Liver microsomal $\Delta 6$ and $\Delta 5$ linoleic acid desaturation in female BB rats

B. Chanussot, M. Narce and J.-P. Poisson

Laboratoire de Physiologie Animale et de la Nutrition, Faculté des Sciences Mirande, Dijon, France

Summary. Rat liver microsomal $\Delta 6$ and $\Delta 5$ desaturation are defective in experimental diabetes, but this defect is correctable with insulin treatment. Rat liver fatty acid composition and $\Delta 6$ and $\Delta 5$ desaturation were studied in the spontaneously diabetic adult female Bio-Breeding (BB) rat. Control Wistar rats and BB rats (4 weeks of diabetes), that received insulin ($1 \text{ IU} \cdot 100 \text{ g body weight}^{-1} \cdot \text{day}^{-1}$), were killed 20 h after the last insulin injection. $\Delta 6$ and $\Delta 5$ desaturase activities were estimated from the incubation of liver microsomes with $(1\text{-}^{14}\text{C})$ 18:2, n-6 or $(2\text{-}^{14}\text{C})$ 20:3, n-6, respectively, and the fatty acid

composition of the liver and microsomal liver lipids were investigated. Under experimental conditions $\Delta 6$ and $\Delta 5$ desaturase activities were unchanged in the BB rats when compared to the control rats. Impairment of the liver fatty acid composition of diabetic BB rats is not consistent with normal desaturase activity and may be explained by factors other than desaturation disturbance.

Key words: Spontaneous diabetes, BB rat, insulin, $\Delta 6$ desaturase, $\Delta 5$ desaturase, liver lipids.

It is well-known that unsaturated fatty acid biosynthesis follows an alternating sequence of desaturation and elongation reactions that take place in the microsomal fraction of the cell [1]. Control of this biosynthesis is evoked mainly by the regulation of different microsomal desaturases which introduce double bonds at $\Delta 9$, $\Delta 6$ and $\Delta 5$ position in the fatty acid chains [1]. Evidence obtained from microsomal rat liver preparations indicates that these enzymes are under hormonal control [2–4].

Insulin, among other hormonal factors, has been specifically studied. In diabetes mellitus several metabolic changes take place which alter the utilization of glucose and fatty acids by the cells. The major reason for these changes is an absolute or relative deficiency of insulin, but exogenous factors such as diet also play a role [4]. A great deal of *in vitro* as well as *in vivo* evidence has been accumulated which indicates an impairment of the $\Delta 9$, $\Delta 6$ and $\Delta 5$ desaturases, which are responsible for the further metabolism of essential fatty acids [4–10]. Diminished microsomal fatty acid desaturation which occurs in experimental diabetes mellitus is correctable with 24 h of insulin therapy [4, 7, 8, 11]. The defect in experimental diabetes results from an effect on the terminal desaturase enzyme rather than an effect on the prior components of the microsomal electron transport system which is responsible for fatty acid desaturation [7, 11]. Insulin is an activating factor of $\Delta 9$, $\Delta 6$ and $\Delta 5$ desaturations [3, 4, 7, 8, 12] according to a mechanism of genetic enzyme induction through the biosynthesis of desaturases [1, 4, 12] even though these are different enzymes [1]. Studies of diabetic animals show alterations of tissue fatty acid composition, consistent with depressed desaturase activity [13–15].

The BB rat is the only reported example of spontaneous insulin-dependent diabetes in the rat, and the only well-characterized spontaneous model in any species with destructive insulinitis resembling the lesions described in human Type 1 (insulin-dependent) diabetes [16–20].

The main purpose of the present experiment was to determine the possible existence of a modulation, by insulin, of liver microsomal $\Delta 6$ and $\Delta 5$ desaturase activities in female spontaneously diabetic BB rats. We have also investigated the possibility that the modulation corresponds to changes in fatty acid composition of the liver, particularly of microsomes. Since the synthesis of unsaturated fatty acids occurs in the microsomal fraction, microsomal fatty acid composition might reflect early changes in fatty acid desaturation.

