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.

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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.

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Effect of pregnancy on hexose monophosphate shunt enzymes in liver and adipose tissue and on the formation of labelled fatty acids from glucose-U-^{14}C by adipose tissue in the rat[a]

RATS	FED			48 h fasted		
	Virgin	Pregnant	*P*	Virgin	Pregnant	*P*
Liver						
G-6-P dehydrogenase (Δ E_{340}/mg protein)	0.050 ± 0.005	0.099 ± 0.007	<0.001	0.030 ± 0.005	0.053 ± 0.010	<0.05
6-PG dehydrogenase (Δ E_{340}/mg protein)	0.036 ± 0.006	0.043 ± 0.009	N.S.	0.020 ± 0.004	0.023 ± 0.005	N.S.
Adipose tissue						
G-6-P dehydrogenase (Δ E_{340}/mg protein)	0.126 ± 0.021	0.203 ± 0.029	<0.05	0.092 ± 0.011	0.153 ± 0.009	<0.01
6-PG dehydrogenase (Δ E_{340}/mg protein)	0.023 ± 0.002	0.034 ± 0.006	N.S.	0.012 ± 0.001	0.016 ± 0.001	<0.05
Formation of ^{14}C-fatty acids (nmoles of glucose carbon/mg protein/h)	7.68 ± 1.45	11.08 ± 1.59	N.S.	0.19 ± 0.07	0.13 ± 0.07	N.S.

[a] Details of incubation procedure and other methods are described in the text. The results are given as means ± S.E.M. of 6–8 rats/group. *P* denotes the significance of the differences between the values for virgin and pregnant animals.

The consistent absence of an increment in adipose tissue fatty acid formation is probably due to the overall decline in lipogenesis that occurs in late gestation. It has been found that both adipose tissue fatty acid synthesis[1, 11] and lipoprotein lipase[13] are elevated in midgestation and decline to subnormal levels by term, and that the 19th day of gestation is an intermediate time where lipogenesis in pregnant and virgin rats is transiently equal. Since maternal lipogenesis in both liver and adipose tissue are maximal in midgestation, could the shunt dehydrogenases be induced at this time and then persist at an elevated level through day 19? Our experiments with fasted rats support this possibility. As shown in the Table, glucose conversion to fatty acids is almost nil in adipose tissue of both pregnant and virgin rats fasted 49 h. Since shunt dehydrogenase induction is unlikely without significant lipogenesis, the persistent elevation of the enzymes in pregnancy after a 48 h fast must reflect their prior induction in the fed state.

If rat pregnancy is viewed as a whole, a good correlation exists between increased food intake[14], plasma hyperinsulinism[12], and accumulation of fat stores[3] on the one hand, and heightened pentose shunt dehydrogenase activity and lipogenesis in liver and adipose tissue on the other. In this respect, pregnancy is similar to other maneouvres that promote lipogenesis such as fasting and refeeding[15, 16], insulin treated alloxan diabetes[17, 18] and meal eating[19].

In pregnancy the maximum stimulus to lipogenesis occurs in midgestation and declines as term approaches. Thus at day 19 the elevated shunt dehydrogenases are somewhat out of keeping with the fall in lipogenesis, particularly in adipose tissue. We suspect that the enzymes are induced earlier in gestation and that an increment over the control levels can persist through day 19 just as after a 48 h fast. A rate of enzyme degradation that is identical with the virgin control, and is probably slow as well, can account for these observations.

Resumen. Se estudiaron las actividades de glucosa-6-fosfato dehidrogenasa y 6-fosfogluconato dehidrogenasa en hígado y tejido adiposo de ratas preñadas, al día 19 de gestación, alimentadas y en ayunas de 48 h, relacionando los resultados obtenidos con la velocidad de síntesis de ácidos grasos en el tejido adiposo de los mismos animales.

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Demonstration of Polysome Disaggregation due to Dimethylnitrosamine by Acrylamide Gel Polymerization After Centrifugation

In studying polysome disaggregation as a parameter of hepatotoxic effect, we examined the postmitochondrial fraction by sucrose gradient centrifugation followed by fractionation and scanning with a spectrophotometer at 260 nm[1]. The fractionation and spectrophotometry procedures were cumbersome requiring several instruments and the ribosome subunit peaks were not well observed because of the large protein peak at the top of the gradient. Therefore, we looked for a simple method to circumvent these difficulties and found that the technique