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

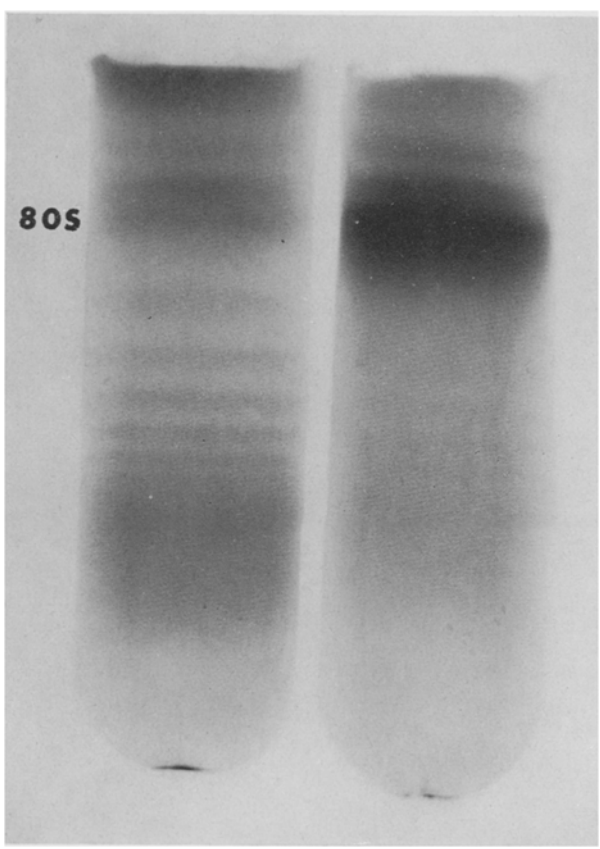
In studying polysome disaggregation as a parameter of hepatotoxic effect, we examined the postmitochondrial fraction by sucrose gradient centrifugation followed by fractionation and scanning with a spectrophotometer at 260 nm[1]. The fractionation and spectrophotometry procedures were cumbersome requiring 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

of acrylamide gel polymerization after centrifugation reported by JOLLEY et al.[2] and COLE and BROOKS[3] can be adapted to the observation of liver polysome disaggregation.

A series of 6 experiments using Swiss albino mice, 1 treated and 1 control for each experiment, was done with the same results. Altogether, 6 mice were injected i.p. with dimethylnitrosamine in saline, 50 mg per kg body weight, and sacrificed after 1 h. The livers were perfused immediately with ice-cold saline and were homogenized in three volumes of ice-cold 0.25 *M* sucrose solution containing 10 m*M* *Tris*-HCl, pH 7.6, 25 m*M* KCl and 5 m*M* $MgCl_2$. Livers of 6 control animals which had been injected with saline were also studied. The homogenate was centrifuged for 10 min at 10,000 × *g* at 3°C and the supernatant (postmitochondrial fraction) was used. A solution of 10% sodium deoxycholate was added to the postmitochondrial fraction to a final concentration of 1% and 0.2 ml of this fraction was layered onto a linear sucrose gradient, 0.5 *M* to 1.2 *M*.

Sucrose gradients were prepared as follows: First, polymerizable acrylamide solution was prepared as described by COLE and BROOKS[2]. The polymerizable solution was prepared by mixing 1 part of a solution containing N, N, N′, N′-tetramethylethylenediamine (12 µl per ml in 0.94 *M* *Tris*-phosphoric acid buffer, pH 6.9), 2 parts of a solution containing 24% (w/v) acrylamide and 5% (w/v) N, N′-methylenebisacrylamide, 1 part of riboflavin 80 µg per ml of water, and 4 parts of water. Secondly, two sucrose solutions, 1 *M* and 2.4 *M*, were prepared each containing additionally 20 m*M* *Tris*-HCl, pH 7.6, 50 m*M* KCl and 10 m*M* $MgCl_2$. By mixing the sucrose solutions with an equal volume of polymerizable solution, 0.5 *M* and 1.2 *M* polymerizable sucrose solutions were obtained. Taking 2.3 ml each of these polymerizable sucrose solutions, linear gradients were made in 50.8 × 12.7 mm cellulose nitrate tubes by using a gradient mixer (Buchler Instruments, Inc., Fort Lee, New Jersey, USA). The gradients were made under dimmed light to prevent premature polymerization.

Centrifugation was done for 1 h in a Spinco L centrifuge with an SW 50.1 rotor at 35,000 rpm at 5°C. After centrifugation, the tubes were placed in a vertical position in an unmarked glass beaker which is stuffed with additional empty cellulose nitrate tubes. The gradients were overlayered with 30 to 50 µl of water to flatten the meniscus. The beaker containing the tubes was then placed equidistant from 2 parallel fluorescent lamps (General Electric daylight fluorescent lamp, 15 Watt) in an X-ray viewing box which was laid horizontally. Polymerization was complete in about 1 h with the tube content becoming slightly opaque. After polymerization, the gels were removed from the tubes by punching a small hole in the bottom and gently blowing the gel into a dish of water. The gel was sliced longitudinally into four slabs by passing it through a snugly-fitting glass tube which had three woman's hairs strung across one end. The gel slabs were fixed in 1 *N* acetic acid for 15 min, stained with 0.2% methylene blue in 0.4 *M* acetate buffer, pH 4.7, for 1 h, and decolorized in running tap water for 8 to 10 h.