Materials and methods

Ten 21-week old female rats were supplied by the Centre de Sélection et d'Élevage d'Animaux de Laboratoire (C.N.R.S., Orléans, France): 5 normal Wistar rats; 5 Wistar BB rats which expressed the spontaneous diabetic syndrome 4 weeks before being killed. All rats were fed U.A.R. A.04 commercial chow (Villemoisson-Sur-Orge, France) and water *ad libitum*. The composition of the diet was as follows: proteins: 17%; glucides: 55.5%; cellulose: 5%; mineral salts: 6%; vitamins: 1%; water: 13%. A simple analysis from one batch of the diet contained 2.5% total lipids with a fatty acid composition of 17.4% palmitic acid (16:0), 2.0% palmitoleic acid (16:1, n-7), 2.1% stearic acid (18:0), 18.8% oleic acid (18:1, n-9), 52.3% linoleic acid (an amount of 18:2, n-6 prevented any essential fatty acid deficiency apt to impair the 18:2, n-6 $\rightarrow$ 20:4, n-6 conversion) and 5.6% α -linolenic acid (18:3, n-3).

The criterion for diabetes was a blood sugar $> 16.7 \text{ mmol/l}$ (Glucose-oxidase Haemo-Glukotest 20–800 strips, Boehringer, Meylan, France). The diabetic rats received subcutaneously 1.0 IU protamine

Fig. 1. Fatty acid composition of liver total lipids (% of total fatty acids). Mean values for normal Wistar rats (■) and for diabetic BB rats (▨) $\pm$ SD (□). * $p < 0.05$

Fig. 2. Fatty acid composition of liver phosphatidylcholines (% of total fatty acids). Mean values for normal Wistar rats (■) and for diabetic BB rats (▨) $\pm$ SD (□). * $p < 0.05$

zinc insulin $\cdot 100 \text{ g body weight}^{-1} \cdot \text{day}^{-1}$ (E. Z. P., gift from Organon, Saint-Denis, France).

After about 3 h of fasting, the rats were killed by cervical dislocation 20 h after the last insulin injection. The livers were quickly removed, rinsed in 0.15 mol/l NaCl and weighed after blotting on filter paper. Total lipids were extracted according to the method of Del-sal [21] and an aliquot weighed; phospholipids and triacylglycerols were separated from another aliquot according to the method of Hirsch and Ahrens [22] and then weighed.

Phosphatidylethanolamines and phosphatidylcholines were isolated by the thin-layer chromatography method of Wagner et al. [23]. A sample laid on the plate was revealed by iodine so as to visualize these lipid classes. Unexposed samples, on the same plate, were scraped according to the different lipid classes. The fatty acids of liver, total lipids, phospholipids, triacylglycerols, phosphatidylethanolamines and phosphatidylcholines were converted to methyl esters according to the method of Slover and Lanza [24] and analysed, in a Packard (Downers Grove, Ill, USA), model 419, gas liquid chromatograph equipped with a flame ionization detector. The 30 m capillary glass column was packed with Carbowax 20 mol/l. The oven, detector and injector temperatures were 198, 250 and 225°C, respectively. Identification of methyl esters was made by comparing with authentic methyl ester standards and heptadecanoic acid (17:0) was used as an internal standard. Retention time and peak areas were determined

electronically using a Delsi Enica 21 integrator (Suresnes, France). Only the fatty acid which was present in greater amounts than 0.1% of the total fatty acids was reported.

The solutions used for lipid extraction and thin-layer chromatography contained 2,6-di-tert-butyl-p-cresol (50 mg/l) as an antioxy-dant and the lipid extracts were stored under nitrogen gas in the dark at -20°C to prevent peroxidation of unsaturated fatty acids.

Three grammes of rat liver tissue from the 10 rats was cut into thin slices and homogenized at 4°C in a Potter-Elvehjem homogenizer with 18 ml of a solution containing 0.05 mol/l phosphate buffer, pH 7.4, and 0.25 mol/l sucrose. The homogenate was then centrifuged at $8,000 \times g$ for 30 min, the pellet then discarded and the supernatant was centrifuged again at $105,000 \times g$ for 60 min (L8-55 ultracentrifuge Beckman, Palo Alto, Calif, USA) to obtain the microsomal pellet. These steps were carried out at 4°C . The microsomal fraction was resuspended in 0.4 ml of homogenizing solution. All subsequent enzymatic assays and composition analysis used these microsomal membrane fractions. Proteins were determined using the Layne method [25]; bovine serum albumin was used as the standard.

