application point. At pH 5.5, the figures were 2.2 cm for MIL and 1.9 cm for HEL. Amino acid analyses performed on 2 different preparations of MIL, yielded almost identical results. The similarity with HEL is striking, although MIL is still more basic than HEL (Table), and the total number of residues is higher. From the amino acid composition a molecular weight of 17,800 is computed, slightly more than accepted molecular weight of hen egg-white lysozyme 14,600. Both lysozymes had identical migration rates in thin layer-gel chromatography.

Discussion. In normal mice the small intestine is a relatively rich source of lysozyme (about 375 µg per mouse). We decided to isolate mouse intestinal lysozyme as a preliminary step in the study of the function of the Paneth cell[9]. A much more convenient model was used by RIBLET and HERZENBERG[12], who isolated lysozyme from the urine of mice bearing a type A reticulum sarcoma, in which the urine contained up to 25 mg/ml. Probably, this urinary lysozyme, as well as that found in some types of human leukaemia[14] is produced by monocytes.

According to RIBLET and HERZENBERG[12], the extinction coëfficient of mouse lysozyme is lower than that of egg white lysozyme, due to a lower amount of tryptophan residues (4 against 6), although more residues of the less absorbing tyrosine (7 against 3) are present. The mouse intestinal lysozyme which we isolated has a high extinction coëfficient due to the presence of 9 tryptophan residues and 5 tyrosine residues. Mouse monocytic lysozyme has the same total number of residues (130) and the same number of basic residues (18) as HEL, but there are 29 acidic residues against 26 in HEL. This monocytic lysozyme moved more slowly than HEL in cellulose acetate electrophoresis. In contrast, MIL has a total of 154 residues, 34 acidic and 28 basic. The surplus of charged residues probably explains why this lysozyme moved faster than HEL in cellulose acetate electrophoresis. It is difficult to estimate how much of the lysozyme in an intestinal homogenate orginates from the Paneth cell, and how much is derived from white blood cells in the intestinal wall. It is possible that monocytic lysozyme was eliminated during the purification procedure (this could explain the low recovery we obtained in the amberlite eluate), or that scraping off mucosa from muscle layers eliminates most of the monocytes. In this context it is worth noting that rabbits, deficient in lysozyme in serum and most other tissues still contain 50% of the lysozyme of normal rabbits in their intestine[15]. The suggestion that structural differences exist between human granulocytic and macrophage lysozymes[6], should be investigated in the light of our observations, as Paneth cells may be considered to function as fixed macrophages[10]. Further research is needed to clarify these points, particularly because the existence of isozymes in sera of patients with inflammatory bowel disease could be the cause of the divergent results obtained by different methods[16–18].

[14] E. F. OSSERMAN and D. P. LAWLOR, J. exp. Med. *124*, 921 (1966).
[15] D. S. PRIEUR, H. M. OLSEN and D. M. YOUNG, Am. J. Path. *77*, 283 (1974).
[16] T. L. PEETERS, K. GEBOES and G. R. VANTRAPPEN, New Engl. J. Med. *292*, 1349 (1975).
[17] T. L. PEETERS, G. R. VANTRAPPEN and K. GEBOES, Gut *17*, 300 (1976).
[18] J. JOLLES and P. JOLLES, Helv. chim. Acta *52*, 2671 (1969).

Galactose Transfer and UDP-Galactose Hydrolysis in Urine from Normal Balb/c and Balb/c YC8 Mice

NICOLE ACHY-SACHOT and EVELYNE MOREL

Institut d'Immuno-Biologie de l'INSERM, Laboratoire Associé 143 du CNRS, Association Claude Bernard et EPHE, Hôpital Broussais, 96, rue Didot, F–75674 Paris Cédex 14 (France), 25 March 1976.

Summary. By chromatographic method we have shown the existence of a complex system for galactose transfer from UDP-galactose and for nucleotide hydrolysis in urines from Balb/c YC8 and normal Balb/c mice. By action of sera from normal and ascitic mice as source of enzyme, we have been able to detect transfer for galactose in urines from ascitic mice and an important inhibitory effect of the nucleotide sugar hydrolysis by the sera with urines from normal mice.

In previous studies, we have demonstrated the presence of soluble glycoprotein-galactosyl transferase activity in sera and in ascitic fluids from Balb/c mice bearing a lymphoma transmitted in ascitic form by i.p. injections of YC8 cells[1]. These results confirm those of BODOLSKY and WEISER[2] who detected galactosyl-transferase activities in human sera and a cancer associated isoenzyme in cancer sera. In our experiments, we have detected, in association with galactosyl-transferase activity, an important ratio of endogeneous transfer in sera and ascitic fluids from YC8 mice.

