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In vitro study of the effect of sea water by-products on the respiratory mucosa

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(Versus sea water diluted to 1/3, isotonic salted serum called "saline", and a specific culture medium on the epithelium of a respiratory mucosa).

The functioning of the nasal mucosa is always on the "front seat" of external atmospheric aggressions : air quality, humidification, particles in suspension causing pollution to various degrees, requiring a good humidification level so as to preserve the quality of the mucus.

The balance of this system is fragile and, in a difficult medium, this is compounded by the fact that the ratio of ciliated cells to brush cells and to mucous and basal cells must remain even at all times.

The increase in number of mucous glands (as often occurs with infants) is a factor of hypersecretion and obstruction, and the deficiency of ciliated cells may jeopardize its elimination by the mucus film, this "disinfecting conveyor belt", while a healthy substructure is essential to the control of the mucosa quality : prevention of congestive and vasodilative reactions or oedemas, etc.

In the range of local treatments of the rhino-pharynx, water, however it may be used, is beneficial during "nose cleaning" and induced nose blowing. Moreover, medications introduced through brush cells in the nasal fossa, have an in-depth effect owing to the cells' highly absorbing effect. Sometimes, they can be destructive (toxic, vaso-constrictive effect) or have a beneficial "eutrophic" effect which can be clinically ascertained but remains always difficult to prove.

Indeed, besides the beneficial effect of water itself, through the cleaning and humidification of the nasal fossa, the other constituents of sea water may also have a positive impact on the mucosa.

The active ingredient of sea water is the hypertonic concentration of salt, but also probably the presence of mineral elements and most of all trace elements which could enhance cell metabolism.

Yet, it remains to be proved :

We wanted to find out whether water really had a beneficial effect on the nasal respiratory mucosa because it is well known that children on the sea side during summer who benefit from bathing in the sea feel incomparably better than they do during the risky winter season.

The use of sea water is far from being recent. It may be deemed trivial to be so interested in such a banal product. However, the concentration in sea water of mineral elements and of trace elements is very close to that of blood serum. This has led some evolutionnists to argument that this would plead in favor of the aquatic origin of man.

Most of the products available are only sea water by-products diluted in bulk to 1/3 so as to obtain an isotonic salinity where mineral salts and trace elements are diluted in the same proportion.

This is the reason why we completed our study with Physiomer (sodium-free non diluted sea water), whose originality resides in keeping normal rates of mineral salts and trace-elements, while reducing the salinity in order to reach an isotonic contents, in contrast with sea water diluted in bulk to one third and with serum salted to 9 per thousand and inappropriately called saline.

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Our study was three-fold :

1) To prove that the product's composition was really the one declared. The study was carried out by Mrs Courtes in the laboratory of hydrology of the university of Bordeaux II.

2) To complete an in-vitro study in order to assess the toxicity or, conversely, the potentially beneficial effect of Physiomer in comparison with the normal culture medium, the dilution in bulk to 1/3 of sea water and the serum salted to 9 per thousand. This study was conducted using tissue cultures of the ciliated respiratory mucosa obtained from samples of mucosa from the human tract. It was carried out by Mrs Bordenave and Mrs Bareille in the CEEMASI, Research Unit 306 of the INSERM (French Institute for Medical Research and Statistics) in the University of Bordeaux II (headed by Ch. Baquey), one of the few laboratories which thoroughly masters this technique, imparting this work its originality.

3) Lastly, to conduct a study in vivo in order to assess there again but in a more clinical context, the potentially deleterious effects of the product and its use in jet and, conversely, its potentially positive effect on the hamster jugal mucosa, on the rabbit nasal mucosa or on superficial skin reactions. The last part of the study has not yet been completed. It is being conducted by M. Philippe Dufour in the laboratory of pharmacodynamics of the university of Bordeaux II.

Findings :

1) Physiomer really associates isotonic salinity and a totally preserved concentration of mineral salts and trace elements (with some variations regarding only some trace elements (copper) or minor elements (silica)).

2) The ability of the INSERM Unit 306 to obtain an extensive and communitive culture of epithelial cells from the respiratory mucosa developed from a sample of human tracheal mucosa made it possible to conduct this in vitro study. The objective was to obtain a confluent culture.