The $\Delta 6$ desaturation of linoleic acid and the $\Delta 5$ desaturation of di-homo- γ -linolenic acid by liver microsomes were measured by estimation of the percentage conversion of ^{14}C linoleic and di-homo- γ -linolenic acids, respectively, to their corresponding product, γ -linolenic and arachidonic acids.

Fig. 3. Fatty acid composition of liver phosphatidylethanolamines (% of total fatty acids). Mean values for normal Wistar rats (■) and for diabetic BB rats (▨) $\pm$ SD (□). * $p < 0.05$

The desaturation activity of the microsomes was determined in a metabolic shaker at 37°C in air, incubating the microsomal protein with the labelled substrate [1^{14}C linoleic and 2^{14}C dihomo- γ -linolenic acid (The Radiochemical Centre, Amersham, Bucks, UK; specific activity: 55 mCi/mmol; radiochemical purity > 96%) diluted with unlabelled fatty acid (Sigma, St. Louis, Mo, USA) to obtain ethanol solution with a specific activity of about 5 mCi/mmol (20 nmol/10 μl of solution) and maintaining 0.02 or 0.04 μmol of radioactive precursor in each flask].

The medium contained 7.4 μmol ATP, 1 μmol CoA, 2.5 μmol NADPH, 10 μmol MgCl_2 in a total volume of 2.1 ml of 0.15 mol/l phosphate buffer pH 7.4. The coenzymes and other reagents were pure products from Sigma or Merck (Darmstadt, FRG). The reaction was initiated by the addition of 5 mg of microsomal suspension protein to the incubation medium. The incubation was carried out for 5 min. Under such conditions, the formation of the product was proportional to the time of incubation and protein concentration according to Narce et al. [26]. The desaturation reaction was terminated by the addition of 5 ml of 12% KOH in ethanol. After 1 h of saponification at 75°C then methylation of fatty acids using boron trifluoride in methanol according to the method of Slover and Lanza [24], the distribution of radioactivity between the substrate and product was determined by gas liquid radiochromatography in a Packard model 470 gas chromatograph according to the method of Bezar et al. [27]. The glass column was packed with 24% diethylene glycol suc-

Fig. 4. Fatty acid composition of liver triacylglycerols (% of total fatty acids). Mean values for normal Wistar rats (■) and for diabetic BB rats (▨) $\pm$ SD (□). * $p < 0.05$

cinate (DEGS) coated on Chromosorb W (80–100 mesh) (Johns-Manville, Celite Division, Denver, Colo, USA). The desaturation activity expressed as pmol of substrate converted per min per mg microsomal protein was calculated from percentage of conversion. A control assay with the addition of microsomes, but also alcoholic KOH, was done at time 0 of the reaction and no desaturation was observed. The fatty acid methyl esters were identified by comparison with pure standards. Microsome total lipids were extracted, converted to methyl esters and analysed for their fatty acid composition as previously described for liver total lipids.

Statistical analysis

Data are reported as mean $\pm$ SD. The significance of differences in a treatment series was determined by the Student's t test ($p < 0.05$).

Results

Table 1 shows the blood glucose levels, body and liver weights and liver total lipid, triacylglycerol and phospholipid weights. Blood samples at the time of killing

Fig. 5. Fatty acid composition of liver microsomal total lipids (% of total fatty acids). Mean values for normal Wistar rats (■) and for diabetic BB rats (▨) $\pm$ SD (□). * $p < 0.05$

Table 1. Blood glucose, body and liver weights and liver lipid weights of normal Wistar rats and diabetic BB rats

	Normal rats <i>n</i> = 5	BB rats <i>n</i> = 5
Blood glucose levels at time of killing (nmol/l)	5.5 $\pm$ 1.1	21.1 $\pm$ 2.7 ^a
Body weight (g)	227 $\pm$ 12	229 $\pm$ 35
Liver weight (g)	9.0 $\pm$ 1.5	16.4 $\pm$ 4.2 ^a
Liver weight (g) Body weight (g) $\times 10$	4.0 $\pm$ 0.6	7.1 $\pm$ 0.9 ^a
Total lipids (mg/g liver)	47.2 $\pm$ 3.4	44.1 $\pm$ 2.9
Triacylglycerols (mg/g liver)	1.3 $\pm$ 0.5	4.0 $\pm$ 1.6
Phospholipids (mg/g liver)	28.3 $\pm$ 5.4	27.0 $\pm$ 6.5

Mean values $\pm$ SD. *n*: number of rats. ^a $p < 0.05$

revealed that blood glucose values of the BB rats were increased 20 h after the last insulin injection compared to the control rats. Although the body weights were similar, the liver weights were significantly increased $p < 0.05$ for the diabetic BB rats. Liver total lipid, triacylglycerol and phospholipid weights of diabetic BB rats were similar to the control rats.

