(200–250 g) by continuous intragastric perfusion (0.5 ml/min via oesophagus) of a simulated gastric juice (0.1 M HCl plus 600 mg pepsin/l) for 3 h. Intragastric pressure was applied by placing the open end of the outlet of the duodenal catheter 120 mm above the stomach level. Two groups of 10 animals each were marked out. One was treated with saline solution. At the end of the experiment, the rats were killed, the abdomen opened and the stomach removed and cut open along the greater curvature. The length (mm) of the erosions in each stomach was measured with the aid of a dissecting microscope (magnification × 10). When punctuate lesions were found, an arbitrary value of 1 mm was assigned to every 3 such lesions and the lesion index was obtained as the total length of the lesions in each stomach. Student's t-test was used to estimate the differences in the mean values of the lesion index between the groups. The research team measuring the lesions was unaware of the treatment being administered.

Results and discussion. In the stress model (table), somatostatin clearly reduced the lesion index (inhibition of 75%, $p < 0.01$). In animals previously treated with indomethacin the somatostatin failed to inhibit ($p > 0.05$) the lesions with regard to the control group. These results back up the observations made by Ligumsky et al.[1] and support the hypothesis that arachidonic acid metabolites from the cyclooxygenase pathway may be mediators of the somatostatin effect on the gastric lesions.

Effect of somatostatin on gastric lesion induced by stress and acid + gastric distension. Values are the mean ± SE of the lesion index (n = 10 for each group)

Gastric lesion model	Control	Somatostatin
Stress	8.81 ± 3.30	2.13 ± 0.85*
Stress + indomethacin	17.66 ± 3.29	12.15 ± 3.85
Gastric distension	10.14 ± 3.12	9.25 ± 4.58

* Significant difference from control group, $p < 0.01$.

In contrast, we observed (table) that somatostatin failed to reduce the gastric lesions brought about by the intragastric distension model in which exogenous prostaglandin E_2 produces a clear cytoprotective effect[2]. This failure backs up the explanation that in the stress model, somatostatin-induced protection was due to inhibition of acid secretion which is mediated by endogenous prostaglandins[1]. But our results do not exclude the possibility of the somatostatin failure being due to an inhibition of the arachidonic acid metabolism mediated by the intragastric acid medium in the rat. This has been observed by Konturek et al.[3] in the cat. In this sense also an identical behavior of somatostatin release induced by intragastric acid medium has been observed in both species[4,5].

We suggest that in the rat, the somatostatin preventive effect on the mucosal gastric lesions may be, at least in the stress model, mediated by endogenous prostaglandins. But further studies concerning the effect of intragastric acid medium on arachidonic acid metabolism at the rat gastric level are still needed to clarify the prostaglandin mediator mechanism.

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Characterization of the third component of pig complement and its utilization in a C3b receptor study

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Summary. The third component of the pig complement system (C3) was isolated in hemolytically active form and characterized. The C3 component is a β-globulin with the molecular weight of 191,000 and is composed of 2 non-identical polypeptide chains of M_r 112,000 and 74,000. The isolated C3 can be used for the detection of the C3b receptor on the membranes of heterologous peritoneal macrophages.

Key words. Complement, pig; complement, system; C3b receptor; β-globulin; macrophages.

It has been suggested that the complement system, especially the third component of complement (C3), is involved in the regulation of such cellular functions as lymphocyte proliferation[1], antibody production[2] and phagocytosis[3]. The C3 probably plays the central role in the activation and functions of the whole complement cascade. By the activation of C3 fragments C3a (anaphylatoxin) and C3b were produced; the latter is capable of binding on the surface of the cell, bacteria, and immune complexes. The C3b mediates immune adherence and thus permits phagocytosis[3,4]. C3 plays a central role in both the classical and alternative pathways[5,6].

The isolation of C3 from the sera of humans[7], cats[8], rats[9], rabbits[10] and pigs[11] has already been described. In this paper the conditions for the isolation and characterization of pig C3 were examined. The isolated pig C3 was used for the detection of the receptor for C3, employing the immunofluorescence technique. The C3 receptors on the macrophage surface are very important structures because of their ability to increase the adsorption of bacteria and other opsonized particles[12].

Materials and methods. The isolation of pig C3 was carried out by the modified method already described[7]. Pig C3 was purified from pooled fresh pig serum by the following sequential steps: PEG 6000 (Lachema, Brno) precipitation 5–12%, DEAE-cellulose chromatography (Reanal, Budapest) with subsequent desalting by Sephadex G-25 (Pharmacia, Uppsala) chromatography, PEG 6000 precipitation to 16%, gel filtration on Sepharose 6B (Pharmacia, Uppsala) and chromatography on hydroxylapatite (prepared by the method of Tiselius et al.[13]). Further ion-exchange chromatography on QAE-Sephadex A-50 equilibrated with 25 mM Tris-HCl buffer (pH 7.8) containing 2 mM EDTA and 100 mM NaCl was used. The column was developed with a linear NaCl gradient to a

limit concentration of 300 mM. This chromatography was used as the final step in C3 purification for the separation of the hemolytically inactive and active forms of pig C3[14].

