## Genesis of Foam Cells: Study in Rats after Administration of Cholesterol-Cholic Acid

Accumulation of foam cells in the lungs is a spontaneous disorder in the rat[1–4]. It has been shown that a diet with a high fat content gives rise to an accumulation of pulmonary foam cells in rats[5,6], rabbits[7], and gerbils[8]. On giving triglycerides (Intralipid®) to rats, an increase in the number of blood monocytes and a pulmonary accumulation of foam cells was found, indicating a relationship between these two parameters[9]. The purpose of the present study was to find out whether there exists a similar relationship in rats given a cholesterol-cholic acid containing diet.

*Materials and methods.* A total of 40 male and female SPF rats of about 3 months of age of the Sprague-Dawley strain were divided into 2 groups, each consisting of 10 males and 10 females. The rats in the experimental group were given a special diet, containing 1% cholesterol and 0.3% cholic acid for 6 months. The animals were weighed once a week and the amount of food consumed was recorded. Blood samples were taken after 2, 4 and 6 months. On sacrificing, the lungs were weighed and examined grossly and microscopically.

Serum and lung tissue were examined for triglycerides, free fatty acids, cholesterol and phospholipids. Total cholesterol and triglyceride values were simultaneously determined with an Auto-Analyzer according to the methods of Block et al.[10] and Kessler and Lederer[11]. Free fatty acids were estimated by a semi-automated method as described by Baird et al.[12]. Phospholipids

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Table I. Differential counting of leukocytes in rats given a cholesterol-cholic acid containing diet

| Length of experiment (months) | | Monocytes[a] | | Lymphocytes | | Granulocytes: Neutrophilic: Band (%) | | Seg (%) | | Eosinophilic (%) | | Basophilic (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | Treated | Control | Treated | Control | Treated | Control | Treated | Control | Treated | Control | Treated |
| 2 | ♂ | 0.2 | 1.7 | 89.9 | 90.3 | 0.0 | 0.0 | 9.3 | 6.7 | 0.6 | 1.3 | 0.0 | 0.0 |
| | ♀ | 0.8 | 1.3 | 92.4 | 87.3 | 0.0 | 0.0 | 6.2 | 10.7 | 1.2 | 0.7 | 0.0 | 0.0 |
| 4 | ♂ | 0.3 | 1.6 | 64.3 | 85.7 | 0.0 | 0.0 | 25.6 | 11.2 | 1.3 | 1.4 | 0.0 | 0.0 |
| | ♀ | 0.3 | 1.1 | 78.1 | 86.2 | 0.0 | 0.0 | 18.4 | 11.4 | 3.1 | 1.2 | 0.0 | 0.0 |
| 6 | ♂ | 0.3 | 2.3 | 78.9 | 80.9 | 0.0 | 0.0 | 18.6 | 15.7 | 2.1 | 1.1 | 0.0 | 0.0 |
| | ♀ | 0.4 | 2.1 | 80.2 | 88.1 | 0.0 | 0.0 | 17.8 | 9.3 | 1.6 | 0.4 | 0.0 | 0.0 |

Each value is the mean of 10 single values. [a] Monocyte increase statistically significant ($P < 0.001$) and caused by treatment (analysis of variance).

Table II. Lipid concentration ± SEM in the blood serum from rats given a cholesterol-cholic acid containing diet

