note également quelques neurones dans le corps mamillaire (Mm) et au-dessus de ce noyau juste au niveau de la commissure supramamillaire ainsi que dans le pédoncule cérébral, particulièrement dans sa région médioventrale (Figure B).

b) Projection du mésencéphale antéroventral vers le CxVis. Après injection de HRP dans le CxVis, nous avons observé un autre groupe de cellules marquées dans le mésencéphale antéroventral homolatéral (Figure B). Dans les plans antérieurs, les neurones sont observés dans une région située entre le nerf du noyau oculomoteur (N III), le noyau interpédonculaire (IP) et le noyau rouge (NR), qui correspond en partie au noyau du tractus optique basal et au noyau tegmentalis ventral de Tsai. Dans les plans postérieurs, les neurones s'étendent dorsalement jusqu'au niveau du noyau linéaire rostral du raphé; la région correspondrait au tractus optico-oculomoteur décrit par GILLILAN[3].

Les régions dans lesquelles nous avons trouvé des neurones marqués après injection de HRP dans le CxVis et le CGLd font partie du système visuel accessoire[3,5]. Il serait donc intéressant de savoir si elles reçoivent des projections directes de la rétine. L'existence d'une projection de la rétine sur les aires hypothalamiques a en effet été démontrée par des études neurophysiologiques[6,7], mais les études anatomiques réalisées jusqu'à présent n'ont pas permit de conclure si cette projection était directe ou non[5,8-10].

D'autre part, KIEVIT et KUYPERS[11] ont récemment démontré chez le singe, par la technique de HRP, une projection directe des aires hypothalamiques et des aires basales du télencéphale ('basal forebrain areas') vers les cortex frontal et pariétal. La topographie des cellules marquées telle qu'elle a été décrite dans leur travail est généralement différente de celle que nous avons trouvée. Il conviendrait donc d'examiner chez le chat les projections possibles des aires hypothalamiques vers d'autres aires corticales que vers l'aire visuelle primaire pour déterminer l'importance et la spécificité de la projection hypothalamo-CxVis que nous avons mise en évidence.

Summary. Using a retrograde tracer technique with horseradish peroxidase, we have revealed some afferent connections from the hypothalamic areas to the lateral geniculate nucleus and visual cortex.

K. SAKAI, L. LEGER, D. SALVERT, M. TOURET et M. JOUVET[13]

Département de Médecine Expérimentale, Université Claude-Bernard, 8, avenue Rockefeller, F-69373 Lyon-Cedex 2 (France), 23 juillet 1975.

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Quantitative Morphological Investigations on Smooth Muscle Cells in Vascular Surgical Specimens and Their Clinical Importance

Besides the typical smooth muscle cell (SMC) of the vessel wall with its well known functions[1-5], there exists a morphologically different type[6,7] mainly described in animal experiments. This fibrocyte-like cell[8] was defined by us as modified or activated smooth muscle cell (ASMC) in investigations[9] analogous to the animal experiment[10,11]. As studies about the function and importance of this cell did not exist in man, we tried to get more detailed information using morphometrical parameter (distribution in %).

Material and methods. 82 freshly fixed surgical specimens of human arteries (splenic, lower extremities) were treated as previously described[9]. Selected histological and semithin sections (PAS, d-PAS, toluidineblue- and Goldner's trichromestaining) were used for quantitative evaluation. ASMC were separately counted. For differentiation of both cell-types, the previously described criteria[9] such as chromophilia, cell shape and PAS-positive membrane were used. For localization, the atherosclerotic plaque (AP) was divided into 7 defined areas, and the media, lying under that plaque, into 18 areas (Figures 1 and 2).

Results. ASMC occurred in different numbers in the inner media, in the distinctly thickened intima and in the AP (Figure 3). The quantitative distribution showed that the greatest part of ASMC was found in the inner media (Figures 1 and 2). When the group of patients was

	15					
	5 24	13	15	25 3		Σ
9	16	8	6	20	10	69
2	6	2	-	6	6	22
1	3	-	-	2	3	9

Fig. 1. Classification of the intimal plaque into 7 areas, as well as of the bordering media lying underneath, in 18 areas; distribution in percent of activated smooth muscle cells (figures in percent of the total number), age group under 40 a, Σ total percentage of media cells.

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divided into 2 age groups, one over and one under 40, the decrease of the frequency of ASMC in percent to the outer layers of the media was particularly marked in the lower age group. The frequency of ASMC was distinctly high in the margin of the AP itself and in the media lying under the margin. We observed an excessive proliferation of ASMC within the areas of the plaque-margin of the lower age group ($^1/_4$ of the total ASMC). When the frequency of ASMC in percent was related to the total number of SMC, 85% were found in the plaque and more than 95% in the inner media of the younger age group. In contrast to this behaviour, only about 15% of ASMC occurred in the plaque and less than 5% in the inner media of the older age group.

