obtained by adjusting the ratio R_A/R_B.) The mirror is removed and the specimen frame containing a sample of the skin being tested is inserted into the cell carrier. Since the intensity of the light reflected (the angle of incidence of the light to the sample is approximately 10° from the normal) from the specimen is much less than that reflected from the mirror, the moving reference cell must be moved away from its original position to receive light of the same intensity as the measuring cell. By measuring this distance and assuming that the lamp is a point source, the percentage reflection may be calculated.

To calibrate the apparatus, the mirror was reinserted into the cell holder and a neutral density filter of known transmission was inserted into the filter mount in front of the mirror. The data presented in table 2 were obtained. Reflection values below 1% were obtained by inserting a 25% neutral density filter in front of the moving reference cell. To calibrate the apparatus, other neutral density filters were then placed in front of the mirror and the data presented in table 3 were obtained.

Specimen frame. Specially built frames were used to hold the samples. They were made of aluminum and were blackened. The frame for the skin consisted of 2 plates with the overall measurements of 5 × 5 cm. The first plate had a ridged hole in its center (1 cm inner diameter) and 2 guide pins for positioning: one in the upper left corner, and the other in the lower right corner of the plate. The top plate had a large hole in its center (1 cm inner diameter) and 2 smaller holes to match the pins of the bottom plate. Once the skin was spread over the ridged hole of the bottom plate, and gently stretched, the top plate was pressed firmly in place and held by 2 spring clips. The specimen frames were made to fit snugly in the slotted frame holder so that the sample was in line with the light source and the photocell. Furthermore, positioning was such that the beam of light always impinged upon the same spot of the sample in all subsequent measurements.

Conclusion. The apparatus described in this paper has been used to investigate the variation of light transmitted through the skull and scalp of the rat, following drying[10]. Small variations in transmissions were measured over a wide range of values. Precision is limited, mainly, by the care exercised in aligning the apparatus. Transmission measurements may be made from 1% to 100% while reflection values ranging from approximately 0.1% to 100% are easily obtained.

Table 3. Calibration of the photometer measuring light less than 1% reflected

Neutral density filter in front of measuring cell (nominal % Reflect)	Theoretical distance of reference cell from source (cm)*	Actual distance of reference cell from source (cm)**
25	20.0	20.0
16	25.0	26.3
13	27.7	28.7
10	31.6	32.4
1	100.0	112.0
0.1	316.0	325.0

$$*d = \frac{20.0}{\sqrt{\frac{\%\ \text{Reflect}}{25}}}.$$

**Measuring cell maintained at 20.0 cm from the light source and 25% filter in front of the moving reference cell.

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On a rabbit hyperlipemia induced by a fungic galactomannane peptide

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Summary. I.v. injection into rabbits of a fungic galactomannane peptide isolated from the culture medium of *Aspergillus oryzae* induced the apparition, 20 h later, of an hypertriglyceridemia, with a concomittant decrease of about 70% of the post-heparin lipoprotein lipase activity. The same effect had been obtained earlier with a carbohydrate-rich fraction purified from a crude papain preparation. The 2 fractions are compared.

It was shown in previous experiments that the i.v. injection into rabbits of some glycoproteins[1-3], glycopeptides[4] or pituitary extracts of peptide nature[5-7] was followed by the apparition of hyperlipemia. In this study, similar results have been obtained by the injection of a fungic galactomannane peptide (FGMP) isolated from the culture medium of *Aspergillus oryzae* (IP 410). We have compared this new result with the hyperlipemia, induced in rabbits, by the injection of a glycopeptide A1 extracted from a crude papain preparation[4].

Materials and methods. The fungic galactomannane peptide (FGMP) was extracted from a culture medium of *Aspergillus oryzae*[8] (strain IP 410, type UF 3981) provided by the Rapidase Society, Seclin, France). The dialyzed culture medium was centrifugated at 6000 rpm for 20 min, and the supernatant filtered through a column of hydroxyapatite according to the method of Benardi and Kawasaki[9]. The FGMP fraction was eluted with a 0.005 M pH 6.8 sodium phosphate buffer[10]. 3 mg of this fraction were injected into the marginal vein of rabbit's ears (Fauves de Bourgogne).

The sugar determination of the FGMP and A1 fractions, the measurement and the ultracentrifugation of the serum lipoproteins, and the estimation of the post-heparin lipoprotein lipase activity (PHLA) of the rabbit post-heparin

plasma (PHP), have been previously described[4,10]. Apparent molecular weight of FGMP was determinated by filtration on Biogel P-150 (Biorad). Glycopeptide A1 was obtained by filtration of the crude papain dialyzate on a Sephadex G-25 column[4].

