^{1}H-NMR spectra[a]) of **I**, **II**, **IV** and **V**

Proton position	I[b])	II[b])	IV	V
CH_3CO	1.90 (s)		1.86 (s)	
H-C(12)	5.31 (m)	4.04 (m)	5.29 (m)	4.10 (m)
H-C(14)	2.43 (bm)	2.42 (bm)	2.39 (bm)	2.46 (bm)
H-C(15)	5.45 (dd, 9.5, 3)	5.53 (dd, 9.5, 3)	5.45 (dd, 10, 3)	5.57 (dd, 10, 3)
H-C(16)	6.28[c])	6.26[d])	6.27[c])	6.28[d])
H-C(19)	5.94 (bs)	6.21[d])	5.93 (bs)	6.21[d])
H-C(20)	6.22[c])	6.32[d])	6.22[c])	6.32[d])
H-C(21)	1.25 (s)	1.01–0.85 (s)	1.25 (s)	1.03–0.87 (s)
H-C(22)–H-C(25)	1.02–0.85 (s)		1.02–0.85 (s)	
H-C(26)	3.57 (m)	3.72 (m)	3.65 (bt, 6)	3.70 (bt, 6)
H-C(29)	1.02–0.85	0.58 (t, 6)[e])		
H-C(30)		0.64 (d, 7)[e])		

[a]) Run at 90 MHz on a Perkin-Elmer R 32 apparatus in $CDCl_3$, using TMS as internal standard. Values are in ppm (δ-scale). Multiplicities are indicated by the usual symbols. Figures in parentheses are coupling constants in Hz. Assignments were confirmed by decoupling; [b]) Added for comparison (see [2]); [c]) H-C(16) and H-C(20) overlap; [d]) H-C(16), H-C(19) and H-C(20) overlap; [e]) In C_6D_6.

(figure 1). The presence of a C_4-saturated chain joining the 2 pentacyclic units A through the nitrogen atoms was deduced from the NMR-spectrum of **IV** which shows a 4 H broad triplet at δ 3.65 [H_2–C(26), H_2–C(26′)].

All these data indicate that molliorin-b is represented most favourably by formula **IV**. This was confirmed by its mass spectrum in which the following peaks are present: 872, M^+; 857, M^+–CH_3; 812, M^+ –CH_3COOH; 797, M^+–CH_3 COOH–CH_3; 752, M^+ –2 CH_3COOH; 737, M^+ –2 CH_3COOH–CH_3; 464, A; 437, B–H; 404, A–CH_3COOH; 377, B–CH_3COOH–H (figure 1).

Definite proof for the proposed structure **IV** was provided by comparison of its properties (IR, NMR, UV, m.p., $[\alpha]_D$) with those of a synthetic sample obtained by reaction of 1,4-diaminebutane with an excess of scalaradial (**III**) (at 60 °C for 5 min), followed by addition of conc. H_2SO_4 (at 60 °C for 5 min.) and chromatography on PLC (SiO_2, eluent benzene/40–70° light petroleum 17:3). It seems reasonable that scalaradial may be a precursor of both molliorin-a and -b; in addition, the central C_4N_2 unit of molliorin-b could derive from ornitine as previously hypothesized for aerothionin[7], a dimeric metabolite present in the sponge Verongia aerophoba.

7 K. Moody, R. H. Thomson, E. Fattorusso, L. Minale and G. Sodano, J. chem. Soc. Perkin *I*, 18 (1972).

Dissociation of obesity, hypercholesterolemia and diabetes from atherosclerosis in ob/ob mice

T. T. Yen, J. A. Allan, D. V. Pearson and M. R. Schinitsky[1]

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis (Indiana 46206, USA), 10 February 1977

Summary. Genetically obese, diabetic and hypercholesterolemic C57BL/6J-ob/ob mice were placed on Purina Laboratory Chow containing 2% cholesterol for up to 4 months. They developed higher plasma cholesterol levels and accumulated an increased quantity of cholesterol in the liver but failed to develop atherosclerotic lesions in the aorta as would be expected in an obese, diabetic and hypercholesterolemic human adult.

The C57BL/6J-ob/ob mice are known to have genetic hypercholesterolemia in addition to being obese[2] and are being used in many laboratories as a model for adult-onset diabetes. Feeding high levels of cholesterol to normal mice has not resulted in aortic atherosclerosis. It is not known, however, if atherosclerosis can be induced in ob/ob mice which have spontaneous hypercholesterolemia. This communication reports an attempt to induce atherosclerotic lesions in this possibly susceptible genotype of mice using high cholesterol diet.

Materials and methods. Male C57BL/6J-ob/ob mice and normal mice (+/+) from the Jackson Laboratory, Bar Harbor, Maine, were 4–6 months of age at the beginning of the experiment. 9 mice per genotype, evenly distributed according to age, were fed either Purina Laboratory Chow or Purina Laboratory Chow containing 2% cholesterol. The diet containing cholesterol was prepared by mixing cholesterol with Purina Chow powder and repelletizing the mixture. The regular diet was processed in the same manner to assure the same consistency as the cholesterol diet.

Mice were housed 3 to a cage in transparent, polycarbonate cages covered with filter bonnets (Filtek, Appleton, Wisconsin). They were fed one of the above 2 diets and water ad libitum. The ambient temperature in the animal room was maintained at about 25 °C. The photoperiod was controlled to provide light from 06.00 h–18.00 h and dark from 18.00 h–06.00 h.

3 mice of each group were sacrificed at the end of 2, 3 and 4 months of treatment, respectively. Blood was collected in heparinized tubes. Total cholesterol in plasma was assayed according to the method of Glick, Fell and Sjølin[3].