Discussion. The ASMC has typical criteria in human samples[9]: a slender cell shape ($^1/_3$ of the typical SMC), long cell-elongations, which can be distinctly differentiated in semithin sections, and an increased chromophilia (CAB-, toluidine blue-, and trichrome staining). It shows a particularly intensive reaction in PAS-staining of normal sections and a PAS-positive membrane[12] in semithin sections. The intracellular PAS-positive granula can be eluted by diastase. In electron microscopy[9], a decreased number of myofilaments and specific cell organelles, abundant perinuclear glycogen and micropinocytotic vesicles along the cell membrane can be found. RES was increased, floccular granular material could be found in the cells and outside near the cell membrane. An increased splitting activity was demonstrated in the subendothelial layer and in the media neighbouring the internal elastic membrane of experimental animals[10, 13–16]. BENDITT[17] presumed analogous conditions to exist in the 'physiologic intimal thickening' of human beings[18]. In 1974 WEBSTER et al.[11] described an undifferentiated cell type, which occurred in early stages of experimental arterial lesions, and showed the morphological criteria of ASMC. He observed these cells frequently in the margin of the AP and in the media near to the AP.

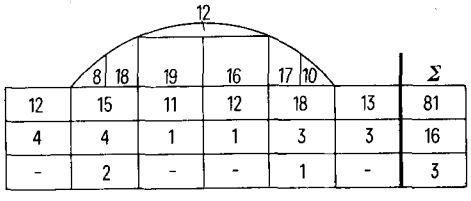

Fig. 2. Age group over 40a; explanation like Figure 1.

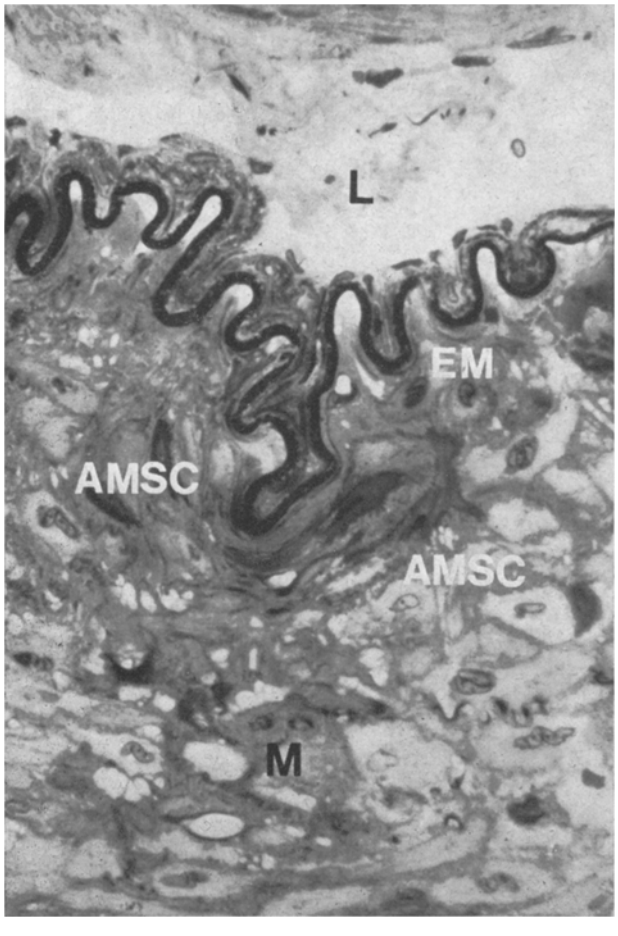

Fig. 3. Activated smooth muscle cells (ASMC) in the inner third of the media of a splenic artery. *M* media; *L* lumen; *EM* elastic membrane. Semithin section, toluidine-blue-staining, 10×40, 30-years-old female.

Our results showed a similar behaviour in human beings. A small quantity of 'activated cells' occurred, however, in the inner media of the youngest examined individuals too. Contrary to this, ASMC could be demonstrated only after experimental damage in young animals. In human beings we found a distribution similar to that in animal experiments[11], but a distinct activation of ASMC within the AP of the younger age group (Figure 1).

As to the explanation of this behaviour, it is rather probable that the proliferation is stimulated by a mild hypoxic metabolic damage[5]. A disturbed passage of plasmatic constituents, which is caused by the increasing thickening of the intima, is responsible for the change of the aerobic to the anaerobic metabolism, which is the mechanism of survival[4]. Morphologically an increased occurrence can be demonstrated of ASMC which show fewer specific characteristics but an increase of unspecific ones[11]. A distinct degree of damage is necessary for an excessive proliferation, which can be optimally demonstrated in the marginal regions of AP of the younger age group. The degree of damage overwhelms the potency of splitting when the intimal thickening is further enhanced in the developing AP. This results in a standstill of the process. The decrease of ASMC in the older age group corresponds to the decrease of the cellular activity of the whole organism[18].

The frequency of the occurrence of ASMC provides a good parameter for the tendency of proliferation of an AP. This seems to be of essential clinical importance. A statement about the prognosis can be made by the examination of arterial specimens (endarteriectomia, bypasses etc.). Vessels with an increased frequency of ASMC in AP show a distinctly higher tendency of proliferation and occlusion and have a rather poor prognosis. The cellular proliferation, however, is only one factor in the rather complex process of atherosclerosis.