SDS-polyacrylamide gel electrophoresis in 8.5% gel in a discontinuous buffer system[15] was used in the C3 preparation purity study.

An analytical ultracentrifugation was performed in a Beckman model E ultracentrifuge by the method described earlier[16].

Specific rabbit anti-pig C3 antiserum and then the IgG fraction were prepared. Following the method of Ouchterlony[17], immunodiffusion experiments and also immunoelectrophoresis were performed in 0.9% agarose (Indubiose A37, Clichy) and 0.1 M veronal-citrate buffer, pH 8.6. Crossed immunoelectrophoresis in 0.9% agarose and the buffer system according to Laurell[18] was used in the C3 preparation homogeneity study. The relative hemolytic activity of pig C3 was tested by the simple alternative pathway assay using methylamine-treated plasma as described by Jessen et al.[19].

The pig C3 did not cross-react with antibody to human or mouse C3, but in biological systems – also in the C receptor study – it could compensate for C3 of a different origin.

3-month-old mice of both sexes of the A/J inbred strain were used in all experiments (Breeding Colony of the Institute). The peritoneal exudate cells were derived from the mice 3 days after stimulation with thioglycollate broth. The peritonea were washed with 4 ml of PBS.

Immunofluorescence techiques: 2×10^6 of the cells were incubated with 10–100 µg of C3 for 45 min at 37 °C with permanent agitation. After a 3-fold washing with PBS the cells were incubated with 0.2 ml of rabbit anti-C3 IgG (0.02%) for 30 min at 4 °C. The incubation was stopped by a 3-fold washing with cold PBS. The cells were then mixed with 0.2 ml of swine anti-rabbit-IgG-FITC (SwAR-FITC conjugate, Institute of Sera and Vaccines, Prague; dilution 1:20) and incubated for 30 min at 4 °C. After 4 washes with cold PBS, the cells were examined in a fluorescence microscope. In the control experiments, the cells were incubated with rabbit-anti-C3 antiserum and SwAR-FITC conjugate alone, respectively. In all these experiments, only 0.2% positive macrophages were observed.

Results. The method of isolation described in this paper permits the preparation of a pig C3 complement component in an immunochemically homogeneous form – that was documented by both immunoelectrophoresis (fig. 1) and crossed immunoelectrophoresis (fig. 2). (The pig C3 developed a single precipitation line against rabbit IgG anti-pig serum in immunoelectrophoresis.) The native form of the pig C3 obtained was veri-

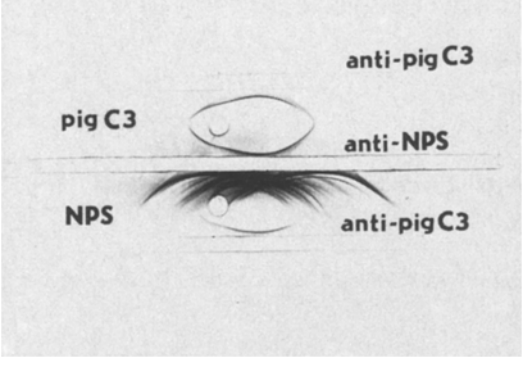

Figure 1. Immunoelectrophoresis of pig C3 on 0.9% agarose in veronal-citrate buffer, pH 8.6, 0.1 M.

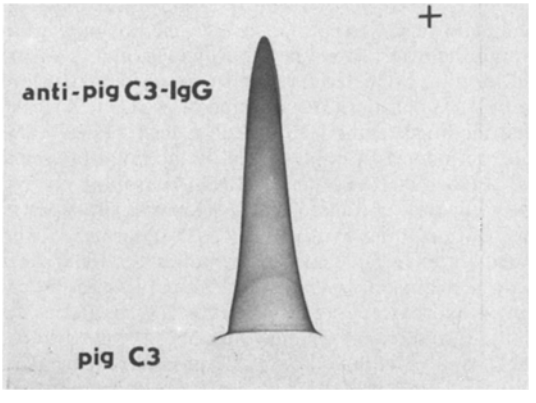

Figure 2. Crossed immunoelectrophoresis of purified pig C3.

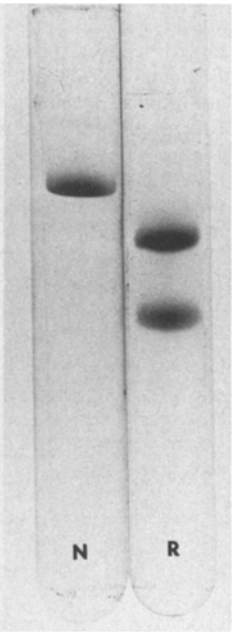

Figure 3. SDS polyacrylamide gel electrophoresis analysis of purified pig C3: in unreduced (U) and reduced (R) form on 8.5% gels.

