ed after the other observations were made. In each case the sterol added to the culture was found in the mycelium[9]. Peak area in GLC for the various free sterols (isolated without saponification) were similar. The amounts were near 0.01% of each sterol on a dry wt basis (wt/wt).

Although naturally occurring sterols display considerable variation in structure, there is a rather narrow distribution of chain lengths attached to C-20 (other than C-21). Only 4–6 C-atoms in a linear array are found even though the total number may be expanded by branches, as in the case of campesterol with a 5-carbon array and 2 methyl branches at C-24 and C-25. With *P. cactorum* both hyphal extension (table 1) and development of oogonia into oospores (table 2) were influenced most by sterols with or very nearly with the natural range. Thus, for hyphal extension maximal stimulation occurred with chains on C-20 of 4–6 C-atoms, and it was chains of 4–7 C-atoms which yielded germinating oospores. However, either a moderate increase (to a 9-carbon chain as in 20(R)-*n*-nonylpregn-5-3β-ol) or a decrease (to a 0-carbon chain as in pregn-5-en-3β-ol) in this distribution of chain lengths remained consistent with production of oogonia (table 2), although maturation was nearly abolished (fig. 2), since most of the oogonia aborted before becoming oospores. The striking retention of regulatory activity (induction of oogonia formation) despite drastic reduction in the size of the side chain is unusual[10] for a sterol but not unknown. For instance, as a mammalian component of the diet pregn-5-en-3β-ol acts qualitatively and quantitatively as cholesterol does to inhibit hepatic sterol synthesis[11]. Androst-5-en-3β-ol is also though somewhat less active[10].

In view of the ability of pregn-5-en-3β-ol to induce formation of oogonia, it seems unlikely that this sterol is normally converted in *P. cactorum* to a hormone with an oxygenated side chain of the sort (the oogoniols)[12] which arises in *Achlya* sp. An alternative regulatory role is for the sterol to modulate membrane structure and function by incorporation into the lipid bilayer of hyphae and spores. The size and shape of the side chain or the nature of unsaturation could then play roles of their own without the occurrence of any metabolism. If such were the case in the pythiaceous fungi, the reproductive similarity of these organisms with those in the genus *Achlya* may be a resultant of an evolutionary history which was convergent with respect to acquisition of oomycetous character. Had the organisms arisen by parallel evolution, one might also have expected the regulatory phenomena to be similar.

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Comparison of circulating lipoprotein lipase activity in Zucker *fa/fa* and *Fa/–*rats

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Summary. Lipoprotein lipase activity was determined in Zucker rats by assaying VLDL radioactivity. Animals were i.v. injected with $^3H_2$-oleic acid and ^{14}C-glycerol with or without Triton WR 1339. This enzymatic activity was higher in *fa/fa* rats than in non-obese *Fa/–* rats.

It is relatively well established[1–5] that hyperlipoproteinemia of the *fa/fa* Zucker rat is due to an increased synthesis of very low density lipoproteins (VLDL)[6]. An eventual decrease of lipid clearance, however, has not been proven. It is known[7,8] that fat cell lipoprotein lipase activity of the *fa/fa* rat is higher than that of the *Fa/–* rat.

We have studied the total activity of circulating lipases with Triton WR 1339 which has the property of inhibiting these lipases[9,10]. VLDL catabolism is suppressed by the action of Triton and lipoproteins accumulate in the circulatory system[11–13]. The quantity of VLDL formed can be determined by the use of radioactive precursors (^{14}C-1-glycerol and $^3H_2$-9,10- oleic acid). The radioactivity of circulating VLDL in *fa/fa* Zucker rats can be compared with that of the *Fa/–*

controls, having or having not received Triton, and it is thus possible to determine the respective degrees of lipoprotein lipase activities.

Materials and Methods. 24 adult male Zucker rats (C.N.R.S., Orléans La Source, France), 6–8 weeks old were fed standard chow (U.A.R., No.A-04) and divided into 2 groups.

Group 1: 12 *fa/fa* obese and hyperlipoproteinemic rats (groups A, B).

Group 2: 12 normal *Fa/–* rats (groups C, D).

There were 6 rats in each lettered sub-group.