Figures 1 to 5 show the fatty acid composition (%) of liver total lipids, phosphatidylcholines, phosphatidylethanolamines, triacylglycerols and microsomal total lipids. Linoleic and oleic acid levels of diabetic BB rats increased (except 18:2, *n*-6 for triacylglycerols) compared to control rats, while arachidonic and stearic acids decreased (except for phosphatidylethanolamines and triacylglycerols). $\Delta 6$ and $\Delta 5$ desaturation measurements of diabetic and control rats appear in Table 2. We observed no significant difference between the two groups with respect to $\Delta 6$ and $\Delta 5$ desaturation for the two quantities of radioactive precursors used.

Discussion

The plasma glucose levels of the diabetic BB rats indicated that they were no longer under the influence of insulin 20 h after their last injection. Diabetic BB rats cannot survive for more than 36–48 h without insulin, and treatment with daily insulin injections restores and maintains the gain in body weight. The results that we have obtained cannot be explained by species differences because the diabetic rats are the products of a selective crossing between animals with an abnormal tolerance to glucose, selected from a normal population of Wistar rats.

The fact that there was no significant difference in food intake between the BB and control rats and that the BB rats gained the same weight as the control rats may be explained by the daily injections of insulin. Consequently, the nutritional variable is not implicated in our experiment and we have only to consider the spontaneous diabetes factor associated with daily insulin injection.

We observed an increase in liver weight in relation to body weight in the diabetic BB rats, which has not been reported in other studies of chemical diabetes [4–6], although there was no increase in lipids. The explanation for the origin of such metabolic transformation may be sought elsewhere, perhaps in the protein and/or water content, due to the high carbohydrate/low fat diet which induces liver hypertrophy. We observed a rise in plasma triglycerides in the BB rats, compared to the control rats, which was lowered by insulin treatment (unpublished results).

The liver phospholipid content of the female insulin-treated BB rats is similar to that of the control rats and may be explained by the daily insulin treatment, streptozotocin diabetic rats having an increase in liver phospholipids [8, 28, 29] and in liver microsomal phos-

Table 2. Liver microsomal $\Delta 6$ and $\Delta 5$ desaturation of normal Wistar rats and diabetic BB rats

	Radio- active precursor (nmol)	Conversion	Normal Wistar rats <i>n</i> = 5	Diabetic BB rats <i>n</i> = 5
Linoleic acid	20	%	2.7 $\pm$ 1.4	3.0 $\pm$ 0.6
$\Delta 6$ desaturation (1- 14 C 18:2, <i>n</i> -6)	40	pmol $\cdot$ min $^{-1}$, mg protein $^{-1}$	21.6 $\pm$ 11.2	24.0 $\pm$ 4.8
		%	2.7 $\pm$ 1.0	3.3 $\pm$ 1.5
		pmol $\cdot$ min $^{-1}$, mg protein $^{-1}$	43.2 $\pm$ 16.0	52.8 $\pm$ 24.0
Dihomo- γ - linolenic acid	20	%	18.6 $\pm$ 7.5	19.2 $\pm$ 7.5
$\Delta 5$ desaturation (2- 14 C 20:3, <i>n</i> -6)	40	pmol $\cdot$ min $^{-1}$, mg protein $^{-1}$	148.8 $\pm$ 60	153.6 $\pm$ 60
		%	15.4 $\pm$ 5.0	12.6 $\pm$ 3.9
		pmol $\cdot$ min $^{-1}$, mg protein $^{-1}$	246.4 $\pm$ 80	201.6 $\pm$ 62.4

Desaturation activity expressed as pmol of substrate converted per min per mg microsomal protein calculated from percentage of conversion. Mean values $\pm$ SD. *n*: number of rats

phatidylethanolamine content which accounts for a slight increase in total microsomal phospholipids [14].