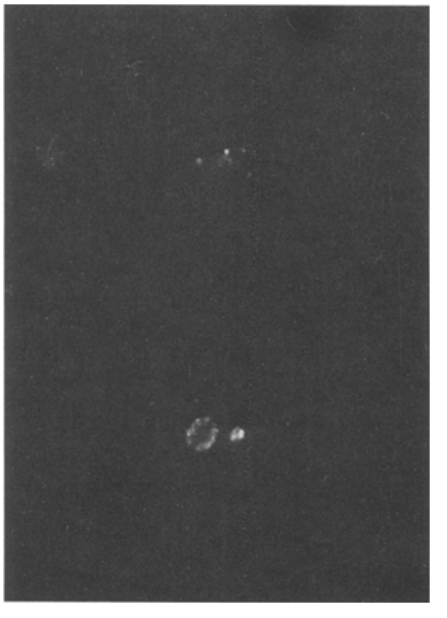

Figure 4. C3b receptors on murine macrophages. Spotty fluorescence of cell surfaces ranging from numerous patches (lower left) to negativity (upper left). C3b-anti-C3-FITC. × 430.

fied by a simple alternative pathway using methylamine-treated plasma.

The homogeneous form of the isolated preparation of pig C3 is documented in figure 3. In SDS-PAGE in 8.5% the pig C3 has a molecular weight of 185,000 ± 18,000 under non-reductive conditions; under reductive conditions, the C3 consists of 2 non-identical polypeptide chains with M_r 112,000 ± 10,000 and 74,000 ± 7,000. Using analytical ultracentrifugation, we have obtained the molecular weight of native pig C3 as 191,000 ± 6,000. The average yield from this method was about 12–17 mg/100 ml of fresh serum. The isolation and characterization of C3 followed the preparation of monospecific rabbit IgG. The antibody obtained did not cross-react with the human, guinea pig, mouse or rat C3 and vice versa. Using this antibody, we tried to detect the C3b receptor on the surface of the macrophages. We found that the C3b receptor is present on the plasma membrane of tested macrophages (fig. 4). The percentage of C3b receptor-bearing macrophages in the peritoneal cavity of stimulated mice is 84.2%. The C3b receptors on the macrophage plasma membrane are spread in the form of clusters, which is documented by figure 4 and also by our electronmicroscopical observation[20]. The distribution of the C3b receptor seems to be independent of temperature, because it has not changed during a 90 min incubation of cell suspension at 37 °C.

Discussion. The pig C3 was isolated in an immunochemically pure, homogeneous and native form and was characterized. Our data agree with the findings of other authors[11,7] and show a high level of homogeneity of the third component of the complement during its evolution. The described method of isolation provided a high yield of pure C3 from the available porcine serum. In spite of the similarity of the C3 structure from various sources, a monospecific IgG was prepared. An interesting finding was that the murine macrophages are able to bind pig C3b, probably due to the high degree of relationship among the C3 components from various species. C3b fragment, previously prepared by limited trypsin cleavage, as described by Tack et al.[14], was also used for studies of C3b receptor. In both cases virtually identical counts of C3b receptor-positive cells were noted and therefore the C3 component only was used in further experiments. The process of the cleavage of C3 to C3b by endogenous macrophage proteases and subsequent binding of C3b on their receptors was blocked by the protease inhibitors PMSF and ε-amino caproic acid. As expected, the native C3 is incapable of binding to the C3b receptors, as has been recently proved by other authors in lymphocytes too[21]. The temperature-independent low lateral redistribution of these receptors seems very likely to have been caused by low mobility of the membrane components of the stimulated macrophages[22]. However, the low rate of C3b receptor redistribution is in contrast to the results of Michl et al.[23] and Griffin and Mullinax[24], who found a normal lateral mobility in stimulated macrophages.

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Immunogenicity of fatty acid anilides in rabbits and the pathogenesis of the Spanish toxic oil syndrome[1]

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Summary. Fatty acid anilides, the major xenobiotic found in the cooking oils responsible for the Spanish toxic oil syndrome, are immunogenic for rabbits as ascertained by a skin test reaction, the characterization of specific antibodies against anilides and the immunofluorescent detection of 'anilide dependent antigens' in tissue slices from treated animals.
Key words. Spanish toxic oil syndrome; oleylanilide; linoleylanilide; hypersensitivity; immunofluorescence; immunopathogenesis.

The Spanish toxic oil syndrome (TOS) resulted from the large scale manufacture and distribution of cooking oil mixtures containing the refining product of imported rape-seed oil denaturated with 2% aniline[5]. Although the identity of the toxic or toxic components in these oil mixtures is yet to be definitely established, fatty acid anilides are likely candidates in view of their high concentration (up to 0.2%) in most samples of suspicious oil[6]. A study of the potential immunogenic properties of fatty acid anilides was undertaken after the consideration of the early clinical features of TOS[7], such as fever, eosinophilia, high IgE titres, skin rash, myalgia, arthralgia, dyspnoea and enlargement of lymph nodes, which were reminiscent of known allergic drug reactions[8]. The rationale for this study was also based on the structural relationships between fatty acid ani-