In the present study, we have investigated a glycoprotein-galactosyl-transferase activity in urines from healthy and ascitic mice on endogeneous and exogeneous acceptors, and we have demonstrated a galactose transfer in urines from ascitic mice by use of enzymes from sera. Hydrolysis of UDP-galactose has also been studied.

Materials and methods. UDP [(^{14}C)]-galactose (specific activity 274 mCi/m*M*) was purchased from New England Nuclear Corporation. Radioactivity was counted in a toluene PPO-POPOP as scintillant liquid. Glycoprotein acceptor in these studies was ovomucoid prepared as indicated by JAKUBCZAK and MONTREUIL[3]. Strain of YC8 ascite cells was carried in adults Balb/c mice by i.p. injections of 2×10^5 cells. Cells suspensions were harvested weekly. Sera from ascitic mice and from normal Balb/c mice were collected.

Urines from ascitic and normal Balb/c mice were collected for 12 h daily. Experiments were carried with 80 ascitic mice and 20 normal mice. Protein and sugars were determined in total urines according to PIRONNEAU et al.[4].

The assay mixture for galactosyl-transferase activity included, 10 µl urine (60 µg protein), 10 µl 1 *M Tris* pH 7.2, 5 µl 0.4 *M* $MnCl_2$, 100 µg ovomucoid for exogeneous

transfer or 10 μl H_2O for endogeneous transfer, 5 μl UDP [(^{14}C)]-galactose. In experiments where urine has been taken as the acceptor, ovomucoid was omitted and 10 μl of sera were added as enzyme source. Incubations were carried out at 37 °C for 60 min. Reaction was stopped in an ice-bath with addition of 10 μl EDTA 0.4 *M*. Chromatography of reaction mixture and detection of radioactive spots were made as previously described[1].

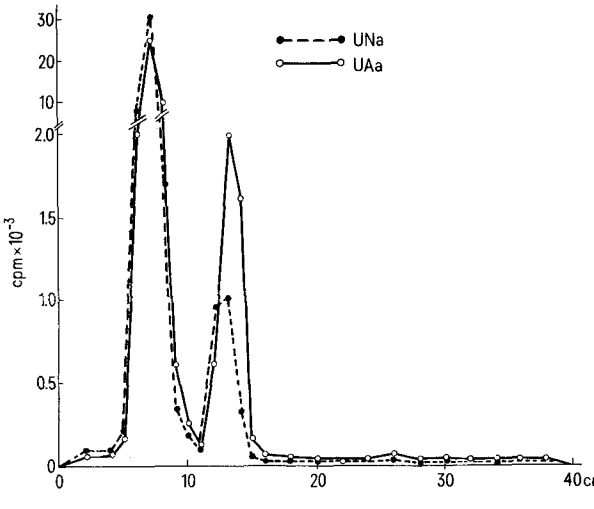
Results and discussion. Measurements of galactosyltransferase activity in urines from normal (UN) and ascitic Balb/c mice (UA) are given in Table I.

In incubation mixtures without any exogeneous acceptor molecule, there is no transfer either in UN or in UA. Furthermore, no more transfer may be detected with ovomucoid as exogeneous acceptor. This latter result shows that there is no glycoprotein galactosyltransferase able to transfer galactose on ovomucoid at the opposite of sera. It does not exclude the possibility of presence of other kind of enzymes able to transfer galactose, either on low weight molecules, as it has been demonstrated by CHESTER[5] in human urine, or on glycolipid acceptors.

Table I. [^{14}C]-galactose transferred from UDP-[^{14}C]-galactose in sera and urines from normal Balb/c and Balb/c YC8 mice

Enzyme	Acceptor			
	H_2O	Ovomucoid	UN	UA
UN	61	39	62	ND
UA	61	53	ND	65
SN	262	24,812	86	1,003
SA	677	39,192	92	1,693

Galactosyl-transferase activity was determined as described in methods with 10 μl of sera and results are expressed in cpm transferred from UDP-[^{14}C]-galactose. UN, urine from normal Balb/c mice; UA, urine from Balb/c YC8 mice; SN, serum from normal Balb/c mice; SA, serum from Balb/c YC8 mice.