In the framework of this study, three tests were carried out : the LDH, the neutral red and the MTT tests which showed a total absence of toxicity on the cell

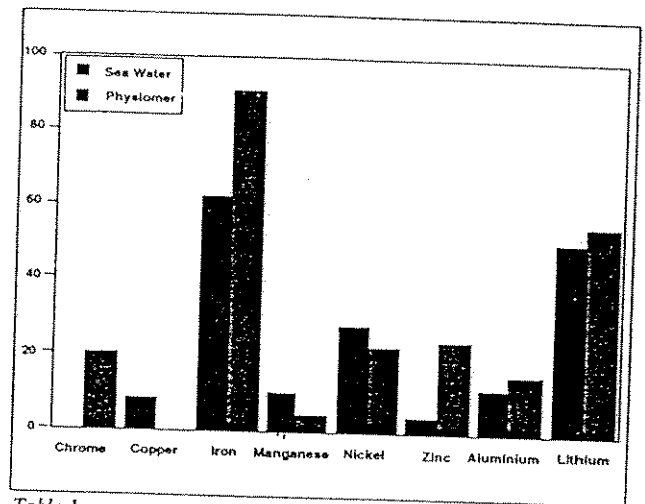


Table 1 :

	Sea Water	Physiomer
Aluminium	11	15
Arsenic	<3	<3
Cadmium	<1	<1
Chrome	<3	20
Cobalt	<3	<3
Copper	8	<1
Iron	62	90
Manganese	10	4
Nickel	28	22
Led	<3	<3
Selenium	<1	<1
Titanium	<3	<3
Zinc	4	24

Table 2 :



Table 3 :

	Sea water	Physiomer
Silica	0,8	5,2
Fluoride	1,4	1,42
Strontium	3,6	3,55
Boron	4,15	3,55

Table 4 :

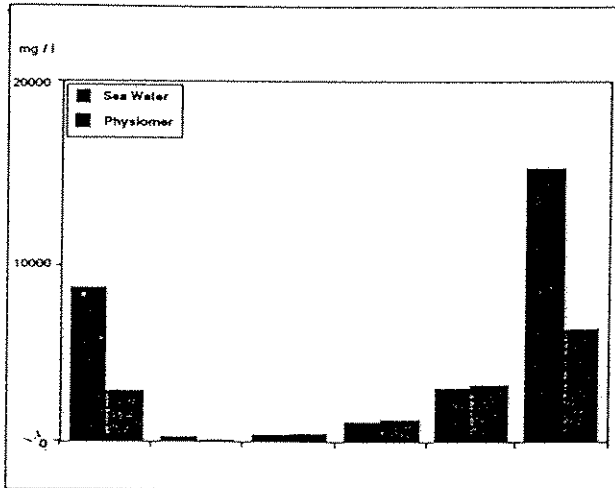


Table 5 :

	Sea Water	Physiomer
Sodium	8500	2720
Potassium	220	89
Calcium	340	345
Magnesium	1110	1200
Sulfate	2830	2950
Chloride	14960	6100

Tableau 6 :

membrane and on the nuclei (the tests were repeated at least twice, sometimes three times, and gave concordant results).

3) The in vitro study on the potentially beneficial effect of Physiomer on the respiratory mucosa was compared with the effect obtained with water diluted in bulk to 1/3 and with isotonic serum salted to 9 per thousand. The result displayed on the diagrammes of Figures 1 and 2 is thoroughly demonstrative and statistically very highly significant : $p < 0,0001$ (see table 6).

The study was completed over a period of 12 days with periodical renewal of the inputs.

Two tests were analyzed :

- The number of cells per culture well, a good reflection of the dynamics of duplication and reproduction of epithelial respiratory cells when in contact with these fluids.

- The protein concentration gives quite a good image of the cell concentration in active metabolic elements. In other words, it is not sufficient to produce cells as these cells should be "functionally vivacious".

Table 7 :

Three experiments A, B and C were conducted to facilitate the comparison between C (usual culture medium), Phy (Physiomer) and EM 1/3 (sea water diluted in bulk to 1/3) which were added to the medium in a proportion of 50% .

The A curve :

- Study of the cell growth through cell count in each culture well : the best effect is obtained in the well containing a specific culture medium, which is only normal, but the results obtained with Phy cdv and EM 1/3 show significantly better results with Phy cdv.

