energy electrons of the redox system and high-energy phosphate bonds in ATP are closely coupled. Therefore the specific determination of an intermediary compound between two such enzymes in a structurally organized system may be a direct way of measuring the interactions (Jardetzky, 1966) and the related permeability properties occurring within the membrane itself.

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The Effects of Theophylline on Mucosubstance Secretion by Rous Sarcoma Cells

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Cell cultures of rat submaxillary gland maintain the secretion of mucosubstances for many months (Gallagher & Marsden, 1969). The rate of secretion is stimulated 28% ($P < 0.02$) by 0.1 mM-adrenaline and 111% ($P < 0.001$) by vasopressin (0.5 i.u./ml.). Similar observations have been made on mucosubstance release by Rous sarcoma cells.

The action of both adrenaline and vasopressin is believed to involve cyclic AMP (adenosine 3':5'-cyclic monophosphate) (Robison, Butcher & Sutherland, 1968).

At 5 mM the dibutyryl derivative of cyclic AMP stimulates the secretion of mucosubstances by Rous sarcoma cells nearly threefold. Derivatives of cyclic AMP have also been found to stimulate secretion in rat parotid gland (Babad, Ben-Zvi, Bdolah & Schramm, 1967) and in rabbit pancreas (Ridderstrap & Bonting, 1969), and in both cases stimulation could also be achieved with theophylline, which, by inhibiting phosphodiesterase, elevates the concentrations of endogenous cyclic AMP (Humes, Rounbehler & Kuehl, 1969). In Rous sarcoma cells, however, 5 mM-theophylline caused a marked inhibition (47%; $P < 0.1$) of mucosubstance release; only at lower concentrations (1.7 mM) was a stimulation (68%; $P < 0.1$) noted. Thus the effect

of theophylline appears to involve rather more than simple inhibition of the breakdown of cyclic AMP.

Theophylline has been found to inhibit protein synthesis in rat adrenal gland (Halkerston, Feinstein & Hechter, 1966), and we have found that puromycin at a concentration of 100 μ g/ml inhibits virtually completely the incorporation of D-[1- 14 C]-glucosamine into mucosubstances by cell cultures; a similar effect on the synthesis of exportable protein by cycloheximide has been reported for guinea-pig pancreas (Jamieson & Palade, 1968). These observations suggest that the use of theophylline to mimic the effects of cyclic AMP should be approached with caution where additional protein synthesis may also be involved.

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The Transformation of Lymphocytes by Adenosine 3':5'-Cyclic Monophosphate, and Consequent Changes in Histone Microstructure

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Cyclic AMP (adenosine 3':5'-cyclic monophosphate) will transform thymocytes (MacManus & Whitfield, 1969) at concentrations in the range 0.01–1.0 μ M. At 0.1–10 μ M no stimulation of human lymphocytes by cyclic AMP was observed (Hirschhorn, Grossman & Weissmann, 1970). In contrast with this it is reported here that cyclic AMP at a concentration of 0.01–0.1 μ M will stimulate cultures of lymphocytes prepared from pig peripheral blood as described by Forsdyke (1968). The timing and extent of the initial polydisperse RNA synthesis (0–1 h), subsequent rRNA synthesis (4–5 h) (Monjardino & MacGillivray, 1968), eventual DNA synthesis (approx. 72 h) and mitosis (approx. 90 h) are identical for cyclic AMP and phytohaemagglutinin. Chlorpromazine at a concentration that is reported to inhibit the adenylate cyclase membrane system (Wolff &

Jones, 1970) prevents transformation of the lymphocytes by phytohaemagglutinin.