Chromatographic profile of enzymatic assay for galactose transfer from UDP-[^{14}C]-galactose in urines from normal Balb/c and Balb/c YC8 mice. 1. [^{14}C]-galactose transferred: UN = 0,1%, UA = 0,1%. 2. UDP-[^{14}C]-galactose: UN = 93,2%, UA = 87,6%. 3. [^{14}C]-galactose-1-P: UN = 6,0%, UA = 11,6%. 4. [^{14}C]-galactose: UN = 0,1%, UA = 0,1%. Incubation mixtures as indicated in the text without any exogeneous acceptor added.

Table II. Chromatographic profiles of transfer of [^{14}C]-galactose from UDP-[^{14}C]-galactose expressed as ratio of radioactivity

Incubation mixture	Radioactivity (%)			
	Galactose transferred	UDP-galactose	Galactose-1-P	Galactose
SNa	0.8	22.2	18.6	57.5
UNc	0.2	92.6	5.9	0.6
UAc[a]	2.5	71.9	12.9	8.6
SAa	2.1	62.2	24.0	11.5
UNe	0.2	91.3	6.7	0.8
UAe[b]	4.4	59.0	13.1	16.0

Sera from normal Balb/c mice (SNa) and urines from normal Balb/c (UNc) and Balb/c YC8 (UAc) mice with SNa as enzyme source; sera from Balb/c YC8 mice (SAa) and urines from normal Balb/c (UNe) and Balb/c YC8 (UAe) with SAa as enzyme source. [a] About 4% of radioactivity as unidentified spots; [b] About 7% of radioactivity as unidentified spots.

To investigate the possibility of the presence of some acceptors molecules in the urines, these fluids have been incubated with sera from normal (SN) and ascitic mice (SA), and these mixtures tested for galactose transfer. In incubation mixtures of these two kinds of sera with normal urines, the galactose transfer is inhibited; with urines of ascitic mice, the transfer is enhanced.

These results may be explained by different mechanisms: first, the presence of acceptors for galactose in urines of ascitic mice, these acceptors being used by sera galactosyl-transferases, and perhaps absence of these acceptors in normal urines; second, occurrence of an activator-inhibitor system for galactose transfer by sera may be imagined in the urines, this system giving either an increase or a loss of transfer amount; third, it may also be assumed that enzymes exist in urines from ascitic mice and that they have transferred galactose on endogeneous serum acceptors, these enzymes having appeared during cancerization process. Enzymatic requirements and kinetic studies are now in progress and will permit us to confirm one of these hypotheses.

By the chromatographic method utilized, both the transfer and the ratio of sugar nucleotide hydrolysis may be measured (SPIK et al. personal communication).

The Figure shows chromatographic profiles for UDP-galactose hydrolysis in UN and UA incubated for transferase assays without exogeneous acceptor. During the incubation, 6 and 12% (respectively for UN and UA) of the total amount of radioactivity is recovered as (^{14}C)-galactose-1-P showing a nucleotide pyrophosphatase activity greater in UA than in UN. On the other hand, no phosphatase activity is seen either in UN or in UA.

Table II indicates the ratio of radioactivity in the different spots for the various incubation mixtures. In the two sorts of sera, the hydrolytic process is quite important. In SN, more than half of the radioactivity is found

[1] E. MOREL, G. SPIK and J. MONTREUIL, C. r. Acad. Sci. Paris *282*, 317 (1976).
[2] D. K. BODOLSKY and M. M. WEISER, Biochem. biophys. Res. Commun. *65*, 545 (1975).
[3] E. JAKUBCZAK and J. MONTREUIL, C. r. Acad. Sci., Paris *273*, 1420 (1971).
[4] F. PIRONNEAU, M. STERNBERG, M. MOISY, J. FERET, P. REBEYROTTE, G. LAGRUE and M. F. JAYLE, Nephron *13*, 434 (1974).
[5] M. A. CHESTER, FEBS Lett. *46*, 59 (1974).

in galactose spot, showing important nucleotide hydrolysis. When UN is incubated with sera, hydrolytic activities of these fluids are inhibited, the importance of galactose 1-P amount being that found in UN only, about 6% of total radioactivity.

These inhibitory processes are less important in assays containing UA; in fact, the ratio of galactose is enhanced in incubation mixtures with sera from ascitic mice. In these mixtures, a fraction of radioactivity is recovered as a new spot migrating faster than galactose. This latter is now being identified.

With further transfer studies those concerning nucleotide sugar hydrolysis in urines and sera from normal and ascitic mice are now being undertaken.

Regulation of Liver Lipogenic Enzymes by Dietary Fats

P. DIVAKARAN

Physiology Department, University of Texas, Medical School at Houston, 6400 West Cullen Street, Houston (Texas 77025, USA), 23 March 1976.

