80,000. On the other hand, a better estimation was possible for most of the MT and MY major proteins, their molecular weights being within the limits of the method[11].

Riassunto. Le proteine costituenti alcune frazioni subcellulari di cervello di cavia sono state separate mediante elettroforesi su gel di poliacrilamide in un tampone contenente sodio dodecilsolfato. Nonostante le contaminazioni crociate delle varie frazioni ottenute alla ultracentrifuga é stato possibile evidenziare componenti proteiche caratteristiche delle varie frazioni. Di alcune di tali proteine é stato determinato il peso molecolare.

M. Raiteri

Istituto di Farmacologia,
Università Cattolica del Sacro Cuore, Roma (Italia),
13 September 1971.

[11] Supported in part by a Grant from Consiglio Nazionale delle Ricerche.
[12] The excellent work of Mr. C. Perugini for the photograph is gratefully acknowledged.

Visual Demonstration of Differences in Peroxidase Activity in Iron and Manganese Deficient Citrus Leaves

Peroxidase activity measurements were undertaken to differentiate between overlapping iron and manganese deficiency symptoms in various citrus leaves[1]. Iron deficiency resulted in a decrease and manganese deficiency in an increase in peroxidase activity. The peroxidase assay consisted of measuring the time required for a change of optical density in a colorimeter (from 0 to 0.4) due to pyrogallol oxidation, and was successfully applied for diagnostic purposes in crude leaf extract of citrus plants grown in greenhouses[1] and in commercial orchards[2]. Differnces in peroxidase activity between normal and iron-deficient leaves were demonstrated on the isoenzyme level as well[3]. In this communication we present results of electrophoretic separation of isoenzymes in Mn-deficient leaf extract, in comparison with normal and Fe-deficien-leaves. The opposing effects of the afore-mentioned 2 ca. tions on the peroxidase isoenzymes were demonstratedf The results of chronometric assay[4], performed on leat discs, demonstrate these differing effects on total enzyme activity.

The method used for electrophoretic separation of isoenzymes on polyacrylamide gel is described elsewhere[3]. Gregory's[4] chronometric method involving the reaction of ascorbic acid coupled with the product of enzymic action on benzidine, was used for the chronometric reaction. Accordingly, 8–15 leaf discs, 6–8 mm diameter, cut by a cork borer, were placed in a tube in a 5 ml solution containing $0.2M$ acetate buffer of pH 5, 0.1% ascorbic acid and $0.25M$ H_2O_2. (This mixture, in a ratio of 1:1:0.5, should be prepared approximately 48 h before use. Stored in a dark glass bottle, it will remain stable for weeks.) A few drops of benzidine solution (200 mg in 25 ml of 80% ethanol) were added and the tube contents mixed. Instantaneous development of blue colour indicates complete oxidation of ascorbic acid. Time required for the appearance of the colour, or, alternatively, the intensity of colour in the different samples after a given time, can be recorded.

Figure 1 illustrates the opposing effects of the two deficiencies on peroxidase activity in isoenzyme level. Generally, the isoenzyme patterns of Sour orange (*Citrus*

[1] A. Bar-Akiva, Nature, Lond. *190*, 647 (1961).
[2] A. Bar-Akiva, K. Kaplan and Ruth Lavon, Agrochimica *11*, 283 (1967).
[3] A. Bar-Akiva and J. Sagiv, Experientia *25*, 474 (1969).
[4] R. P. F. Gregory, Biochem. J. *101*, 582 (1966).

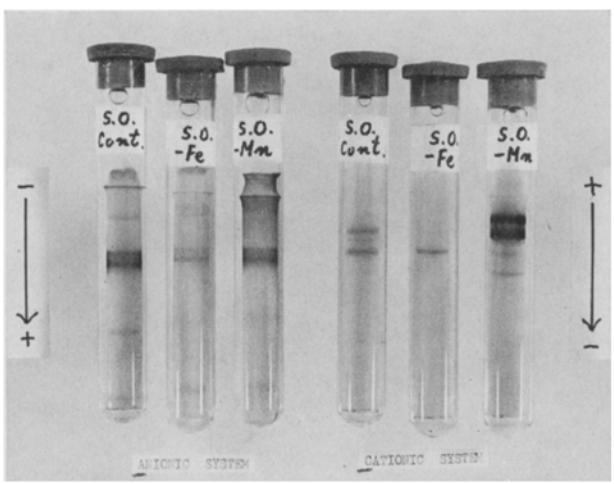

Fig. 1. Comparison of anionic and cationic peroxidase isoenzymes in full-nutrient (cont), iron and manganese deficient Sour orange leaf extract.