| Length of experiment (months) | Sex | Cholesterol (mg/100 ml) | | Triglycerides (μmoles/ml) | | Free fatty acids (μEq/ml | | Phospholipids (mg/100 ml) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Treated | Control | Treated | Control | Treated | Control | Treated |
| 0 | ♂ | 88 ± 3 | 84 ± 2 | 0.78 ± 0.03 | 0.81 ± 0.06 | 0.13 ± 0.01 | 0.08 ± 0.01 | 123 ± 14 | 122 ± 4 |
| | ♀ | 95 ± 3 | 82 ± 4 | 0.65 ± 0.05 | 0.68 ± 0.06 | 0.16 ± 0.01 | 0.06 ± 0.01 | 144 ± 6 | 135 ± 5 |
| 2 | ♂ | 113 ± 2 | 116 ± 2 | 1.03 ± 0.02 | 0.86 ± 0.05 | 0.36 ± 0.02 | 0.22 ± 0.01 | 153 ± 12 | 124 ± 3 |
| | ♀ | 92 ± 5 | 80 ± 7 | 0.51 ± 0.02 | 0.53 ± 0.05 | 0.30 ± 0.02 | 0.16 ± 0.02 | 139 ± 10 | 122 ± 8 |
| 4 | ♂ | 118 ± 11 | 160 ± 5 | 1.02 ± 0.06 | 1.03 ± 0.04 | 0.51 ± 0.02 | 0 48 ± 0.02 | 133 ± 29 | 134 ± 5 |
| | ♀ | 87 ± 10 | 90 ± 5 | 0.57 ± 0.07 | 0.70 ± 0.10 | 0.55 ± 0.04 | 0.48 ± 0.01 | 123 ± 9 | 132 ± 7 |
| 6 | ♂ | 150 ± 2 | 251 ± 13 | 1.00 ± 0.07 | 1.54 ± 0.10 | 0.44 ± 0.01 | 0.38 ± 0.05 | 129 ± 4 | 164 ± 20 |
| | ♀ | 116 ± 6 | 105 ± 6 | 0.68 ± 0.06 | 0.49 ± 0.02 | 0.48 ± 0.02 | 0.32 ± 0.02 | 151 ± 10 | 154 ± 9 |

Each value is the mean of 10 single values.

Table III. Lipid concentration ± SEM in the lungs from rats given a cholesterol-cholic acid containing diet

| Sex | Cholesterol (mg/g) | | Triglycerides (μmoles/g) | | Free fatty acids (μEq/g) | | Phospholipids (mg/g) | |
|---|---|---|---|---|---|---|---|---|
| | Control | Treated | Control | Treated | Control | Treated | Control | Treated |
| ♂ | 2.6 ± 0.2 | 3.1 ± 0.1 | 6.8 ± 0.1 | 5.2 ± 0.5 | 8.8 ± 0.7 | 10.5 ± 0.5 | 8.6 ± 0.4 | 9.4 ± 0.2 |
| ♀ | 2.7 ± 0.1 | 2.8 ± 0.1 | 9.1 ± 0.8 | 4.8 ± 0.5 | 7.4 ± 0.9 | 11.3 ± 0.5 | 10.1 ± 0.2 | 9.7 ± 0.3 |

Each value is the mean of 7 single values.

were determined using a method developed by KRAML[13] for estimation of inorganic phosphate.

The blood samples were examined for the number of white blood cells, and differential counting of leucocytes was performed. The calculated total number of blood cells of each cell type was compared in control and treated animals by means of an analysis of variance, in which the three factors treatment, time and animal were considered.

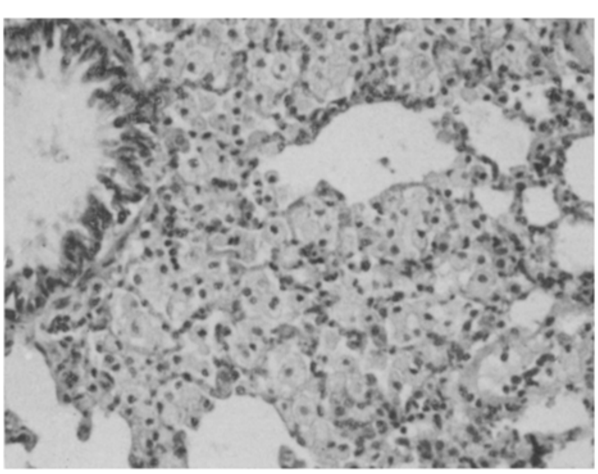
Tissue samples of the lungs were fixed in 10% neutral formalin solution. Paraffin embedded sections were prepared and stained with haematoxylin-eosin. Frozen sections were stained with haemalum and Sudan III for fat.

