achieved, though found with chick-embryo liver on similar culture (Ko et al., 1967; Skea & Nemeth, 1969) and with rat foetal liver on culture with glucocorticoids (Wishart et al., 1976).

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Differential Stimulation of Mono-Oxygenase and Uridine Diphosphate Glucuronosyltransferase Activities in Chick Liver during Natural Development and after Treatment in ovo with Corticosterone

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During natural development the rise in activity of the two NADPH-dependent microsomal mono-oxygenases aniline hydroxylase (EC 1.14.14.1) and aminopyrine N-demethylase differs from that of UDP-glucuronosyltransferase (EC 2.4.1.17), the enzyme which glucuronidates the products of the reaction catalysed by the mono-oxygenases. The transferase activity rises suddenly from negligible embryonic values to a plateau at 'adult' value within 2-3 days of hatching (Dutton & Ko, 1966; Wishart & Dutton, 1975). The two mono-oxygenases are present in late embryonic liver, surge to a sharp peak at day 1 of hatching and fall thereafter to 'adult' values (Drummond et al., 1972; Wishart & Dutton, 1975; Powis et al., 1976; present work).

Aniline hydroxylase and the transferase can develop precociously in the embryonic liver in ovo, after grafting on to the chorioallantoic membrane of anterior pituitary gland from chick or mammal (Wishart & Dutton, 1975) or after continuous application of corticotropin or certain 11β-hydroxy corticosteroids to the membrane (Leakey & Dutton, 1975; Leakey et al., 1976). We have extended these observations to aminopyrine N-demethylase and have noted that the differential developmental pattern of the mono-oxygenases and the transferase persists during precocious stimulation of the development of these enzymes by corticosterone.

Corticosterone was applied by the continuous-flow method (Leakey & Dutton, 1975) to 13-day embryos for 4 days. On each day the three enzymes were measured in the livers of treated embryos and of controls. Assay of transferase and aniline hydroxylase were as previously described (Wishart & Dutton, 1975); assay of the demethylase was that of Poland & Nebert (1973).

At lower corticosterone concentrations (0.03-0.05 μmol applied per 24h), the demethylase activity reached a peak 3 days after onset of treatment (i.e. at day 16). At that time transferase activity was just beginning to rise. At 4 days after onset of treatment, demethylase activity was falling and that of the transferase still rising.

At higher corticosterone concentrations (0.07-0.13 μmol applied per 24h) the precocious development of both enzymes was speeded, but the differential was maintained: demethylase activity reached a peak after 2 days, when the transferase activity had again just begun to rise. Aniline hydroxylase activity paralleled that of the demethylase under these conditions.
Activities reached by the enzymes during this precocious development corresponded to those attained in natural development, e.g. demethylase activity rose to 14–16 nmol of aminopyrine demethylated/h per mg of protein in the treated embryo, compared with a normal value in the embryo (13–18 days) of 6–9 of the above units, a peak at hatching of 15–23 units and a value in the 1-week-old chick of 7–9 units.

Reproduction of this differential pattern in corticosterone-treated and naturally developing embryos supports the suggestion (Wishart & Dutton, 1975; Leakey et al., 1976) that corticosteroids initiate the hatching surge of these enzymes in vivo. The difference may reflect the markedly differing half-lives of the enzymes. The half-life of hepatic transferase, on phenobarbital induction, has been estimated in chick embryo at 24 days (Burchell et al., 1972), and of the mono-oxygenase system in mammalian adult or foetus at 8–10 h (Hilton & Sartorelli, 1970; Gielen & Nebert, 1972).

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Alteration in Macromolecular Glycosylation of Transformed Cells Mediated by Cholera Toxin and Dibutyryl Adenosine 3':5'-Cyclic Monophosphate

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Cholera toxin is known to alter the regulation and growth properties of mammalian cells (Guerrant et al., 1974; Hollenberg et al., 1974), presumably through an alteration of the plasma membrane, which stimulates the activity of the adenylate cyclase (Hollenberg et al., 1974). In addition, some of our studies have demonstrated that the morphological changes mediated by cholera toxin appear related to alterations in the external topography of cells, leading to selective modifications in metabolite uptake (Rieber et al., 1975b). We have now extended our studies on cholera toxin to investigate its possible effect on the relative glycosylation of cells that can be grown under conditions that either restrict or allow the expression of transformation. The system used is the ts-NT3-KR cell line, which is a cloned derivative of normal rat kidney cells transformed by the temperature-sensitive ts 339 derivative of B77 virus. Such cells exhibit an increased agglutinability, deoxyglucose transport, colony formation on agar and growth to a higher saturation density when grown at 33°C, but tend to resemble the untransformed parent cells with regard to the above properties, when grown at 37°C (Rieber et al., 1975a).

When cells are labelled with [3H]glucosamine in medium supplemented with 0.5% serum, we observe a relative decrease in the glycosylation of a Pronase-sensitive component with mol.wt. 250000, previously shown to be decreased in plasma-membrane preparations of cells grown at 33°C (Rieber & Irwin, 1974). This decreased glycosylation in the high-molecular-weight region is reproducibly counteracted by addition of cholera