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Present possibilities for diagnosis in human nasal secretions

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During this morning session we got an excellent theoretical, detailed and almost complete view of the pathophysiology of nasal mucosa. As an ENT physician however, I would like to emphasize that one is often confrontated with patients suffering from chronical sniffing without the possibilities of a complete cure, if I might use this open statement. In other words we are able to treat symptoms by prescribing nosedrops, antihistamines or even corticoids mostly without an effective causal therapy.

Therefore we have attempted in our clinic in Münich during the last years to achieve secretion specific hints in various nasal disorders by investigation of nasal secretions. Several years ago my colleagues, Drs. Hochstrasser, Reichert and Schorn reported on protease inhibitors and enzymatic patterns in nasal secretions.

Diagnostics on secretions however, were limited up to now as the way of collection of nasal secretions was very complicated and the amount mostly too small. These difficulties led so far only to a very restricted number of publications on nasal secretion investigations in patients. Nearly all reported results in the past varied in the mucus collection, which gave only an incomplete composition of human nasal secretions.

At first our intentions were concentrated on the method of collecting nasal secretions. We tried to harvest nasal secretions in a very simple way without any further physicochemical treatment, and attempted to establish a procedure as an routine chemical examination. In contrast to the complicated technique of weighing an elution - as described by Mygind - we used dry cotton or filterpaperstrips respectively of defined measures which are placed into the nasal cavity. Figure 1 shows two cotton strips, which are located in the left middle and lower nasal cavity. These cotton or filterpaperstrips are removed after 20 min. and centrifuged over a sieve into a small plastic tube (Figure 2).

The pure secretions are collected on the bottom of the tube and can be analysed immediately or frozen until used. By this method of collection the further analy-

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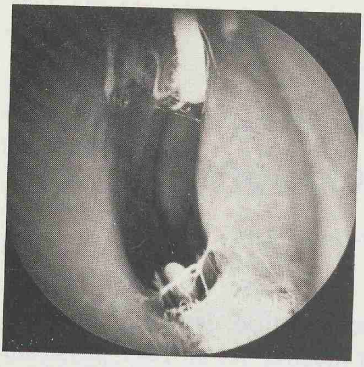

Figure 1. Two cotton strips, which are located in the left, middle and lower nasal cavity

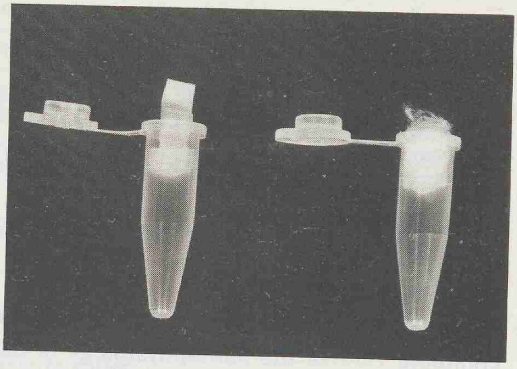

Figure 2. These cotton- or filterpaperstrips are removed after 20 minutes and centrifuged over a sieve into a small plastic tube. On the left you see a filterpaperstrip in the sieve, on the right a cotton strip on the sieve in the plastic tube.

sis of the secretions becomes comparably easy to that of plasma or serum. Nevertheless the analytical possibilities are still restricted because of the extreme small amounts of secretions. Table 1 shows the volume of secretions harvested in healthy donors. Depending on the use of cotton or filterpaperstrips and on the nasal region the average amounts of collected secretes varied between 70–135 microliter.

Table 1. Secretion rate (healthy donors n = 40).

	cotton	filterpaper
left upper cavity	$\bar{x} = 130$ µl	$\bar{x} = 80$ µl
left lower cavity	$\bar{x} = 110$ µl	$\bar{x} = 85$ µl
right upper cavity	$\bar{x} = 135$ µl	$\bar{x} = 75$ µl
right lower cavity	$\bar{x} = 110$ µl	$\bar{x} = 80$ µl

During the past 4 years we examined a total of 1150 nasal secretions. About 600 samples were collected from healthy donors. From October 1980 to August 1982 434 samples of patients with allergies were examined. Furthermore secretions from patients suffering from a dry nose and patients after laryngectomy were analysed. Our experiments were directed towards the question: Which parameters should routinely be examined and which one can be examined microanalytically. Since Schorn and Hochstrasser had reported on alterations of the intermediary enzymes LDH, CPK, GOT, GPT, we tried to get a broad screening of protein and electrolyte alterations in the secretions with our technique.

Table 2 shows those parameters which we have been concentrated on so far. Especially I like to mention the discelectrophoretic separation of proteins as

Table 2. Examined parameters.

Secretion rate	Lysozyme
Protein	Protease inhibitor
Discelectrophoretic separation	Sodium
Immunoglobulin-A	Potassium
Immunoglobulin-E	Calcium

micromethod. In addition IgA as the most important immunoglobulin in mucosal secretion, IgE as an indicator for allergic defence reactions, lysozyme as a well known protector of the mucosa, proteinase inhibitors as newly known protectives against bacterial and leucocytic proteases and finally electrolytes like sodium, potassium and calcium.