Free lung cells were determined in 3 males and 3 females from each group. A blunt syringe needle was introduced into the trachea, and 7 ml of sterile saline were slowly injected into the lungs and withdrawn. This washing process was repeated 10 times. A sample of the fluid was mixed with Türk's stain to count free lung cells, mainly foam cells.

Table IV. Free cells/mm$^3$ in lung washings from rats given a cholesterol-cholic acid containing diet

| Sex | Number of cells | |
|---|---|---|
| | Control | Treated |
| ♂ | 400 | 700 |
| ♀ | 300 | 600 |

Each value is the mean of 3 single values.



Foam cells in lung of rat receiving a cholesterol-cholic acid containing diet. Haematoxylin and eosin. ×250.

*Results.* The body weight gain and food consumption was the same for control and treated rats during the whole experimental period. The haematological examination revealed no important differences between the 2 groups as to the total number of leukocytes. Concerning the monocytes the differential counting of the leukocytes showed differences between control and treated animals (Table I). The number of monocytes was higher in treated rats than in controls. The monocyte increase was statistically significant in the treated rats according to an analysis of variance ($P < 0.001$).

Examination of the serum lipids showed that in males there was an increase in cholesterol after 4 and 6 months and in triglycerides after 6 months (Table II). Otherwise, nothing of note was observed. As to the lipid content of the lungs, there was no essential difference between control and treated rats (Table III).

There was an increase in the number of free cells, mainly as foam cells, in the lung washings (Table IV). The number of free cells was about twice as many in treated as in control rats. The mean weight of the lungs was comparable in control and treated rats. Grossly the lungs of treated rats showed occasional very small greyish foci. No such observation was made in the lungs of control rats. Microscopically a slight focal accumulation of foam cells was found in all the treated rats and in 2 male and 1 female control rats (Figure). On staining with Sudan III, fine fat droplets were found in the cytoplasm of the foam cells.

*Discussion.* A focal accumulation of foam cells in the lungs was observed in all rats receiving the cholesterol-cholic acid containing diet and in 3 control animals. Accumulation of foam cells is a spontaneous disorder in the rat[2,3]. The incidence in the controls in this study is lower than has been found for a corresponding age group[4]. The investigation thus indicates that the accumulation of pulmonary foam cells is much more frequent in treated than in control rats. This indication is also supported by the results from the lung washing showing a greater number of foam cells in treated than in control animals. Thus, the results of the study show that a cholesterol-cholic acid containing diet can give rise to an accumulation of pulmonary foam cells in the rat. This finding is in agreement with observations in other similar investigations of lipids[5,6,14].

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Moreover, the results show a statistically significant increase in the number of monocytes in rats receiving the cholesterol-cholic acid containing diet. An elevated number of monocytes is also observed, if an accumulation of pulmonary foam cells is induced by cloforex, an anorogenic drug[14] or Intralipid®, a triglyceride preparation[9]. Thus, experimental studies give evidence that there is a monocytosis in connection with an accumulation of pulmonary foam cells. This might also be true of related conditions in which foam cells are involved.

*Zusammenfassung.* Cholesterol-cholsäurehaltiges Futter verursachte bei der Ratte einen statistisch sichergestellten Anstieg der Monozyten im Blut und eine fokale Anhäufung von Schaumzellen in der Lunge.

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## The Branching Pattern in Apical Dendrites of Cortical Neurons

The morphometry of dendrites is important for functional modelling[1] and for the question of functional adaptation of neurons to environmental conditions[2,3]. Therefore, our analysis for segment lengths and bifurcation probabilities per order of the basal dendrites[3] is supplemented here with the similar analysis of the apical dendrites. The analysis was carried out with the same 30 pyramidal neurons from laminae II, III and IV of the striate area of the adult rabbit[3]. This provides the basic material for the above-mentioned questions.