The *in vitro* activity of $\Delta 6$ and $\Delta 5$ desaturase in the female diabetic BB rats was similar to the control rats during the hyperglycaemic period which followed the daily injection of insulin. The daily treatment with insulin, which maintained the influence of the hormone, may explain the fact that $\Delta 6$ and $\Delta 5$ desaturase activity of diabetic BB rats was no different from control rats. The activities of these microsomal enzymes are partially inhibited by chemical diabetes; insulin abruptly reverses and even overcorrects them [4–8, 12, 30, 31]. Therefore, we hypothesise that $\Delta 6$ and $\Delta 5$ desaturase activity could be depressed during the short hyperglycaemic period which precedes the insulin injection to the diabetic BB rats.

The experimental conditions for insulin injections were sufficient to normalize $\Delta 6$ and $\Delta 5$ desaturase activity. We observed that $\Delta 6$ and $\Delta 5$ desaturations required similar amounts of insulin and time of treatment as for experimental diabetes [4]. This phenomenon may have an effect on protein synthesis [7]. Insulin has been shown to stimulate fatty acid desaturation, probably via an increase in enzyme synthesis. On the other hand, a decrease in the level of desaturation through glycolysis has also been reported [2, 4]. It is interesting to note that Oshino and Sato [32] reported a peak of desaturase activity 17 h after refeeding starved rats, which is the time required for restoration after insulin was given to diabetic rats and consistent with the appearance of a protein synthetic effect [7]. Appel et al. [33] demonstrated marked variations in the activities of a number of hepatic glycolytic enzymes in diabetic BB rats. Warren et al. [34] examined the effect of diabetes on the activities of several hepatic microsomal enzymes and found differential alterations which could be corrected by insulin treatment.

Furthermore, the activation of acyl-CoA desaturases by soluble proteins has been reported previously [35, 36]. We cannot exclude an opposing effect of diabetes and insulin on such protein factors. This phenomenon might partially explain why we observed no change in fatty acid $\Delta 6$ and $\Delta 5$ desaturase activities.

The level of linoleic acid was increased in the liver phospholipids of the insulin-treated BB rats, as during experimental diabetes [15], but a low level of arachidonic acid was seen. The observed changes could be related to a changed pattern of fatty acid incorporation or to a change in the composition of the fatty acid pool. The decrease in stearic and increase in oleic acid are probably due to insulin activation of $\Delta 9$ stearic acid desaturase, an important factor able to change the pool of fatty acids available for incorporation in phospholipids.

The fatty acid composition of the diabetic BB rat liver triacylglycerol fraction may be explained by the normal food intake associated with the daily insulin treatment. Some differences, previously shown during experimental diabetes [28, 37], may be related to lipolysis or perturbations of the intestinal absorption of these fatty acids.

Unchanged $\Delta 6$ and $\Delta 5$ desaturase activity and the suggestion of increased $\Delta 9$ desaturase activity led us to suppose that the corresponding microsomal fatty acid composition was changed in a similar way. Daily insulin treatment of diabetic BB rats was insufficient to maintain normal microsomal lipid fatty acid composition. While most of the fatty acid levels were similar to that of the control rats, oleic and linoleic acids increased when stearic and arachidonic acids were significantly decreased in the insulin-treated BB rats. The amounts of fatty acid seemed to indicate an inhibition of $\Delta 6$ and $\Delta 5$ desaturations inconsistent with the measured desaturase activities. This phenomenon could reflect an increased utilization of arachidonic acid not compensated for by increased synthesis. The decreased ratio of arachidonic to linoleic acid suggested a decreased synthesis of arachidonic acid.

The stearic and oleic acid composition of liver microsomal total lipids strengthens our hypothesis of a stimulated $\Delta 9$ desaturase activity. $\Delta 6$ and $\Delta 5$ desaturase activity could be less depressed in spontaneous diabetes and less responsive to insulin than is $\Delta 9$ desaturase activity, as previously observed in streptozotocin-induced diabetes [7].

Spontaneous diabetes, even insulin-treated, results in fatty acid alterations in the lipid composition of microsomes. In addition to alterations following changes in desaturation other factors such as fatty acid elongation, membrane lipid degradation and synthesis, and fatty acid oxidation may all be important. Further work is necessary to delineate the cause and physiological consequences of the abnormal microsomal phospholipid composition, more or less restored by insulin therapy, in the spontaneously diabetic rat.

