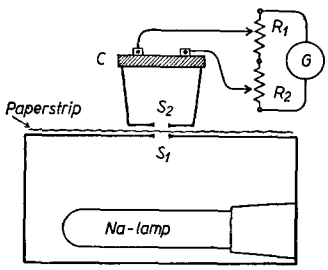

Fig. 1. Paper-photometer, schematic.

C = seleniumcell.
G = mirrorgalvanometer
S_1S_2: slits 1 mm wide
R_1 = sensitivity, coarse
R_2 = sensitivity, fine
R_1+R_2 = critical damping resistance of G

The strip is driven along the slit by a screw with a speed of 1 mm.

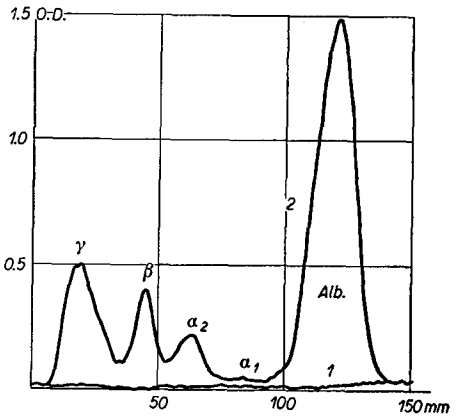

Fig. 2. Normal serum
curve 1: blank paper
curve 2: after electrophoresis
subtraction of 1 and 2 gives the corrected curve.

In using such methods as this and GRASSMAN's it is essential that the testing fluid be applied very evenly perpendicularly to the long side of the paper. This can be shown by the following example. Suppose that so much stain has been sprayed equally over the breadth of the paper in a certain place that the transmission is 50%. Now if the same quantity of stain has been distributed over half the breadth, nothing is absorbed in the unstained half, so that this half alone transmits 50% of the incident light. The total transmission is therefore greater than 50%.

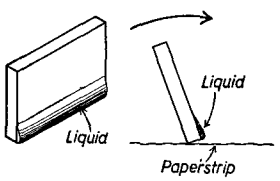

Fig. 3. Method for obtaining equal distribution of the serum

An equal distribution is obtained as follows: A rectangular glassplate a little narrower than the paper-strip is made free of grease and dried. By means of a pipette the required quantity of serum (0.02–0.04 ml) is applied to the glass in a small area on one side (Fig. 3). The glass plate is then placed on the paper in the manner indicated and by tilting in the direction of the arrow the liquid is now brought into contact with the paper over the whole breadth.

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