Immunization of rabbits: 1 mg of FGMP mixed with complete Freund adjuvant was injected intradermally on day 0, and i.m. on day 7. An i.p. booster injection of 1 mg was given on day 21 and the animals were sacrified on day 28. The anti-FGMP anti serum was used to check the presence of a commune antigenicity between the FGMP and A1 purified fractions by the double diffusion precipitation test (method of Ouchterlony).

Results. Sera analysis: Results are shown in table 1. About 20 h after the FGMP injection, the total lipids and the triglycerides levels are markedly increased and the serum is milky. After ultracentrifugation we found that the hyperlipemic serum is rich in very light particles of density about 1.006. They are probably lipomicrons since the phenomenon occurs even if the animals are fasting. Generally the hyperlipemia disappears after a few days, but if it persists, the animals die.

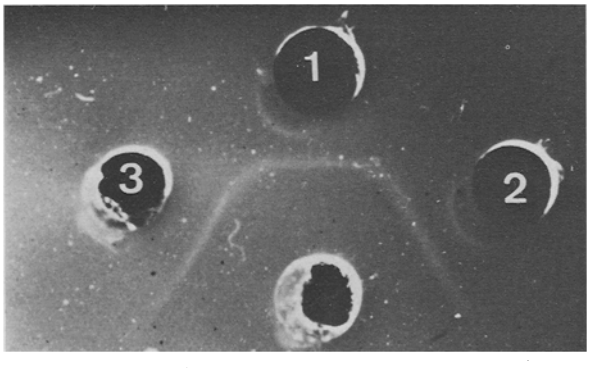
Table 1. Effect on the rabbit serum lipid level of the i.v. injection of the fungic galactomannane peptide (FGMP) isolated from the culture medium of *Aspergillus oryzae*

Time after injection	Serum Lactescene	Total lipids (g/l)	Triglycerides (g/l)
0 h	–	3.5	0.5
20 h	+ + +	22.9	13
24 h	+ + +	27.4	16.8
48 h	+	7	2.3
66 h	+	7.2	2.3
6 days	–	4	0.7

Table 2. Carbohydrate composition of the FGMP and A1 fractions

Carbohydrates*	FGMP	A1
Glc	13	38
Man	49	30
Gal	32	17
Glc NH_2	2	4
Gal NH_2	1	3
Pentoses	3	8
% of total weight	50–70	35

* Expressed as percentage of total sugars.



Ouchterlony double diffusion of the A1 fraction tested against the anti-FGMP anti-serum. Center well contains the A1 purified fraction. Wells number 1, 2 and 3 contain the anti-FGMP anti-serum.

PHLA measurement: The lipase activity of rabbit post-heparin plasma (PHP) is decreased (of about 70%) 20 h after the FGMP injection and returns to normal in the following days. PHLA expressed in µequivalents of free fatty acids released per min and per ml of PHP was found to be: 0.229 before the injection, 0.0066 20 h later, and 0.207 6 days after the injection.

Comparison between the fungic galactomannane peptide (FGMP) and the glycopeptide A1 isolated from the crude papain. The figure shows that in Ouchterlony double diffusion, the A1 purified fraction gives a precipitin line with the anti-FGMP anti-serum. No line was observed with a normal rabbit serum. This result suggests that the FGMP and the A1 purified fractions have a common antigenicity. Moreover, the 2 fractions are both rich in carbohydrates (35 and 50% respectively), though some differences can be noticed in their mol. wt (150,000 daltons for FGMP and 15,000 for A1) and their sugar composition (table 2).

Discussion. The data reported here show that hypertriglyceridemia was observed in rabbits 20 h after the injection of a fungic galactomannane peptide (FGMP) isolated from the culture medium of *Aspergillus oryzae*. A concomittant decrease of PHLA was found. The same phenomena have been observed after the injection of a glycopeptide A1 isolated from a crude papain preparation[4]. It suggests that the described hyperlipemias result from the inhibition of the clearing action of both hepatic triglyceride lipase and extra-hepatic lipoprotein lipase (released by heparin). Some peptides injections were found to have a similar action[11] in rabbits. Moreover, it was previously reported that some glycoproteins[12,13] and peptides of some lipoproteins such as apolipoproteins C1 and E[14], inhibit in vitro the lipoprotein lipase activity.

In this view, the role played by the structure of the FGMP and A1 fractions in the mechanism of the lipoprotein lipase inhibition has to be established. Preliminary studies have shown an important content of sugars, but differences in carbohydrates composition in the 2 fractions. Moreover, they possess some antigenic determinant which is not yet identified. Thus we cannot conclude what part of the fractions is involved in the mechanism of inhibition.

Experiments are now being undertaken to show whether the presence of sugars is necessary to induce the hyperlipemia, and whether it is related or not to the inhibiting effect on the lipoprotein lipase activity.

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