Summary. The hepatic lipogenic enzyme levels are more in rats on a fatfree diet and less in unsaturated fat-fed rats, the saturated fat-fed ones remaining in between.

There have been many reports of nutritional and hormonal effects on enzyme systems associated with the metabolism of lipids[1–4]. For some time, our laboratory has been interested in studying various factors controlling lipid metabolism, and the present paper summarizes our studies so far on the effects of dietary fats on some of the liver enzymes involved in fat metabolism. The enzymes studied are α-glycerol *p*-dehydrogenase, glycerol kinase, citrate cleavage enzyme, malic enzyme, malate dehydrogenase and isocitrate dehydrogenase.

Materials and methods. 40 weanling male albino rats were divided into 5 groups of 8 each and were fed ad libitum. The composition of the diet is shown in Table I. The feeding experiment was conducted for a period of 2 months, after which the animals were killed by decapitation and the liver enzymes estimated. The procedures employed were essentially those of BALDWIN et al.[5]. Liver sample was homogenized in 9 volumes of ice-cold 0.14 *N* potassium chloride solution in a Potter-Elvehjem type homogenizer. This was centrifuged at 0–4 °C for 30 min at 27,000 *g*. The supernatant was removed and used as the enzyme source for all the assays. All the enzyme measurements were carried out in a Carl-Zeiss Spectrophotometer PM Q II Model using quartz cell of 1 cm light path by standard procedures[6].

Results and discussion. The results of the various enzyme levels are shown in Table II. The activity of α-glycerol *p*-dehydrogenase in the safflower oil group of rats is lower than the other group of rats; the fat-free and the fat-free plus cholesterol group show the maximum activity. The glycerol kinase activity seems to be not much affected by the different dietary treatments, although the values for the last 2 groups are slightly higher than the rest. The changes in the activities of these 2 enzymes, although not very significant, appear to represent an increased capacity of the liver for triglyceride synthesis in the fat-free groups. Even the nature of the dietary fats affects the levels of these 2 enzymes at least to a certain extent. This could be a physiological necessity so that the synthesis of fatty acid takes place in the presence of a dietary deficiency of the same. BALDWIN et al.[5], however, showed that the changes in the activities of α-glycerol *p*-dehydrogenase and glycerol kinase took place only in the guinea-piglets and not in the rats.

Table I. Diet composition (%)

Fat source	Fat-free casein	Fat	Corn starch.	Cane sugar	Salt mixture	Vitaminized starch dextrose
Safflower oil	15	30	39	10	4	2
Coconut oil	15	30	39	10	4	2
Completely hydrogenated vegetable fat	15	30	39	10	4	2
Fat free	15	..	60	19	4	2
Fat free + 1% cholesterol	15	..	59	19	4	2

The activities of the citrate cleavage enzyme and the malic enzyme are very much affected by the dietary fat. Our results are in agreement with those reported by BALDWIN et al.[5] who showed that the activities of the citrate cleavage enzyme and the malic enzyme were 7.0 and 9.1 times greater with the fat-free diet than with the high fat diet (15.1% lard). ABRAHAM et al.[1] also noted that the citrate cleavage enzyme was dependent on the nutritional status of the animal. Our results, in addition to supporting the above views, also point out that the amount of unsaturated fat in the diet helps to regulate these enzyme levels, the rats fed unsaturated fat showing a lower level than the coconut or the hydrogenated vegetable oil groups. Formation of acetyl Co-A from citrate is the first step in the extramitochondrial fatty acid synthesis, and therefore an increase in the level of citrate cleavage enzyme obtained in fat-free group of rats shows that the fatty acid synthesis is more in these rats. KORNACKER and LÖWENSTEIN[7] suggested that the citrate cleavage reaction could be the rate controlling reaction with respect to the fatty acid synthesis in vivo. YOUNG, SHRAGO and LARDY[8] showed that the increase in malic enzyme when lipogenesis is high represents a mechanism for retrieval of the large amounts of oxalacetate fragments generated by the citrate cleavage enzyme. Both PANDE et al.[9] and WISE and BALL[10] suggested that some of the NADPH required for fatty acid synthesis may be generated by the malic enzyme-catalyzed reaction.

The malate and isocitrate dehydrogenase activities also are less in safflower oil group compared with the others. The higher dehydrogenase activities of the mitochondria as noted here, as well as the higher swelling rates of liver mitochondria from rats fed a diet deficient in EFAs[11],