Acknowledgements. Thanks are due to Ms. M.-T. Divry-Aellen, Ms. T. Duboucarre and J. Gresti for technical assistance, to E. Christiansen (Department of Nutritional Research, University of Oslo, Norway) for helpful suggestions and to C. Straker for correcting the English.

References

- Brenner RR (1977) Metabolism of endogenous substrates by microsomes. *Drug Metab Rev* 6: 155-212
- Brenner RR (1981) Nutritional and hormonal factors influencing desaturation of essential fatty acids. In: Holman RT (ed) *Progress in lipid research*. Pergamon Press, Oxford New York, pp 41-47
- Jeffcoat R, James AT (1984) The regulation of desaturation and elongation of fatty acids in mammals. In: Numa S (ed) *New comprehensive biochemistry*. Elsevier, Amsterdam New York Paris, pp 87-112
- Poisson J-P (1985) Comparative in vivo and in vitro study of the influence of experimental diabetes on rat liver linoleic acid $\Delta 6$ and $\Delta 5$ desaturation. *Enzyme* 34: 1-14
- Poisson J-P, Lemarchal P, Blond J-P, Lecerf J, Mendy F (1978) Influence du diabète alloxanique sur la conversion des acides linoléique et γ -linoléique (^{14}C) en arachidonate chez le rat in vivo. *Diabete Metab* 4: 39-45
- Poisson J-P, Blond J-P, Lemarchal P (1979) Influence du diabète streptozotocique sur la conversion des acides γ -linoléique et di-homo- γ -linoléique (^{14}C) en acide arachidonique chez le rat in vivo. *Diabete Metab* 5: 43-46
- Eck MG, Wynn JO, Carter WJ, Faas FH (1979) Fatty acid desaturation in experimental diabetes mellitus. *Diabetes* 28: 479-485
- Faas FH, Carter WJ (1980) Altered fatty acid desaturation and microsomal fatty acid composition in the streptozotocin diabetic rat. *Lipids* 15: 953-961
- Riisom T, Johnson S, Hill EG, Holman RT (1981) Effects of experimental diabetes on the essential fatty acid-deficient rat. *J Lab Clin Med* 98: 764-775
- Poisson J-P, Blond J-P (1985) Influence du diabète streptozotocique sur la conversion in vivo des acides γ -linoléique et di-homo- γ -linoléique ^{14}C en acide arachidonique dans les reins et le rat entier. *Diabete Metab* 11: 289-294
- Prasad MR, Joshi VC (1979) Regulation of rat hepatic stearoyl coenzyme A desaturase. *J Biol Chem* 254: 997-999
- Brenner RR (1977) Regulatory function of $\Delta 6$ desaturase-key enzyme of polyunsaturated fatty acid synthesis. *Adv Exp Med Biol* 83: 85-101
- Worcester NA, Bruckdorfer KR, Hallinan T, Wilkins AJ, Mann JA, Yudkin J (1979) The influence of diet and diabetes on stearoyl coenzyme A desaturase (EC 1.14.99.5) activity and fatty acid composition in rat tissues. *Br J Nutr* 41: 239-252
- Faas FH, Carter WJ (1983) Altered microsomal phospholipid composition in the streptozotocin diabetic rat. *Lipids* 18: 339-342
- Huang YS, Horrobin DF, Manku MS, Mitchell J, Ryan MA (1984) Tissue phospholipid fatty acid composition in the diabetic rat. *Lipids* 19: 367-370
- Marliss EB, Nakhoda AF, Poussier P, Sima AAF (1982) The diabetic syndrome of the "BB" Wistar rat: possible relevance to Type 1 (insulin-dependent) diabetes in man. *Diabetologia* 22: 225-232
- Bell RH Jr, Hye RJ (1983) Animal models for diabetes mellitus: physiology and pathology. *J Surg Res* 35: 433-460
- Chappel CI, Chappel WR (1983) The discovery and development of the BB rat colony: an animal model of spontaneous diabetes mellitus. *Metabolism* 7 [Suppl 1]: 8-10
- Like AA, Rossini AA (1984) Spontaneous autoimmune diabetes mellitus in the Bio-Breeding Worcester rat. *Surv Synth Path Res* 3: 131-138