*Methods.* All data were calculated from the 3 cartesian coordinates of all the initial points, the bifurcation points and the end points, measured in 80 µm thick Golgi sections. A centrifugal ordering[3] of segments was applied, since some branches are inevitably cut. The difficulty that the apical dendrites do not have a symmetrical branching structure[3], was met by distinguishing 2 parts in the apical dendrites (ref.[4]): 1. the main branch, ordered starting at the proximal segment; 2. the oblique branches, ordered separately starting at the segment arizing from the main branch. Along with order, also type was determined: i.e., end segment or intermediate segment. Cut segments were not considered for segment length calculations. The ith order branching probability, $p(i)$, is defined, irrespective of the length of the segment, as the quotient of the number of intermediate segments ($N_I$) and the total number of segments ($N_I + N_E$) of that order.

*Results and discussion.* The lengths of the segments of the basal dendrites[3] and the lengths of the segments of the apical main branches and of the apical oblique branches all show frequency distributions that are skewed to the right for all orders (e.g. Figure 1).

It is remarkable that the lengths of the intermediate segments of the apical main branches show the maximum in the same class (10–20 µm) for the orders 1, 2 and 3, as do the intermediate segments of the basal dendrites[3]. The intermediate segments of the apical oblique branches

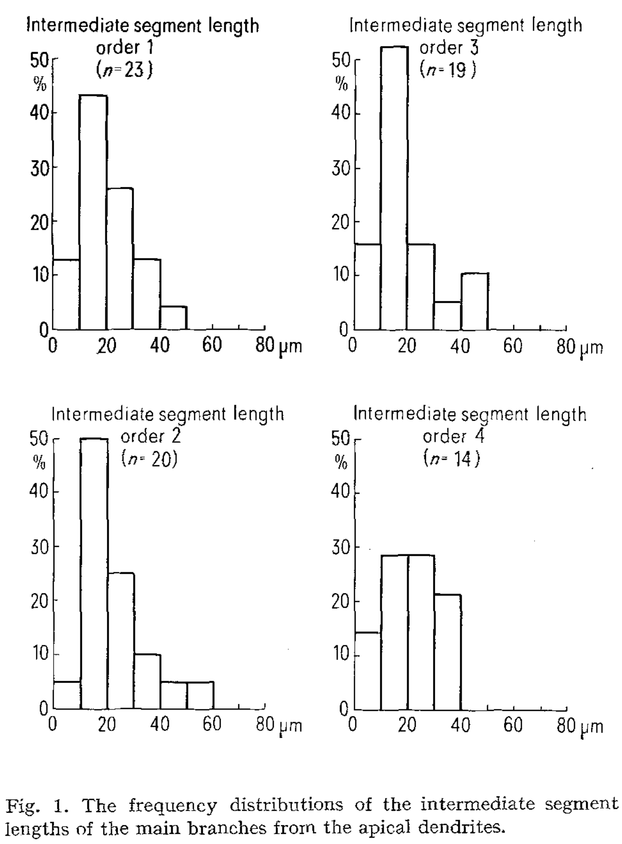


Fig. 1. The frequency distributions of the intermediate segment lengths of the main branches from the apical dendrites.

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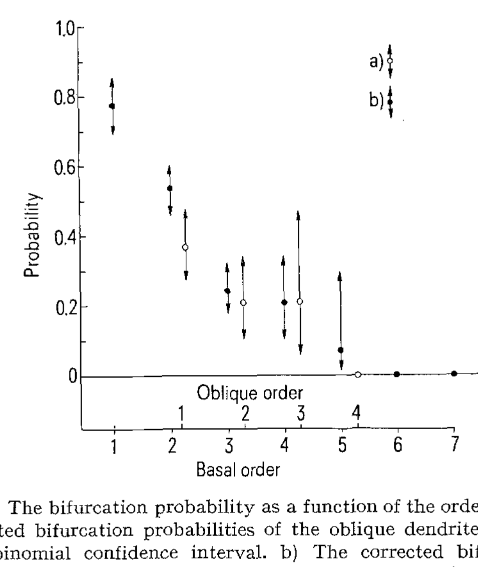


Fig. 2. The bifurcation probability as a function of the order. a) The corrected bifurcation probabilities of the oblique dendrites with a 0.95 binomial confidence interval. b) The corrected bifurcation probabilities of the basal dendrites with a 0.95 binomial confidence interval.