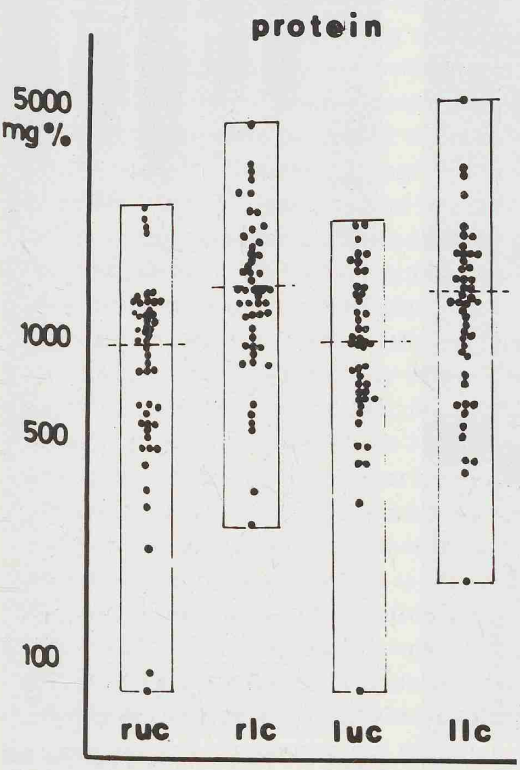

Figure 3. Protein concentration varied individually by a high degree. Noticable was a dependance on the place of collection. Usually the lower cavity gave higher concentrations on proteins.

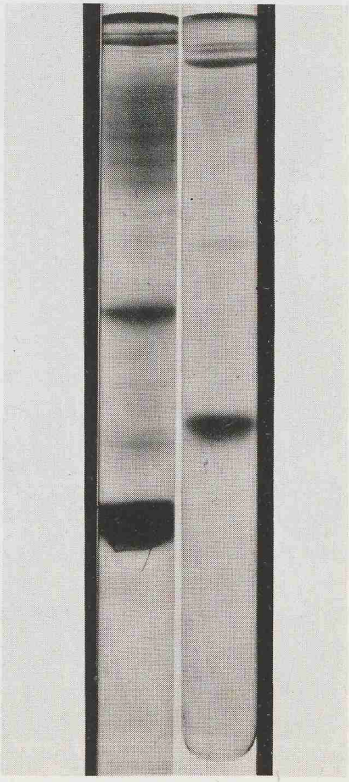

Figure 4. Two discelectrophoretic separations. In the first gel you see a separation of one to ten diluted serum with the marked bend of albumin classicly closed to the gel. In the right gel nasal secretion is separated.

The results are as follows: The concentration of protein varied individually by a high degree. Noticable was a dependance on the place of collection. Usually the lower cavity gave higher concentrations on proteins, most likely due to thickening of the nasal secretions (Figure 3). Discelectrophoresis with its high resolution power showed a complicated pattern of bands. In the first gel you see a separation of 1 : 10 diluted serum with the marked band of albumine classically close to the anode. In the middle region of the gel the band of transferrin and in the region close to the cathode among others the immunoglobulin fractions (Figure 4). In pathological situations nasal secretions showed significant change in their band patterns in comparison to serum. A significant band near the band of transferrin appeared in allergic and vasomotorical rhinitis. In secretion of patients suf-

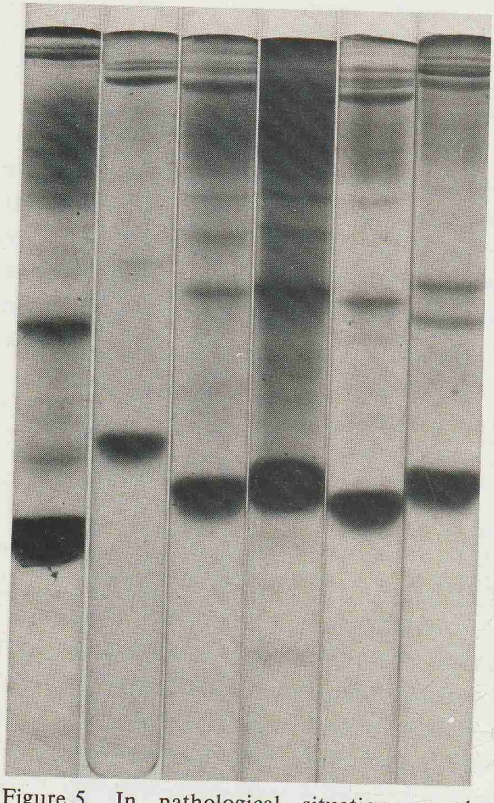

Figure 5. In pathological situations nasal secretions showed significant change in the band patterns in comparison to serum. From left to right: Serum separation, normal nasal secretion, two gels from patients suffering from polyposis nasi and two gels from patients suffering from allergic rhinitis.