- The study of the cell protein concentration shows that sea water, under the form of Physiomer or diluted sea water yields better results than the culture medium. However, the difference is only highly significant with Physiomer. This fact demonstrates that Physiomer yields a better protein cell contents.

The B curve :

- Study of the cell growth through cell count in each culture well : the result is to be compared with the previous one since it yields a significantly better result with Phy cdv.

- The study on the protein cell concentration yields a better result with sea water than with the usual culture medium and impars the use of Physiomer a highly significant advantage.

The C curve :

The exact same findings were obtained.

Conclusion :

When the beneficial effect of Physiomer on cell growth is compared with the one obtained with sea water diluted in bulk to 1/3 and compared with the effect of the specific culture medium on protein concentration, the scores obtained with Physiomer are by far and significantly better.

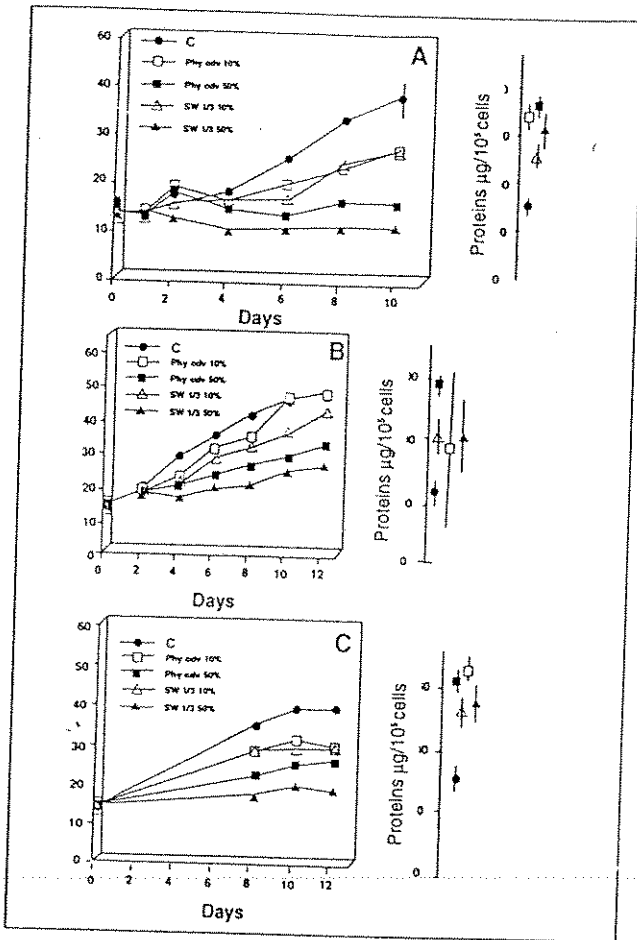


Table 7 : number of cells $\times 10^6$ /well

Table 8 :

Two experiments were carried out, A and B, in order to compare the C culture medium with Physiomer and the isotonic serum salted to 9 per thousand inadequately called saline.

The A curve :

- Study of cell growth :

The result yielded is spectacularly better with Physiomer. It would even seem that with the saline no cell growth occurs !

- Concentration of protein cells :

The difference between Physiomer and "saline" is highly significative.

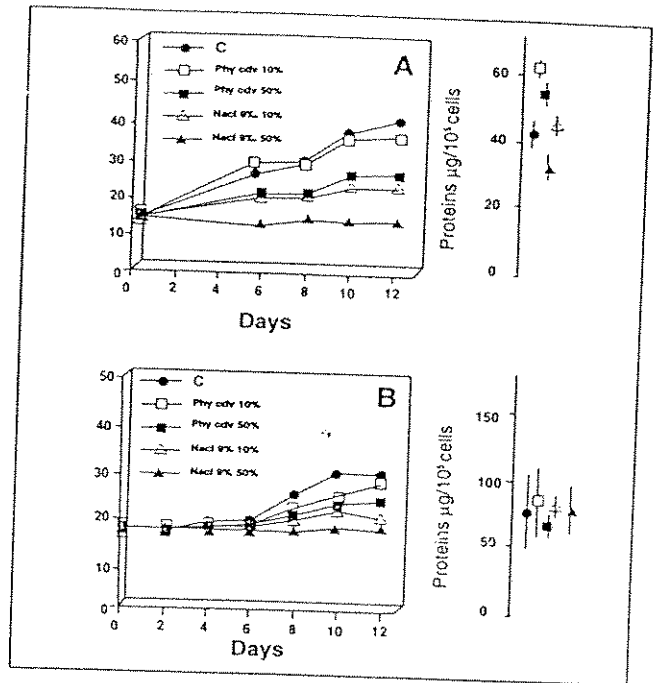


Table 8 : number of cells $\times 10^6$ /well

The B curve

Study of cell growth :

The result is practically identical to that of the A curve. Almost no cell growth generated with the "saline".