1 Acknowledgments. We wish to thank Mr. Willis M. Overton for his excellent technical assistance.

2 J. Mayer and A. K. Jones, Am. J. Physiol. *175*, 339 (1953).

3 D. Glick, B. F. Fell and K. Sjølin, Analyt. Chem. *36*, 1119 (1964).

Table 1. Body weight of C57BL/6J-ob/ob mice and normal mice (+/+) on Purina Laboratory Chow (PLC) and PLC containing 2% cholesterol

Months of treatment	ob/ob* Cholesterol	Regular	+/+* Cholesterol	Regular
0	60.9 ± 0.9 (9)	60.7 ± 2.5 (9)	34.8 ± 1.1 (9)	35.0 ± 0.5 (9)
2	63.8 ± 0.9 (9)	65.3 ± 2.2 (8)	34.3 ± 1.1 (9)	35.7 ± 0.7 (9)
3	64.2 ± 0.9 (6)	64.3 ± 2.9 (6)	34.5 ± 0.9 (6)	36.2 ± 0.5 (6)
4	62.5 ± 2.5 (3)**	52.0 ± 0.5 (2)	33.3 ± 0.4 (3)	34.8 ± 0.7 (3)

* Mean ± SE in g (N); ** Significantly different from corresponding ob/ob mice on regular diet at $p < 0.025$.

Table 2. Plasma and liver cholesterol concentrations of C57BL/6J-ob/ob and normal mice (+/+) on Purina Laboratory Chow (PLC) and PLC containing 2% cholesterol[a])

Diet	ob/ob Cholesterol	Regular	+/+ Cholesterol	Regular
Plasma cholesterol (mean ± SE in mg%)				
2 months	358 ± 11***	269 ± 11	126 ± 15	101 ± 10
3 months	428 ± 38**	295 ± 21	131 ± 5*	119 ± 3
4 months	385 ± 20	309	110 ± 5	116 ± 3
Liver cholesterol (mean ± SE in mg/g wet tissue)				
3 months	16.1 ± 1.4****	7.6 ± 0.6	4.3 ± 0.8	2.7 ± 0.3
4 months	34.6 ± 1.7	7.5	4.7 ± 0.5**	2.6 ± 0.1

[a]) All values were from 3 mice except that from the ob/ob mice on regular diet for 2 months, N = 2, and that from same for 4 months, N = 1, due to death and sickness in that group. Comparing with mice of same genotype on regular diet for same length of treatment. * $p < 0.05$; ** $p < 0.025$; *** $p < 0.01$; **** $p < 0.005$.

Livers of mice sacrificed at the end of 3 and 4 months of treatment were extracted by the method of Emerson and Van Bruggen[4]. The cholesterol content in the liver extracts was assayed according to the method of Zlatkis, Zak and Boyle[5]. Tissues were fixed with 10% neutral formalin and stained with hematoxylin and eosin for microscopic examinations. In addition to the hematoxylin and eosin staining, aorta was also stained with Gomori's trichrome, specific for connective tissue. Slides of all tissues were coded before examination and read blind. Student's t-test was used in all statistical analyses of the data.

Results and discussion. Mean body weights of all mice before the beginning of the experiment and at the end of 2, 3 and 4 months of treatment are shown in table 1. 2 deaths occurred in the ob/ob group on regular diet. One of the 2 remaining mice in that group lost a significant amount of weight during the last month of the experiment which accounted for the significant difference between the 2 groups of ob/ob mice. Otherwise, both ob/ob mice and normal mice on cholesterol diet maintained similar weights as corresponding mice on regular diet.

The ob/ob mice on cholesterol diet had a significant average increase of 34% in plasma cholesterol (table 2). This is in contrast to the normal mice on cholesterol diet which had an average increase of only 8% and statistically, only the difference after 3 months of treatment was significant (table 2).

In the liver, the cholesterol content of ob/ob mice on cholesterol diet for 3 months doubled and that of the mice on the same diet for 4 months quadrupled (table 2). The liver cholesterol concentration of normal mice on cholesterol diet increased only 60–80%. This difference in the increase confirms the observation of Jansen, Zanetti and Hutchison[6] that ob/ob mice are deficient in their ability to remove cholesterol from the liver.

Histologically, none of the normal mice on regular diet had any observable fatty deposition in the liver. However, 1 normal mouse fed cholesterol diet for 3 months showed a moderate fatty deposition in the liver. After 4 months on cholesterol diet, all 3 normal mice showed small fat nodules profusely spread throughout the liver.

The ob/ob mice normally had fatty livers. Varying the cholesterol content of the diet for 3 months did not result in an observable increase in fat histologically. However, ob/ob mice on cholesterol diet for 4 months did appear to have an increase in the liver lipid deposition. In spite of the significant increase in plasma as well as liver cholesterol concentrations in the ob/ob mice on cholesterol diet, no pathological evidence of atherosclerosis was observed in the aorta of the mice.

This study does not point towards a reason for the lack of correlation between increased plasma and liver cholesterol concentrations and lack of histological evidence for atherosclerosis in genetically obese, diabetic and hypercholesterolemic mice. The lack of correlation and the apparent dissociation of diabetes, obesity and hypercholesterolemia from complications of atherosclerosis in this animal model is perhaps itself significant, indicating the possible importance of factors other than diabetes, obesity and hypercholesterolemia in the etiology of atherosclerosis.

4 R. J. Emerson and J. T. Van Bruggen, Archs Biochem. Biophys. *77*, 467 (1958).

5 A. Zlatkis, B. Zak and A. J. Boyle, J. Lab. clin. Med. *41*, 486 (1953).

6 G. R. Jansen, M. E. Zanetti and C. F. Hutchison, Biochem. J. *102*, 870 (1967).