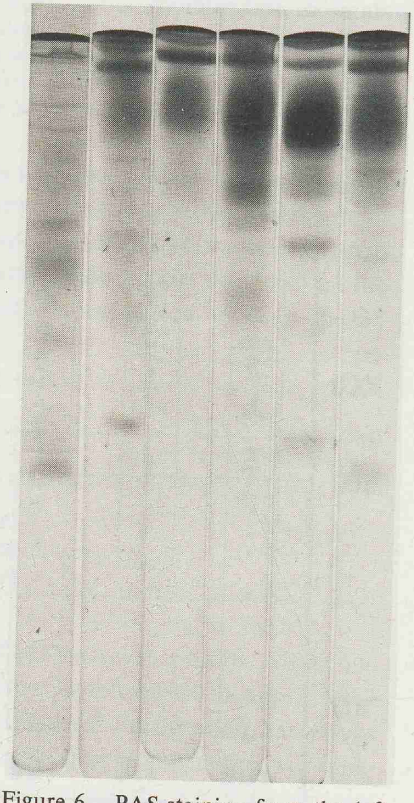

Figure 6. PAS-staining from the left to the right serum, 1 : 2 diluted next normal nasal secretions. Two patients suffering from polyposis nasi and two patients suffering from allergic rhinitis. In contrast to serum strong PAS-positive bands are striking in the upper third of the gel.

fering from polyposis nasi besides the increased concentration of protein in the secretion a strong staining band close to the cathode was found (Figure 5). Since the main compounds of the dry substances of nasal secretion are mucopolysaccharides, i.e. glycoproteins, we tried to apply PAS stainings for these fractions. On Figure 6 you can see in the first gel a serum separation with a dilution of 1 : 2, followed by normal nasal secretion and further to the right a secretion of a patient suffering from polyposis nasi and finally from patients with allergic rhinitis. In contrast to serum strong PAS-positive bands are striking in the upper third of the gel, which need further investigations. As the resolution of total protein by coomassie and glycoproteins by PAS staining showed a strong superposition by serum proteins, secretion specific bands are difficult to distinguish.

Therefore we started to precipitate the serum proteins by chromatographically purified IgG antibodies directed against human serum on small columns, in order to be able to separate selectively secretion specific proteins. To speculate from our recent experiments this method seems to render a possibility to analyse specifically different protein compositions in various nasal disorders.

Quite interesting are our results of the immunoglobulin content. IgA varied in healthy donors between 10–15 mg/100 ml. Significantly lower was the concentration in secretions of patients after laryngectomy, significantly higher in patients suffering from vasomotor rhinitis and with patients suffering from allergic rhinitis. The percental IgA fraction in relation to the total protein content was higher in patients suffering from allergies.

Very striking were the results of the IgE concentrations. In 232 patients suffering from allergic rhinopathia nearly 90% of the patients showed IgE values in the secretions clearly above 10 I.U., the 201 non allergic patients in contrast gave more than 90% IgE values below 10 I.U. (Table 3). By determination of the IgE concentration in secretions rather than in serum the analysis seems to give more reliable results to substantiate an allergic disease in the nasal mucosa. Theoretically this is not too surprising since the amount of IgE in secretions is measured directly from the nasal mucosa as the shock organ.

This is true for patients suffering from polyposis nasi, which often show a negative skin test, but still have high IgE values in their secretions. These results demonstrate, that the skin test is not as specific as the mucosa in nasal allergies (Table 4).

During the last one and a half years we determined the IgE concentrations with an enzyme-linked immunoassey, which is equally sensitive as the RIST but which is more simple and less expensive to perform.

Table 3. IgE (enzyme assay).

	total n	IgE > 10 IU	IgE < 10 IU
allergic	234	209	24
non allergic	201	20	181

Table 4. IgE (enzyme assay).

	total n	IgE > 10 IU	IgE < 10 IU
nasal polyps	33	31	2

The concentration of lysozyme appeared to be increased in patients suffering from allergies and polyposis nasi.

The electrolytes like sodium and potassium showed a great variability: sodium levels were between 60–150 mval and potassium 5–20 mval. Both of them did not show any pathognomonical changes in all examined mucosal disorders. Calcium behaved differently. In patients with allergies it usually gave increased levels compared to other diseases or normal secretions.

Finally I like to mention the protease inhibitors: In the preceeding article Dr. Hochstrasser stressed that there might evolve more diagnostic parameters by the determination of the protease inhibitor spectra. So far we examined routinely the free antitryptic activity in secretions. Its determination is easy, but shows no pathognomonical alterations in different nasal disorders. Far more important are the free and bound elastolytic activities. Elastase when liberated from leucocytes is able to digest tissue and cause and maintain chronic inflammation. In acute pussy inflammation of the nose and the sinuses the bound and liberated anti-elastolytic activity shows values between 5–10 fold above normal. In our laboratory investigations just have started in examining the behaviour of antielastolytic activity in secretions by using as substrate PMN-elastase.

Summarizing I like to stress that despite of having an excellent collection system which allows us to measure quite a number of parameters, many questions are still open. Especially the investigation of secretion specific proteins and the various mucopolysaccharide fractions as well as long term studies on inhibitor spectra and the antielastolytic spectra in different nasal disorders should be studied in the future.

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