- Concentration of protein cells :

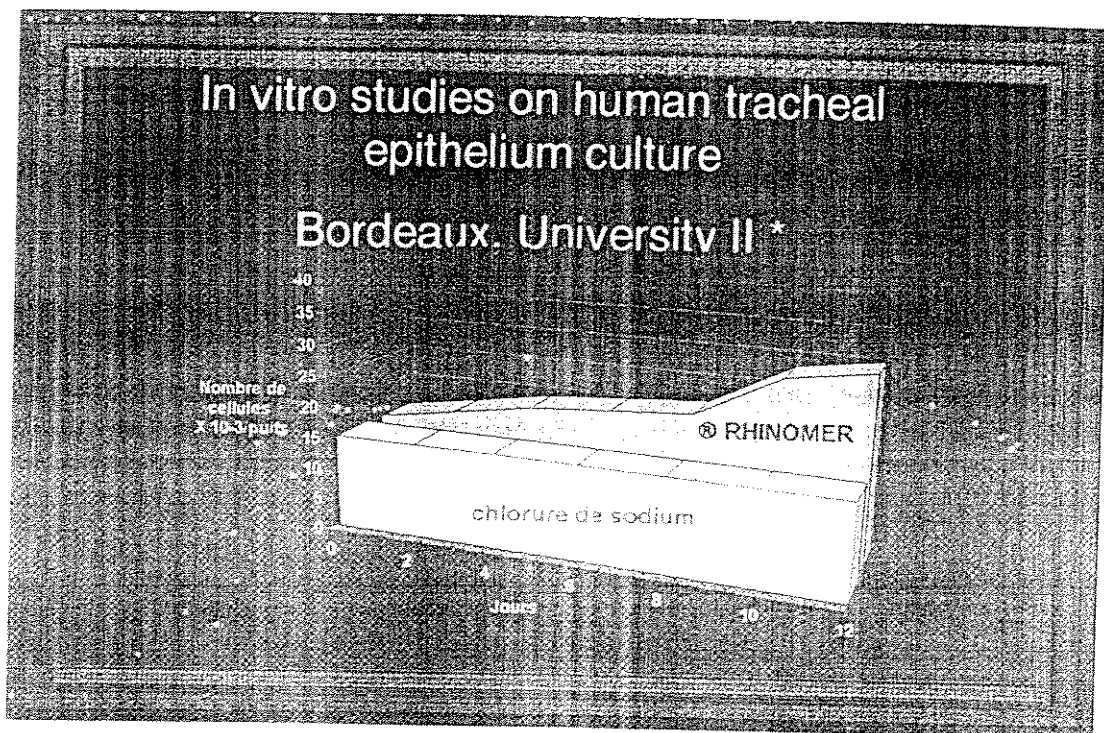
The results are not significant owing to the great variability of results obtained from one culture well to another one.

Summary :

Although this study is an in vitro study, it seemed all the more interesting to analyze the effect of sea water on the cells of the respiratory epithelium that this work had never been undertaken.

The reliability of the results obtained is guaranteed as a stringent experimenting protocol was followed and the study scrupulously complied with the generally admitted rules of good practice in biomedical research applied in the INSERM laboratory.

These results give evidence that, generally speaking, sea water, as compared to "saline" and Physiomer in particular, play a eutrophic role on the epithelium of the respiratory mucosa. ■



- The tests performed on the respiratory mucosa in vitro allowed the effect of RHINOMER to be compared with that of 0.9% sodium chloride. The following curve shows the number of cells per culture well reflecting the “dynamics” of duplication and reproduction of epithelial cells in contact with these liquids.

- The results are in favour of RHINOMER and highly significant ($p < 0.001$).

- Analysis of the protein concentration, a fairly reliable indication of the cellular concentration of active metabolic elements, gives similar results.

* L. Bordenave, R. Bareille, G. Janvier, L. Traissac, Ch. Baquey, 1993