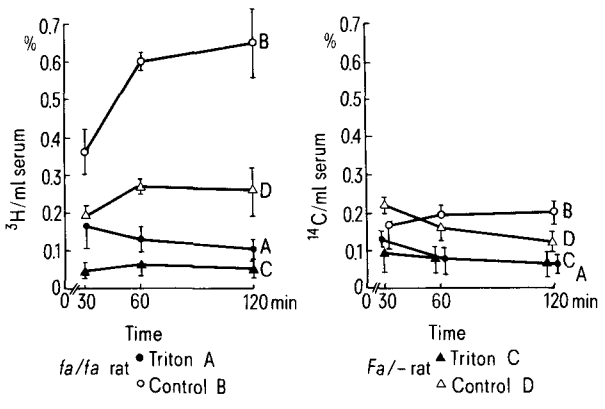
After an 18 h fast, rats were anesthetized by i.p. injection of sodium pentobarbital at 5 mg/100 g b.wt. Groups B and D received via slow injection in the right jugular vein 0.5 ml of Triton (generously supplied by Winthrop Laboratories, Z.I. Dijon-Longvic, France) as 100 ml dissolved in 0.9% NaCl. Groups A and C (controls) received the same volume of saline alone.

5 min later, all the rats received an injection of 0.5 ml of saline containing 1.8 µCi (12.8 µmoles) of ^{14}C-l-glycerol (C.E.A., Saclay, France) and 5.4 µCi (22.8 µmoles) of $^{3}H_2$-9,10-oleic acid (C.E.A., Saclay, France) fixed on 15 mg of albumin (Calbiochem fatty acid depleted albumin, San Diego, Ca., USA). Injection was in the right jugular vein. Two rats in each group were sacrificed by abdominal aortic puncture at times 30, 60 and 120 min post-injection.

VLDL were isolated by ultracentrifugation[14] in an MSE 72 rotor at 120,000 × g for 20 h. This technique enables the VLDL fraction to be separated from plasma albumin containing the oleic acid. The latter is found in the centrifugation pellet. Tritium and ^{14}C radioactivities were determined with a model ABAC Intertechnique SL 40 Scintillation Spectrometer.

Results and discussion. The methodology used should first be discussed. The distribution between hepatic oxidation and esterification pathways is not necessarily equivalent for the 2 rat phenotypes. It has in fact been shown[15] that this distribution was different between males and females of the same strain. Because of this finding, we used only male rats. If a differential distribution exists between the 2 phenotypes, it is not a factor in the present work, since lipoprotein lipase activity was not evaluated from the basal VLDL secretory rate, but rather from the different VLDL levels of rats having or not having received Triton.

The level of VLDL precursors in *fa/fa* rats is much higher (group A–B) than in *Fa/–* rats (group C–D) ($^{3}H_2$-oleic acid at all points, ^{14}C-glycerol at 120 min). Thus, circulating lipoprotein lipase activity is higher in obese rats than in controls. Radioactive glycerol disappears from the bloodstream more rapidly than tritiated oleic acid as a result of its higher rate of oxidation and extra-hepatic capture. This explains why it is present in VLDL in lower quantities that is oleic acid. Triton decreases hepatic lipid anabolism[16] by reducing the activities of lipogenesis enzymes (especially the dehydrogenases). Thus the differences (A–B) and (C–D), representing the resultant of lipase activities, are evaluated by default.



Incorporation of $^{3}H_2$-9,10-oleic acid and of ^{14}C-1-glycerol in the VLDL of Zucker *fa/fa* and *Fa/–* rats. Results expressed as percent of injected radioactivity/ml of serum. Results are expressed as the means (N = 2). Each vertical bar represent the difference between the 2 experimental values.

Triton inhibition of extra-hepatic lipoprotein lipase activities has been demonstrated[17]. To our knowledge, however, its effect on hepatic lipase activity has not been clearly elucidated. This latter action has relatively little importance in the present study because it does not require the participation of hepatic lipase in order that the substrate, albumin-bound oleic acid, be captured by the liver.

The present results obtained in vivo agree with those of other workers[7,8] who studied fat cell lipase. This activity, coupled with that of muscle cell lipase[17], is responsible for the majority of total lipase activity. The former authors[7,8] concluded that adipose tissue lipoprotein lipase activity of the *fa/fa* rat was much greater than that of the same tissue of non-obese rats.

A hypothesis to explain these results was formulated by Schonfeld et al.[7], according to which the plasma apoproteins synthesized by obese rats in higher quantities than by controls could be lipase activating factors. This would result in an increased catabolic capacity of circulating lipoprotein lipases in obese rats.

Conclusion. Total circulating lipoprotein lipase activity determined indirectly in the *fa/fa* rat in vivo is higher than that of *Fa–* rats. A defective VLDL catabolism resulting from decreased lipoprotein lipase activity is thus excluded as being a cause of hyperlipoproteinemia in *fa/fa* rats.

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