

criterion, I am not yet prepared to accept it as an intermediate of respiratory-chain phosphorylation. However, although it does not fulfil my criterion, a special circumstance does not allow us as yet to exclude it as an intermediate. Further experiments are required. Incidentally, there was no time lag of labelling of ATP in this experiment.

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### Carbamyl Phosphate as an Essential Component of the Flattening Factor for Cells in Culture

A COMPOUND of low molecular weight which causes attachment and flattening of cells in culture was prepared from the growth-promoting  $\alpha$ -globulin<sup>1</sup>. The protein from which this low-molecular compound was isolated was prepared by chromatography on 'DEAE'-cellulose<sup>2</sup>; in further experiments it was found that this protein could be inactivated by filtration through 'Sephadex G-25'. Reactivation occurred after incubation of this protein with  $\text{NaH}_2\text{PO}_4$  or with  $\text{NaHCO}_3$ ; if not incubated with salts, the protein caused flattening of cells in 4–8 h, so that the process of flattening was prolonged.

These findings suggested that the active factor contained a compound of low molecular weight, capable of binding an inorganic salt. For this reason, the active protein was chromatographed in a butanol-acetic acid-water mixture (4:1:5) without deproteinization. Staining with bromocresol green disclosed two components,  $R_F$  0.08 yellow and  $R_F$  0.16 blue; this active system also contained a protein which remained at the starting spot. On chromatograms treated with ninhydrin one-colour reaction only was obtained. The same  $R_F$ -values and the same reactions were obtained with carbamyl phosphate prepared according to the method of Spector, Jones and Lippman<sup>3</sup>.

To investigate the significance of carbamyl phosphate for cultivation, HeLa cells were used. The cells were incubated in a synthetic medium<sup>4</sup> containing carbamyl phosphate in a concentration of 20–200  $\mu\text{g}$  per ml. At 37° C the cells attached to glass within 1 h and within 20 h they were all flattened, but they did not flatten as well as in a medium with  $\alpha$ -globulin. It was further observed that on supplementation of this synthetic medium with insulin the cells flattened well.

These results were not unexpected, because the crystalline carbamyl phosphate prepared by direct crystallization from the active protein behaves in the same way. In contrast to the crystalline carbamyl phosphate, a low-molecular compound prepared from the same protein after incubation with erythrocytes, and causing a flattening of cells to the same extent as the growth-promoting  $\alpha$ -globulin, contained at least ten amino-acids in acid hydrolysate<sup>1</sup>.

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### Decomposition of Benzhydrol catalysed by Boric Acid

In a recent investigation of the secondary decomposition mechanisms in the pyrolysis of esters, it was discovered that the borate of benzyl alcohol decomposes to anthracene and resinous substances<sup>1</sup>. This result supports the contention of Chapman and Borden<sup>2</sup> that the elimination involves an ionic mechanism.

In an attempt to extend the synthetic capabilities of this reaction, the pyrolysis of benzhydrol was undertaken. The pyrolysis was conducted in the usual manner: the alcohol (1.84 g, 0.01 m) and boric acid (0.62 g, 0.01 m) were mixed in a 'Pyrex' tube and slowly heated to 140°. After 2 h the temperature of the heating bath was slowly raised to 280–290°. Decomposition was complete after 1 h. The crude products were then dissolved in benzene and chromatographed on alumina. Elution with benzene-petroleum ether gave three products; benzophenone (0.133 g), diphenylmethane (0.085 g) and *s*-tetraphenylethane (0.248 g). Neither 9,10-diphenylanthracene nor other fluorescent derivatives were located.

To explain the different pyrolytic routes of benzhydrol and benzyl alcohol, it was suggested that benzhydrol may have undergone ether formation rather than the expected borate ester formation as in the case of benzyl alcohol. This explanation is reasonable, since benzhydrol ether does give rise to these products under thermal conditions<sup>3,4</sup>, and, theoretically, the rate of ether formation in the benzhydrol reaction would be expected to be faster than benzyl ether formation, since the activation energy required for the formation of diphenylmethyl carbonium ion would be lower than the energy required for the benzyl cation. In an attempt to support this concept, the pyrolysis was repeated; however, in this experiment the degradation was conducted only at the lower temperature for 2 h. At the end of this time, the contents of the tube were cooled and then extracted with ether. On evaporation of the solvent, crystals of benzhydrol ether (1.50 g, 85 per cent) were deposited. The compound was identified by infra-red analysis and mixed melting point determinations.

Under these conditions, therefore, the pyrolysis of benzhydrol appears to involve the formation of benzhydrol ether, and the final products are produced from the decomposition of this intermediate.

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### Pregnant Mare Serum Gonadotrophin Potency: Effect of Single and Multiple Injections

It has been demonstrated that a single subcutaneous injection of pregnant mare serum gonadotrophin (PMSG) is as effective as multiple injections of the same amount of PMSG on the weight increase of the immature rat ovary<sup>1,2</sup>. However, it was later shown that multiple injections of PMSG were more effective in producing an increase in testis weight of three-day-old cockerels than a single injection of the gonadotrophin<sup>3</sup>. Because of these opposing views, it was of interest to re-investigate the chick testis response with a more sensitive assay method.

An assay was developed that made use of the incorporation of inorganic phosphorus-32 into testes of two-day-old cockerels as the end-point for gonadotrophin activity<sup>4</sup>. The phosphorus-32 end-point was more sensitive than gonad weight change, and the incorporation of this isotope