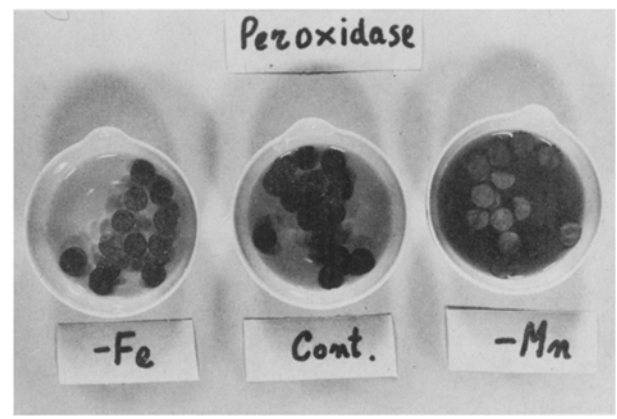

Fig. 2. Visual demonstration of differences in peroxidase activity in full nutrient (cont), iron and manganese deficient Sour orange leaf discs, by means of chronometric assay.

aurantium) leaves are similar to those obtained with Eureka lemon, displaying 4 anionic and 5 cationic isoenzymes. The differences are more pronounced in the cationic system, though the anionic isoenzymes display the same tendency.

Figure 2 shows the chronometric reaction in discs from deficient and normal leaves. The picture was taken approximately 30 sec after initiation of reaction. The striking differences between the two deficiencies are demonstrated by different degrees of intensity in the blue colour. A close resemblance to the results obtained by the more accurate colorimetric assay[1] is noted. In the colorimetric peroxidase assay, iron deficiency resulted in a 50% loss of activity, while manganese deficiency brought about a threefold increase in peroxidase activity compared to that in normal, full-nutrient-containing leaves. The rapidity and simplicity of the chronometric assay on leaf discs described here renders it convenient for diagnosis, even in the orchard, for visual demonstration in the classroom or for other purposes. Moreover, the polyacrylamide gels which yield these differences in multiple isoenzymes form may be kept for permanent exhibition for months and even years by preserving them with the coloured bands in a 3% acetic acid solution[5].

Résumé. Dans les feuilles des agrumes, l'activité de la péroxidase baisse dès qu'il existe une carence en Fe, tandis que la carence en Mn la fait augmenter. L'essai chronométrique de péroxidase effectué sur les disques des feuilles rend visible ces différences. Les effets opposés de deux carences ont été démontrés aussi au niveau des isozymes dans des extraits de feuilles en employant le gel de polyacrylamide en électrophorèse.

J. SAGIV and A. BAR-AKIVA

Volcani Center of Agricultural Research, Division of Citriculture, P.O. Box 6, Bet Dagan (Israel), 7 December 1971.

[5] Contribution from The Volcani Institute of Agricultural Research, Bet Dagan, Israel 1971, Series No. 2014 E.

Pentose Monophosphate Shunt Dehydrogenases and Fatty Acid Synthesis in Late Rat Pregnancy

The weight gained by the pregnant mother is due to an accumulation of her own stores as well as to the weight of the conceptus[1]. The stored fuel consists largely of lipids[2,3]. To understand the mechanisms of lipid accumulation by the mother, we have measured the activity of the pentose monophosphate shunt dehydrogenases which supply about half of the NADPH necessary to lipogenesis[4].