Although no change in net phosphate on histones was found during the period of RNA synthesis immediately after stimulation, a change in labelling with [^{32}P]P_i (Cross & Ord, 1970) was detected. It was therefore decided to look at the sizes and specific radioactivities of the ATP and P_i pools immediately after stimulation. Lymphocytes were given a continuous uridine label 30 min before stimulation. In addition a [^{32}P]P_i pulse was administered 30 min before the animals were killed. P_i and acid-hydrolysable phosphate were determined by the method of Berenblum & Chain (1938) and the radioactivity of the butanol extracts was measured. Net values for ATP were confirmed by the firefly luciferase system (Holton, 1959). Intracellular P_i increased immediately after stimulation, returning to control values within 10 min. The specific radioactivity of P_i also showed a correspondingly large increase. It is suggested that this represents an influx of labelled P_i from the medium into the cell as a result of a change in membrane conformation on stimulation. ATP concentrations were lowered during the first 30 min and then rose rapidly above the control value. The specific radioactivity of the ATP reflected this.

Changes in the specific radioactivity of total histone minus histone F1 were directly proportional to changes in the specific radioactivity of the cellular ATP pool. The specific radioactivity of histone F1, however, was not directly related to the specific radioactivity of the ATP pool. It would appear that a rapid increase in labelling with [^{32}P]P_i occurred on this protein within the first 30 min after stimulation, when the rate of RNA synthesis was high. Only a small incorporation of [^{32}P]P_i occurred thereafter.

The changes in histone specific radioactivity and in ATP and P_i concentrations within 30 min of lymphocyte stimulation demonstrate the rapid effect on cellular metabolism produced by transforming agents such as phytohaemagglutinin or cyclic AMP.

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Influence of Reaction Medium on the Behaviour of a Hydroxy Steroid-Nicotinamide-Adenine Dinucleotide Oxidoreductase

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Molecules of diverse structures are frequently substrates for hydroxy steroid oxidoreductases, and analogues are often inhibitors. Because of the importance of these phenomena both in general enzymology and steroid biochemistry, it was decided to investigate the recognition and reaction of various steroids by using a crystalline enzyme of microbiological origin (EC 1.1.1.53). Properties are now described of a reaction medium in which various non-polar and most polar steroids are sufficiently soluble to allow detailed study.

The system consists of sodium phosphate buffer, pH 5.2 (0.1 M with respect to phosphate), containing 20% (v/v) of 2-methylpropan-2-ol. Increasing buffer molarity from 0.1 M to 0.2 M decreased the reaction rate with 5 α -androstan-3-one as substrate. Replicates (eight at each of five steroid concentrations) were closer in the 0.10 M than in the 0.15 M or 0.20 M systems, possibly indicating some instability at higher molarities. The high K_m value for 5 α -androstan-3-one (436 μM at 25°C with 115 μM -NADH) made it difficult to discern whether changes in reaction rate arose from small changes in K_m or V_{max} ; K_m for NADH (with 100 μM -5 α -androstan-3-one) increased from 14.8 μM at 0.10 M to 39.8 μM at 0.2 M. Increasing the concentration of 2-methylpropan-2-ol from 18% to 22% decreased the reaction rate fivefold (with 75 μM -5 α -androstan-3-one and 115 μM -NADH).

In the 0.10 M-phosphate-20% 2-methylpropan-2-ol system reaction rates ($\mu\text{mol/min per mg}$) for 90 μM -steroid and 115 μM -NADH were: 5 α -androstan-3-one, 4.8; 5 β -androstan-3-one, 1.0; the corresponding 17 α -hydroxy compounds, <0.1.

In the same reaction medium containing 10% (v/v) of dimethyl sulphoxide instead of 20% 2-methylpropan-2-ol, 5 α - and 5 β -androstan-3-one were substrates, but came out of solution. Reaction of the 5 β -isomer was slower, but exact comparison was not possible. 17 β -Hydroxy-5 α -androstan-3-one and 17 β -hydroxy-5 β -androstan-3-one (90 μM , with 115 μM -NADH) reacted at 3.8 and 0.3 $\mu\text{mol/min per mg}$ respectively, and were much better substrates in this than in the 2-methylpropan-2-ol system.

Reduction of 5 α -androstan-3-one in the 2-methylpropan-2-ol system was inhibited by the hydrocarbon 5 β -androstan-3-one and by 5 α -androstan-17 β -ol. Some implications of these findings are discussed.