- Nasso R, Moutier R (1984) La souche de rats BB modèle d'étude du diabète juvénile humain insulino-dépendant. *Symbioses* 4: 226-232
- Delsal JL (1944) Nouveau procédé d'extraction des lipides du serum par le méthylal. Applications aux microdosages du cholestérol total, des phosphoaminolipides et des protéides. *Bull Soc Chim Biol* 26: 99-105
- Hirsch J, Ahrens EH Jr (1958) The separation of complex lipid mixture by the use of silicic acid chromatography. *J Biol Chem* 233: 311-320
- Wagner H, Hörhammer L, Wolff P (1961) Dünnschichtchromatographie von phosphatiden und glykolipiden. *Biochem Z* 334: 175-184
- Slover HT, Lanza E (1979) Quantitative analysis of food fatty acids by capillary gas chromatography. *J Am Oil Chem Soc* 56: 933-943
- Layne E (1957) Spectrophotometric and turbidimetric methods for measuring proteins. In: Colowick SP, Kaplan NO (eds) *Methods in enzymology*. Academic Press, New York, pp 447-454
- Narce M, Poisson J-P, Belleville J, Chanussot B (1988) Time-course effects of protein malnutrition on hepatic fatty acids $\Delta 6$ and $\Delta 5$ desaturation in the growing rat. *Br J Nutr* 60: 389-402
- Bézar J, Boucrot P, Clément G (1964) Collecte d'esters d'acides gras marqués au tritium et au carbone 14 élués par chromatographie gaz-liquide. *J Chromatogr* 14: 368-377
- Mukerjee SS, Murthi PSR, Garg NK, Mukerjee SK (1979) Effect of alloxan-diabetes on some aspects of lipid metabolism in albino rats. *Acta Diabetol Lat* 16: 111-118
- Poisson J-P, Blond J-P (1983) Effets d'une supplémentation en acide γ -linoléique sur la composition en acides gras des lipides du foie et du plasma du rat diabétique. *Arch Int Physiol Biochim* 91: 361-378
- Lee TC, Baker RC, Stephens N, Snyder F (1977) Evidence for participation of cytochrome b in microsomal $\Delta 6$ -desaturation of fatty acids. *Biochim Biophys Acta* 489: 25-31
- Mercuri O, De Tomas MA, Itarte H (1979) Prenatal protein depletion and $\Delta 9$ -, $\Delta 6$ - and $\Delta 5$ -desaturases in the rat. *Lipids* 14: 822-825
- Oshino N, Sato R (1972) The dietary control of the microsomal stearyl CoA desaturation enzyme system in liver. *Arch Biochem Biophys* 149: 369-377
- Appel MC, Like AA, Rossini AA, Carp DB, Miller TB Jr (1981) Hepatic carbohydrate metabolism in the spontaneously diabetic Bio-Breeding Worcester rat. *Am J Physiol* 240: E 83-87
- Warren BL, Pak R, Finlayson M, Gontovnick L, Sunhara G, Bellward GD (1983) Differential effects of diabetes on microsomal metabolism of various substrates. Comparison of streptozotocin and spontaneously diabetic Wistar rats. *Biochem Pharmacol* 2: 327-335
- Nervi AM, Catala A, Brunner RR, Peluffo RO (1975) Dietary and hormonal effects upon activity of "soluble" protein and particulate fraction of fatty acid desaturation system of rat liver microsomes. *Lipids* 10: 348-352
- Leikin AI, Brenner RR (1986) Regulation of linoleic acid $\Delta 6$ desaturation by a cytosolic lipoprotein-like fraction in isolated rat liver microsomes. *Biochim Biophys Acta* 876: 300-308
- Brenner RR, Nervi AM (1965) Kinetics of linoleic and arachidonic incorporation and eicosatrienoic depletion in the lipids of fat deficient rats fed methyl linoleate and arachidonate. *J Lipid Res* 6: 363-368

Received: 16 November 1988
and in final revised form: 24 July 1989

Prof. J.-P. Poisson
Laboratoire de Physiologie Animale
et de la Nutrition
Faculté des Sciences Mirande
B.P. 138
F-21004 Dijon Cedex
France