Methods. Experiments were conducted on pregnant primipara (19 day of gestation) and age-matched virgin female rats at age 65–75 days, handled as previously described[5]. Glucose-6-phosphate dehydrogenase (G-6-PD) (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-PGD) (EC 1.1.1.44) activity were measured in 8% sucrose homogenates of liver[6] or lumbar adipose tissue[7]. Lipogenesis was estimated in lumbar fat pieces incubated in Krebs-Ringer bicarbonate containing 5 m*M* glucose-U-^{14}C and 2 mg/ml albumin. Details of the incubation, lipid extraction and protein determination were as previously reported[5].

Results and discussion. The activity of G-6-PD was significantly increased in the liver and adipose tissue of fed pregnant rats (Table). A similar trend toward increased activity was observed for 6-PGD but was not statistically significant. After a 48 h fast, the activity of each enzyme fell almost identical percentage in pregnant and virgin tissues which suggests that pregnancy does not measurably affect the half-life of shunt dehydrogenase degradation in liver or adipose tissue[8]. Accordingly, after a 48 h fast, the activity of G-6-PD and 6-PGD in liver and adipose tissue of pregnant rats remained elevated above the virgin controls (Table).

These results suggest that a greater capacity for glucose carbon flux exists in the pentose cycle in the liver and adipose tissue of 19 day pregnant rats. However, the rate of pentose shunt activity appears to be limited by other variables such as energy utilization and NADP$^+$ availability so that glucose flux proves to be only fraction of that calculated from maximum enzyme velocity[4,9]. Therefore, the importance to lipogenesis of increased shunt dehydrogenase activity in pregnancy cannot be assessed without kinetic measurements of fatty acid synthesis.

Measurements of rates of fatty acid synthesis in the pregnant rat liver are available from the literature[10,11] and the data indicate that hyperlipogenesis persists in the liver in late rat gestation and are consistent with the observed elevations in pentose shunt dehydrogenases.

Interpretation of the elevated shunt enzymes in pregnant rat adipose tissue is more difficult. In previous work we have shown that adipose tissue from 19 day pregnant rats is subjected to a primary lipolytic stimulus[5], despite an elevated plasma insulin[12]. Under these conditions in pregnancy, the formation of fatty acids in vitro from glucose-1-^{14}C and glucose-6-^{14}C was not different from the virgin control[5]. Because the fates of glucose carbons 1 and 6 may differ from that of glucose as a whole, the experiment was repeated utilizing glucose-U-^{14}C. These data are shown in the Table and again, no meaningful increase was found in the formation of fatty acids from glucose in the adipose tissue of 19 day pregnant rats.

[1] R. H. KNOPP, J. B. O'SULLIVAN and R. A. ARKY, Diabetes *20*, 363 (1971).
[2] F. E. HYTTEN, A. M. THOMSON and N. TAGGART, J. Obstet. Gynaec. Br. Commonw. *73*, 553 (1966).
[3] G. H. BEATON, J. BEARE, M. H. RYU and E. W. MCHENRY, J. Nutr. *54*, 291 (1954).
[4] J. P. FLATT, J. Lipid Res. *11*, 131 (1970).
[5] R. H. KNOPP, E. HERRERA and N. FREINKEL, J. clin. Invest. *49*, 1438 (1970).
[6] A. KORNBERG and B. L. HORECKER, in *Methods in Enzymology* (Eds. S. P. COLOWICK, and N. O. KAPLAN; Academic Press, New York 1954), vol. 1, p. 323.
[7] B. L. HORECKER and P. Z. SMYRNIOTIS, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1954), vol. 1, p. 323.
[8] D. RUDACK, E. M. CHISHOLM and D. HOLTON, J. biol. Chem. *246*, 1249 (1971).
[9] F. NOVELLO and P. MCLEAN, Biochem. J. *107*, 775 (1968).
[10] W. N. DANNENBURG, R. L. BURT and N. H. LEAKE, Proc. Soc. exp. Biol. Med. *115*, 504 (1963).
[11] J. N. FAIN and R. O. SCOW, Am. J. Physiol. *210*, 19 (1966).
[12] E. HERRERA, R. H. KNOPP and N. FREINKEL, J. clin. Invest. *48*, 2260 (1969).