Mouse liver polysome profiles demonstrated by acrylamide gel polymerization after sucrose gradient centrifugation. Left, normal; right, dimethylnitrosamine treated. 80 S indicates 80 S monosome band.

The Figure shows 2 gel slabs, the left showing the polysome profile of normal mouse liver and the right the polysome disaggregation 1 h after the i.p. injection of dimethylnitrosamine, 50 mg per kg body weight. The broad band at the top is due to deoxycholate. The band labeled 80 S corresponds to the monosome band and the 2 bands above it are presumed to be 40 S and 60 S ribosome subunit bands judging from the distances from the top. The other distinct bands are presumed to be of oligosomes and they are followed by a broad confluent zone of polysomes. Dimethylnitrosamine treatment decreased oligosomes and polysomes and greatly increased the monosomes without a concomitant increase in subunits. This is similar to other cases of liver polysome disaggregation previously pointed out by WEBB and MORRIS[4].

Thus, this procedure is useful to observe liver polysome disaggregation. The staining with methylene blue at pH 4.7 allowed the selective binding of the dye to nucleic acids among the cell constituents and the observation was not interfered with by proteins and other substances. Perhaps this is one of the reasons for the sharp resolution of the bands and also the distinct demonstration of the bands presumed to be of ribosome subunits. The reduced handling of the gradient after centrifugation also contributed to the sharp resolution. These advantages coupled with the simplicity has made this method useful in studying liver polysome disaggregation even though it is not a rapid one[5].

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Résumé. La désagrégation des polysomes du foie provoquée par des agents toxiques peut être mise en évidence par polymérisation, après centrifugation, d'un gradient de saccharose auquel des monomères d'acrylamide ont été additionnés. Ce procédé simple et souple donne de bons résultats et exige moins d'instrumentation que les méthodes habituelles.

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Absence of Renin Secretion in the Portal Venous System in Rats

Barnardo et al.[1] reported an unexpected higher plasma renin activity in the portal venous plasma as compared to peripheral venous plasma in patients with various abdominal and liver diseases. The mean difference of 34%, with a range up to three times the peripheral venous level, was considered to indicate an extrarenal site of renin production in the splanchnic area. In dogs[2,3] and rats[4,5] similar differences in renin activity could not be detected. To clarify whether a splanchnic source of renin can be demonstrated in animals without renal renin secretion, studies were performed on nephrectomized rats subjected to acute hemorrhagic hypotension.

Methods. In male and female Wistar rats, ranging in weight from 340 to 500 g (average 392 g), both kidneys were removed under light ether anesthesia. Two to 24 h later urethane anesthesia was induced by i.p. injection of 0.5 ml 25% solution/100 g body weight and a thin heparinized polyethylene tube was inserted into the carotid artery. The catheter allowed sampling of arterial blood and measurement of the blood pressure by connection to a mercury manometer. After opening the abdominal cavity, a canula (No. 12) was passed through a mesentric vein to the hilar part of the portal vein. Avoidance of external blood loss and fixation of the canula could be readily achieved by use of Histacryl®.

After reading the blood pressure, hemorrhagic hypotension was induced by sampling blood simultaneously in heparinized tubes from the carotid artery and the portal vein, serving also for the determination of plasma renin activity. The total blood loss reached 3.5 to 4.5 ml, ranging from 10 to 12 ml/kg body weight, with a decrease in blood pressure of 25.3 $\pm$ 12.2 mm Hg ($n = 17$). Second sampling of blood from either site was performed 60 to 165 min later when the blood pressure had decreased below 30 mm Hg in 4 and between 30 and 50 mm Hg in 10 animals.

Plasma renin activity was determined after dialyzing the plasma against a solution of ethylene-diamintetraacetic acid 0.22% in 0.9% saline for 20 h and then against

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Renin activity in portalvenous and arterial plasma in 18 nephrectomized rats subjected to hemorrhage

Animal No.	Sex	Weight (g)	Time interval between nephrectomy and bleeding (h)	Blood pressure after bleeding (mm Hg)	Time interval between bleeding and blood sampling (h)	Renin activity Portovenous	Arterial
15	♂	345	2	35	1	n.d.[a]	n.d.[a]
16	♀	350	2	not registrated	1	n.d.[a]	n.d.[a]
17	♀	380	2	25	1	n.d.[a]	n.d.[a]
18	♀	340	2	25	1.2	n.d.[a]	n.d.[a]
19	♀	340	2	25	2	n.d.	n.d.
20	♀	340	2	22	1	n.d.	n.d.
21	♀	340	7.5	30	2	n.d.	n.d.
22	♀	380	12	42	1.4	n.d.	n.d.
23	♀	370	18	28	1.45	n.d.	n.d.
25	♀	370	17	85	1.45	n.d.	n.d.
26	♂	400	12	40	1.3	n.d.	n.d.
27	♂	420	8	60	2	n.d.	n.d.
28	♂	410	24	30	1	n.d.	n.d.
29	♂	420	18.5	26	2	++	n.d.
30	♂	420	17.5	32	2	n.d.	n.d.
31	♂	440	24	50	2	n.d.	n.d.
32	♂	490	24	45	2.45	n.d.	n.d.
33	♂	500	24	80	1.30	n.d.	n.d.

[a]Renin activity determined after 1 h incubation time; ++material with a slight depressor effect; n.d. = non detectable.