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Short Communications

Guanosine Triphosphate and Uridine Triphosphate from Muscle

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Upon subjecting adenosine triphosphate (ATP) which has been prepared [1] as the barium salt from rabbit muscle to ion exchange chromatography on the strong base anion exchange resin Dowex-2 according to Cohn and Carter [2], the presence is revealed of a contaminating phosphate fraction in ATP preparations, amounting to 2—4 % of the ATP. By application of the ion exchange procedure directly to trichloroacetic acid extracts of muscle (after removal of the bulk of trichloroacetic acid by ether), similar amounts of the contaminating phosphate fraction are obtained. Phosphorus analysis shows the presence of easily hydrolysable (10 minutes in 1 *N* hydrochloric acid at 100° C) and difficultly hydrolysable phosphate in the ratio of 2 : 1, corresponding to that of a triphosphate. The elution position in ion exchange chromatography is that of inosine triphosphate [3], *i.e.* it is eluted by 1 *N* hydrochloric acid following the removal of ATP. Ultraviolet absorption measurements, however, give an absorption curve differing from that of inosine nucleotides. Paper chromatography in the saturated ammonium sulphate solution — water — *iso*propanol (79 : 19 : 2) solvent system [4] — locating the spots both by ultraviolet photography and spraying with the molybdate reagent of Hanes and Isherwood [5] — reveals the presence of two ultraviolet-absorbing and phosphorus-containing components in the unknown phosphate fraction contaminating ATP. Analyses on the eluted spots give the ultraviolet absorption curve of a guanosine derivative for the slower moving spot and that of a uridine derivative for the faster moving one, and an approximate ratio of 1 mole base : 3 atoms phosphorus for each spot. Pentose estimations [6] indicate the presence of 1 mole pentose per mole base in the slower moving spot and the absence of directly estimable pentose in the faster moving one, as expected for guanosine and uridine derivatives respectively. Paper chromatography after hydrolysis for 60 minutes in 1 *N* hydrochloric acid at 100° C gives two spots, the positions of the spots being identical with those of guanine and uridylic acid (four solvent systems). After hydrolysis for 60 minutes in 70 % perchloric acid [7] at 100° C, two spots with positions identical with those of guanine and uracil — chromatographed side by side with the experimental solution — are obtained (four solvent systems). The eluted guanine spots from the experimental solution and the authentic guanine sample gave identical absorption spectra in acid and alkali. The absorption spectra of the eluted spots from the experimental solution were likewise identical with the corresponding spots from authentic uracil and uridylic acid respectively, both in acid and alkali, and after treatment with bromine. After hydrolysis in 1 *N* sulphuric acid (60 minutes, 100° C) guanine could be isolated as the sulphate [8] in the form of colourless needles, indistinguishable from the sulphate prepared under identical conditions from authentic guanine. The ultraviolet absorption curve, identical for both preparations, gave the guanine content calculated for $(C_5H_5ON_5)_2$, H_2SO_4, $2H_2O$.

Summarising, the results of paper chromatography before and after acid hydrolysis indicate the presence of guanosine triphosphate and uridine triphosphate in ATP preparations and in trichloroacetic acid extracts of muscle. Roughly equimolar amounts of the two triphosphates are found, corresponding to approximately 2—4 mg of each phosphate, compared with approximately 200 mg of ATP per 100 g of fresh muscle.

By precipitation of the barium salt in pooled fractions of the effluent from ion

exchange chromatography followed by fractional reprecipitations, we were able to obtain a nearly complete separation of the two components. The less soluble barium salt fraction gave the absorption spectrum of a guanylic acid derivative and a ratio of 1 : 1 : 2 for guanine : difficultly hydrolysable phosphate : acid labile phosphate. Only a trace of the uridylic acid derivative could be detected by paper chromatography before and after hydrolysis. The most soluble barium salt fraction gave the absorption curve of the uridylic acid derivative and a ratio of 1 : 1 : 2 for uracil : difficultly hydrolysable phosphate : acid labile phosphate. Paper chromatography revealed the presence of traces of the guanylic acid derivative. Pentose estimations using the orcinol reagent before and after bromination [9] for the determination of purine- and pyrimidine-bound pentose, gave confirmatory results. Experiments on the periodate oxidation and copper complex formation of guanosine and uridine triphosphates in comparative tests with ATP, and adenosine 5′- and 3′-phosphates showed that the two new triphosphates behaved as if the phosphate groups were in the 5′-position. Although location of the acid labile phosphate groups in the purine or pyrimidine part of the molecule is not excluded, we consider that their rates of acid hydrolysis as compared with ATP render their formulation as 5′-triphosphates most likely. The structural analogy of ATP coupled with the isolation of uridine 5′-diphosphate derivatives [10], from which uridine triphosphate may be formed [11,12] supports this view. In spite of many attempts, no carbohydrate other than pentose and no ninhydrin-reacting material has so far been detected in our triphosphate preparations.

An unequivocal proof of the constitution of guanosine triphosphate and uridine triphosphate must await the results of further investigations at present in progress. Fuller details together with a consideration of possible biochemical implications will be given in a separate communication.

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Molecular Structure of Perhydroanthracene, M. P. 90° C

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If the "*cis*"connection between the six-membered rings in the perhydroanthracene is of the same kind as that observed for *cis* decalin [1] the "*trans-trans*", "*trans-cis*" and "*cis-cis*" forms of perhydroanthracene would be represented by the forms I, II and III of Fig. 1. Judging from the physical constants of these substances J. W. Cook *et al.*[2] have suggested that the substance melting at 90° C is the "*trans-trans*" form (I).

Crystals of this substance are usually twinned, but in two cases we succeeded in growing single crystals suited for X-ray measurements. The crystals are triclinic:

$a = 5.46$; $b = 5.60$; $c = 10.20$ (Å)
$\alpha = 93.9°$; $\beta = 94.6°$; $\gamma = 102.0°$

The space group is $P\bar{1}$ and the unit cell contains *one* molecule which must therefore have a centre of symmetry. Suggesting that the strong 008 reflexion corresponds to the 1.26 Å spacing of the "*trans-trans*" form and taking into account the pseudo-symmetry respective to the *a* and *b* axes it proved possible to find approximate carbon atomic coordinates which explained in a