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Summary. A distinct distribution of activated SMC could be demonstrated in atherosclerotic plaques and the neighbouring media of human beings. An increased proliferation was found in the younger age group and generally in the marginal regions of the plaques. The occurrence of activated SMC is thought to be a sequel of metabolic hypoxic damages. A high frequency of activated SMC is a bad prognostic sign in surgical specimens indicating a tendency for proliferation and occlusion.

W. FEIGL, H. SINZINGER, O. WAGNER and CH. LEITHNER[19]

Department of Pathology, 1st Department of Anatomy, 1st Department of Surgery and 2nd Department of Internal Medicine, Faculty of Medicine, University of Vienna, Währingerstrasse 13, A-1090 Wien (Austria), 26 June 1975.

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Further Evidence of a Positive Correlation Between Exposure to Nitrate Fertilizers ($NaNO_3$ and KNO_3) and Gastric Cancer Death Rates: Nitrites and Nitrosamines

Chile and Japan have the highest age-adjusted mortality rates per 100,000 population for stomach cancer in the world[1]. A survey of 15,300 necropsies performed at Santiago[2] has confirmed the high death rates. The relative frequency of stomach carcinoma amongst males with cancer ranked first with 20.60% and amongst females ranked second with 10.05% (ZALDÍVAR, unpublished observations). The morbid anatomy of gastric cancer in Chileans has been previously described[3-6]. The regional variation of oesophageal and gastric cancer in Chile was reported[7,8]. The predominantly agricultural provinces, which have the highest consumption of nitrate fertilizers, showed the highest death rates for stomach cancer. To explain this fact, a hypothesis of a nitrosamine synthesis in vivo in the stomach was advanced[8]. Recently, an association between exposure to sodium nitrate and gastric cancer mortality for 1960 was reported[9,10].

To study further this environmental problem, more stable death rates for stomach cancer in the 25 Chilean provinces were used (mean age-adjusted death rates per 100,000 pop. for 1960, 1962 and 1964), relating this variable with sodium nitrate exposure as well as with exposure to nitrates ($NaNO_3$ and KNO_3).

Material and methods. The figures on the general population and farm workers by province were taken from the 13th National Population Census. Numerical data on metric tons of nitrates used by province for 1960–61 were provided by the Chemical & Mining Society of Chile. Gastric cancer death rates per 100,000 pop., standardized for age for 1960, 1962 and 1964 by province, were taken from a previous communication[8]. The exposure to nitrate fertilizer in each province was expressed as metric tons of $NaNO_3$ or nitrates per person (general population) or per farm worker (agricultural pop.). The index of general population exposure was obtained by dividing the metric tons of fertilizer for each province by the number of persons in the province. The index of farm worker exposure was obtained in a similar way; the metric tons of fertilizer used in each province were divided by the number of farm workers in the province.

Results and discussion. The regression of death rates on the general population exposure to sodium nitrate showed a highly significant association ($p < 0.00005$). The equation of the regression line is $y = 37.01 + 910.2\,x$, where y is mean age-adjusted death rate for stomach cancer and x is metric tons of sodium nitrate per person. The correlation coefficient was 0.745. The regression of death rate on the farm worker exposure to sodium nitrate exhibited a significant association ($p < 0.0001$). The regression equation is $y = 35.87 + 154.0\,x$, where y is mean death rate and x is metric tons of sodium nitrate per farm worker. The r-value was 0.714. When the death rate was regressed on the general population exposure to nitrates, a significant association was found ($p < 0.0001$). The equation is $y = 36.77 + 454.0\,x$, where y is mean death rate and x is metric tons of nitrates per person. The correlation coefficient was 0.718. The regression of mortality rates on the farm worker exposure to nitrates showed again a significant association ($p < 0.0003$). The equation is $y = 35.53 + 74.88\,x$, where y is mean death rate and x is metric tons of nitrates per farm worker. The r-value was 0.668. The behaviour of the 4 independent variables in the regressions was quite similar, since the r-values were close to each other.

As previously postulated[8-10], the most probable explanation for this relationship is the nitrosamine formation in vivo. According to this hypothesis, nitrates undergo reduction in plants and are transformed into nitrites by nitroreductases present in many parasitic and saprophytic bacteria. Chilean farm workers would be exposed to dietary nitrates and nitrites, primarily from water supplies. The general population of Chile is exposed to nitrates and nitrites from food additives (meat products) and vegetables. SANDER[11] estimated a mean daily intake of nitrite in man of 22 µmoles, equivalent to 1.5 mg of $NaNO_2$. Secondary amines (R′–NH–R″), such as piperidine formed by heating from cadaverine present in partially decayed meat and fish, and pyrrolidine formed by heating from putrescine present in such foodstuffs, may be formed during pyrolysis of protein and therefore during cooking of protein food. Thus, ingested nitrite and certain secondary amines (piperidine, pyrrolidine) may lead to the in vivo formation of carcinogenic nitrosamines. Many amines, including pyrrolidine, have been detected in wine[12,13], a dietary item consumed in considerable

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