Johnson, 1963; Hunt, Hughes & Lowenstein, 1958; Hayaishi, 1969). A similar hydrophilic substrate requirement perhaps dictates the formation of 2-furoyl-CoA as substrate for hydroxylation by *P. putida* F2.

The soluble enzyme system that catalyses the conversion of furan-2-carboxylate into 2-oxoglutarate has been purified 30-fold by $(\text{NH}_4)_2\text{SO}_4$ fractionation (0–31% saturation fraction), dialysis, protamine sulphate treatment and chromatography on Sephadex G-200, which separates the system into two fractions. The first fraction (mol.wt. about 200 000) contains the 2-furoyl-CoA hydroxylase, which can be assayed with synthetic 2-furoyl-CoA as substrate and Methylene Blue as electron acceptor in an anaerobic cuvette. The second fraction (mol.wt. about 100 000) contains the 2-furoyl-CoA synthetase and the 5-oxo- Δ^2 -dihydro-2-furoyl-CoA (lactone) hydrolase. We have not yet ascertained which fraction contains the 2-oxoglutaryl-CoA hydrolase or whether the two obligatory keto-enol tautomerizations are assisted by the agency of enzymes.

Spheroplasts (Repaske, 1956) prepared from furan-2-carboxylate-grown cells yield membrane preparations that facilitate a CO- and KCN (1 mM)-sensitive transport of electrons from the 2-furoyl-CoA hydroxylase reaction to oxygen. Membranes prepared under identical conditions from glutamate- or nutrient-broth-grown cells are quite inactive in this capacity. Reduced minus oxidized cytochrome difference spectra show the presence of cytochromes a_1 , b_1 and c-551; neither they nor the ubiquinone and flavin content of membrane fractions show detectable variations consistent with change of growth substrate. Although crucial differences may be masked in crude preparations, the apparent requirement for a furan-2-carboxylate-induced membrane-bound component, specifically required if electrons from the 2-furoyl-CoA hydroxylase are to be accepted, is an interesting phenomenon.

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Some Biochemical Studies on Warfarin Resistance in the Rat

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Warfarin [3-(α -acetylbenzyl)-4-hydroxy-coumarin] has been used as an anticoagulant rodenticide for over 20 years. The first reported case of failure to obtain field control of rats (*Rattus norvegicus* L.) due to warfarin resistance was reported from Scotland by Boyle (1960). Genetic selection in this laboratory has resulted in the isolation of a strain of rat that is homozygous for warfarin resistance of Scottish origin (Greaves, personal communication). Ikeda, Conney & Burns (1968) have shown that warfarin is metabolized by a microsomal enzyme capable of induction by phenobarbitone, chlordane or DDT. We have investigated the possibility that resistance may be associated with increased microsomal enzyme activity.

A comparative study has been made of the liver drug-metabolizing enzyme systems of susceptible and resistant male rats. Their activities *in vivo* were assessed by comparing the 'sleeping times' of animals receiving an intraperitoneal injection of 30 mg of pentobarbitone sodium/kg body wt. The warfarin-resistant rats showed a significantly shorter sleeping time. The aromatic hydroxylation of aniline *in vitro* was assayed in postmitochondrial supernatants by using a modification of the method described by Kato & Gillette (1965). The liver microsomal cytochrome P-450 concentrations were determined by a method based on that of Omura & Sato (1964). The concentrations of both systems were significantly higher in the resistant strain. Other differences were noticed in the relative liver weight of both resistant and susceptible animals. Microsomal enzyme activities were affected to a different extent in both strains when DDE was incorporated in the diet.

Warfarin detoxication in susceptible rats has been shown to involve several metabolites, including four hydroxylated products (Barker, Hermodson & Link, 1969). Although large interstrain differences have been demonstrated with microsomal drug metabolism in four laboratory rat strains (Furner, Gram & Stitzel, 1969), it is possible that warfarin resistance in this species may involve a greater or more rapid metabolism of this compound.

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Some Modifications to the Technique of Two-Dimensional Immunoelectrophoresis

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The technique of two-dimensional immunoelectrophoresis as described by Minchin-Clarke & Freeman (1968) is very effective for the quantitative analysis of a mixture of proteins. The method consists of running the mixture first in a strip of agarose on a 10 cm × 10 cm glass plate, when a partial linear separation of the proteins occurs. The strip is then transferred to a second plate of similar size, the empty portion of the plate being filled with agarose containing an appropriate mixture of antibodies. The current is run at right-angles to the previous direction and the antigens are driven into the bed of antiserum. This results in the formation of antigen-antibody curves, the area under which is proportional to the amount of the protein present.

The choice of 10 cm × 10 cm glass plate appears to limit the use of the method, since it conditions the volumes of expensive reagents and the size of equipment. However, the size of the plate can be reduced to one-quarter of the original size without loss of clarity or detail. As a result, the electrophoretic tank can be completely redesigned to produce a more compact and flexible unit in which the volume of barbitone buffer is reduced to 10% of that originally used.

Redesigning the tank necessitates a change in the preparation of the plate, in that the two dimensions have to be prepared before commencing electrophoresis. This involved the construction of a new pattern of carrier and wick-electrode system.

A further improvement, which involved superimposition of plates one on top of the other, made it possible to increase the number of plates developed in a single run three- or four-fold.

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The Low-Molecular-Weight Components of Bovine Skeletal Myosin

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Low-molecular-weight components (light components) released from rabbit skeletal myosin by chemical modification, urea and alkali treatment have been well characterized and more recently implicated in the functioning of the intact myosin molecule (Stracher, 1969). The presence of light components in myosins of other species has been demonstrated (Locker & Hagyard, 1967; Perrie, Perry & Stone, 1969) and an examination of those of bovine skeletal myosin was therefore made.

Myosin was prepared from muscle in rigor and was free of nucleoprotein and adenylate kinase activity. Low activities of adenylate deaminase present could be removed by batch treatment with DEAE-cellulose. No cathepsin-like proteolytic activity (Gordon & Zak, 1963) could be detected. On chromatography on DEAE-cellulose, Sephadex G-200 and Sephadex G-100 and on ultracentrifugation the preparation appeared as a single component. Amino acid analyses, molecular weight (540 000, by sedimentation-diffusion) and enzymic characteristics closely correspond to those of rabbit (J. Thomas, unpublished work).

Acetylation, maleylation or treatments with 0.1 M-Na₂CO₃, 1% sodium dodecyl sulphate or 8 M-urea were shown to release the light components. The acetylated and alkali-treated forms (best characterized in rabbit) and the maleylated form were examined in more detail. Light components obtained by acetylation and maleylation treatments were separated from the high-molecular-weight material (heavy components) by (NH₄)₂SO₄ fractionation, whereas the light components from alkali treatment were separated by dilution. In no case was the separation complete: the light components were contaminated by the heavy component. Further purification on Sephadex G-100 gave a component representing about 10% of the total protein that had consistent amino acid analyses and gave a single peak ($s_{20,w}^0$ 1.6S) on ultracentrifugation. The results of polyacrylamide-gel (7.5%) electrophoresis at pH 9.0 were complex, but in light components prepared with thiol groups protected by *N*-ethylmaleimide three major bands were detected.

The acetylated, maleylated and alkali-treated light components behaved differently on Sephadex G-100 calibrated for molecular weight and Stokes radius (the latter derived from diffusion coefficients of standard proteins): each gave a single peak, though at different elution volumes. In view of the sedimentation data and the work of Habeeb (1967)