was extracted twice with ether. The ether was removed under reduced pressure and the residual liquid was taken up in acetonitrile. The acetonitrile solution was washed with petroleum ether (b.p. 30–60°) to remove mineral oil and then dried over anhydrous magnesium sulfate. After removing the solvent the residual liquid was distilled to obtain ethyl 1,2-diphenylcyclopropanecarboxylate, b.p. 130–135°/0.1 mm Hg, in 78.6 % yield.

The foregoing ester was dissolved in 200 ml of ethanol and aqueous potassium hydroxide (0.2 mole dissolved in 25 ml of water) was added to it. The mixture was refluxed for 15 h. The ethanol was distilled off under reduced pressure, the residue was diluted with water and the mixture was extracted with ether to remove unhydrolysed material. The aqueous solution was made acidic and the resulting precipitate was filtered. Fractional crystallization from ethanol yielded two acids: (i) m.p. 145–150°; yield, 12.65 g. (ii) m.p. 220–222°[5]; yield, 6.2 g.

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# Induced Heterogeneity of Lactoperoxidase

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An earlier study,[1] employing chromatography on Sephadex-DEAE, demonstrated several enzymically active fractions of peroxidase prepared from cow's milk. The existence of multiple forms of LPO* was later confirmed by the separation of this enzyme into six zones by means of electrophoresis in a stable pH-gradient [2] and into five zones by disc electrophoresis.[3] Morrison and Hultquist [4] have suggested that the two earlier known forms of LPO, A and B, might be the result of the proteolytic activity of rennin, which is used in the preparation procedure, or due to genetic differences among the cows. However, the heterogeneity pattern was not affected by the omission of rennin from the preparation procedure,[1,2] neither did milk from a single cow yield a homogeneous enzyme.[1] The ratio between the light absorption at the Soret-band, 412 mμ, and at 280 mμ is different for the subfractions. The highest value is obtained for that fraction which has the highest isoelectric point, fraction 1. Upon storage in 80 % saturated ammonium sulfate the light absorption ratio of chromatographically pure fraction 1 decreased to a value resembling that of fraction 2. This observation suggested a conversion of fraction 1 into fraction 2.[1] The purpose of the present investigation is to determine, if such a conversion can be demonstrated, using disc electrophoresis.

*Materials and methods.* LPO was prepared as described previously.[1] Disc electrophoresis was carried out according to Ornstein and Davis [5] and Broome [6] with some modifications.[3] "Preparative" disc electrophoresis was performed in glass tubes with an internal diameter of 0.9 cm compared to 0.5 cm for the analytical separation. All chemicals used were of analytical grade.

*Results and discussion.* LPO was separated into three major and two minor zones by disc electrophoresis in polyacrylamide gel.[3] These zones are designated, from cathode to anode, LPO 1 – LPO 5. The main fraction, LPO 1, corresponding to the fraction with the highest isoelectric point,[2] was collected from "preparative" runs by cutting out the zone from the polyacrylamide. The gel was homogenized in a Potter-Elvehjem homogenizer and then extracted with 0.06 M potassium acetate buffer, pH 6.5. The enzyme thus obtained, which moved as a single zone upon repeated electrophoresis, was dialysed at room temperature for 36 h against 40 % saturated ammonium sulfate, adjusted to pH 9.4 with ammonium hydroxide. After an additional dialysis against 0.06 M potassium acetate buffer, pH 6.5, the enzyme was analyzed by disc electrophoresis. The peroxidase was now heterogeneous and was composed of the original frac-

* Abbreviation: LPO, lactoperoxidase

tion, LPO 1, and a minor zone with lower mobility towards the cathode. By subjecting enzyme, treated in this way, to electrophoresis together with isolated LPO 2, the second minor component was shown to have a mobility indistinguishable from LPO 2. No heterogeneity was demonstrated after dialysis against the potassium acetate buffer only. Dialysis of homogeneous LPO 2 against ammonium sulfate, pH 9.4, also resulted in a heterogeneity, the minor component corresponding to LPO 3.

These results strongly indicate that LPO 1 and LPO 2 can, under certain conditions be converted to LPO 2 and LPO 3, respectively. The convertibility of LPO 3 and LPO 4 have not yet been investigated. However, before the identity is finally established between the conversion products and LPO 2 and LPO 3 further characterization of the conversion products is necessary.

Thus it has been shown that a heterogeneous LPO, corresponding to LPO 1, LPO 2, and LPO 3, can be produced from a homogeneous enzyme. It is not yet known, however, if conversion is effected during the purification of the enzyme. But even so, it is still quite possible that the peroxidase is heterogeneous in native cow's milk and that there is some process *in vivo* that corresponds to the effect of the alkaline ammonium sulfate. It is known that cytochrome c from beef heart is heterogeneous in fresh muscle extract [7] and it has been shown in this laboratory that this protein is split into subfractions at condition remarkably similar to those effective for LPO.[8] The cytochrome c subfractions appear to differ, like the subfractions of insulin and corticotropin, only in the number of amide groups.[8-10] There are reasons to believe that a similar explanation can also account for the heterogeneity of LPO, as hydrolysis of amide groups under relatively mild conditions should not be an isolated phenomenon for corticotropin [10,11] and cytochrome c.[8]

There are, however, other possible explanations that must be investigated and work is continuing in this laboratory to further study the heterogeneity and convertibility of LPO.

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## Crystalline Cytochrome c Peroxidase

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Altschul *et al.*[1] were the first to demonstrate the presence of cytochrome *c* peroxidase (cytochrome *c* $H_2O_2$:oxidoreductase, EC 1.7.11.1) in baker's yeast, assigning a method for its partial purification. Later, Abrams *et al.*[2] improved the purification procedure, which gave a highly pure preparation. However, the enzyme was obtained in a poor yield. By introducing ion exchange chromatography on cellulose-ion-exchangers as a step in the purification procedure, the yield of the enzyme was improved [3,4] and the method of purification simplified. In this communication a procedure for crystallization of cytochrome *c* peroxidase (CcP) is reported, as well as some preliminary observations on its properties.

Dried baker's yeast was ground and allowed to autolyze mainly as described by Altschul *et al.*[1] The turbid brown solution obtained after centrifugation was first