cells are in cycle and thus are sensitive to cytotoxic agents; goblet cells are not in cycle and thus are resistant. Their decrease in absolute number is probably due to killing of their proliferative precursors, coupled with continued migration out of the crypt.

Differences in sensitivity may also explain WIERNIK's[5] data, which describes a wave of goblet cells migrating out of the crypt following irradiation. Since goblet cells and proliferative cells are normally intermingled in the crypt, an agent which kills a portion of the intervening proliferative cells, creates a group of goblet cells which may then migrate together as a wave.

All previous work on goblet cell kinetics has been done with tissue sections, which may give a misleading impression of dramatically increased goblet cell number because of an increase in the size of these cells following irradiation. Most investigators have been correct in reporting a goblet cell increase, but have failed to distinguish between absolute and relative numbers.

Résumé. Les modifications apparaissant dans les cellules caliciformes de l'iléum des souris après irradiation ou l'administration d'actinomycine D sont en premier lieu la répercussion des changements survenus dans la zone de prolifération.

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Effect of Hyperventilation on the Platelet Aggregation Induced by ADP

It was found previously[1] that circulating platelets show a higher responsiveness to ADP before their passage through the pulmonary vessels than after it; in fact, the platelets of the arterial blood show a lesser extent in the maximal aggregation compared to the platelets of the venous blood. This different behaviour is related to a plasmatic component released or metabolized during the passage of blood through the pulmonary vessels[2]. This result gives an account of the interaction between the lungs and the platelet's functions also observed by other authors[3-8]. Additional evidence of these connections is provided by the effect of an artificially produced hyperventilation on the aggregative behaviour of the platelets, which is reported in this paper.

Methods. Adult rats of both sexes were anaesthetized with ether and then with urethan (Carlo Erba, Milano, 400 mg/kg body wt.) and Na Nembutal (Abbot, Aprilia, 30 mg/kg body wt.)

Ventilation was performed by inflating the lungs with air by means of a small-animal respiratory pump (Scientific and Research Instruments LTD, Croydon, Surrey) connected to a cannula inserted in the trachea. The volume/min was adjusted to 0.6 l/min (85 breaths/min for 5 min), in rats hyperventilated and to 0.3 l/min (35 breaths/min for 5 min) in the controls, a value which agrees with normal quiet respiration. Then the chest of the animals was opened and the arterial blood was collected by cardiac puncture from the left ventricle. Sodium citrate (3.8% mixed 1:9) was used as the anticoagulant.

The preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) and also platelet count were carried out as previously described[1].

The platelet aggregation was studied by means of an aggregometer (169 Platelet Aggregation Meter-Evans Electroselenium Ltd) to measure the changes in the optical density (O.D.) of PRP during aggregation induced by ADP (Na_3ADP – C.F. Boehringer and Söhne H-Mannheim – final concentration 9.2×10^{-6} *M*).

In two groups of experiments, sodium lactate (The British Drug Houses Ltd – B.D.H. Laboratory Chemical Division – final concentration 20 mEq/l) was added to blood just before the PRP preparation, or to PRP and incubated for 3 min at 37 °C in the aggregometer before adding ADP.

The aggregation curve was measured in the following way: the maximum curve height was measured from the baseline to the midpoint of the highest segment of the curve, to estimate the extent of aggregation. Statistical analysis was performed with the 2 sample *t*-test for the limiting value of 0.05 probability[9].

Results. Platelets of hyperventilated animals show a greater degree of responsiveness to ADP in comparison with platelets of normally ventilated animals. The values obtained in the experiments are reported in the Table; the mean value of the maximal extent of aggregation was 49.72% (S.D. ± 4.62) in the hyperventilated rats

Maximal extent of platelet aggregation by ADP in PRP obtained from hyperventilated rats (8 experiments) and from normally ventilated rats (8 experiments)

Hyperventilation	Control
51.40	34.80
49.04	30.80
50.00	41.60
49.20	40.40
46.20	36.00
41.32	41.00
55.40	39.20
55.32	40.92
Mean 49.72 (S.D. ± 4.62)	Mean 39.21 (S.D. ± 2.48)

$t = 5.664 > 2.120$ for P 0.05.

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(8 experiments) whereas it was 39.21% (S.D. ± 2.48) in the normally ventilated rats (8 experiments). The values were statistically analyzed with the 2 sample t-test and were significant with $t = 5.644 > 2.210$ for the limiting value of 0.05 probability. From the mean of the values obtained in each group of experiments the curves of the Figure were drawn.

Since it is known that during hyperventilation there is an increase of the lactate concentration in blood[10,11], in one group of experiments sodium lactate was added to the arterial blood obtained from normally ventilated rats, before the PRP preparation. The final concentration was 20 mEq/l. No statistically significant difference in the response to ADP could be detected in PRP so treated in comparison with the controls.

In another group of experiments, sodium lactate (final concentration 20 mEq/l) was added to the sample of PRP in the aggregometer and incubated at 37 °C for 3 min before adding ADP. Also in these experiments, no statistically significant difference could be detected in comparison with the controls.

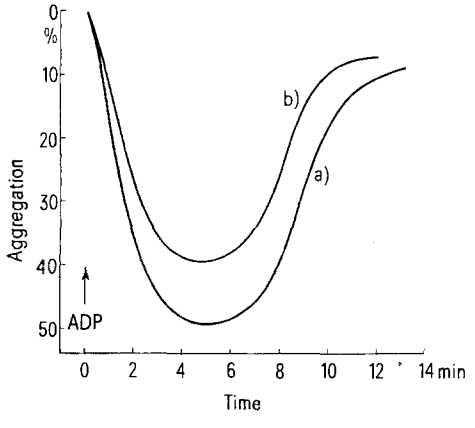
Discussion. From the results reported it appears that hyperventilation increases the responsiveness of the platelets to ADP in the arterial PRP.

Many processes, contemporaneously occurring during hyperventilation, may contribute to the enhancement of the platelet aggregability. One of them, i.e. the rise of the hematic lactate concentration[10,11], may be excluded because the addition of sodium lactate to the whole blood or to PRP was not followed by any statistically significant modification of the platelet aggregation.

The importance of the other changes occurring during the hyperventilation may be considered as follows. It is known that the platelet aggregability is sensitive to modifications of tension of respiratory gases, as previously investigated (work in preparation). However, these changes may be disregarded because the handling of the blood for the PRP preparation and the prolonged stirring of the PRP allows the gases in the sample to equilibrate with the air.

In our opinion, an important contributor to the phenomenon may be the changes of the pulmonary circulation occurring during the hyperventilation. The result of previous work[2] supports the view that, in a normally ventilated rat, a plasmatic component enhancing the platelet aggregability is cleared from the venous plasma by the pulmonary vessels, or alternatively, that the pulmonary vessels pour out a substance inhibiting the platelet aggregation in the arterial plasma. This hypothesis is supported also by the knowledge that the lungs metabolize or release many biologically active substances, as during the normal respiration[12,13], and as during hyperventilation[14,15]. Considering that, following hyperventilation the hemodynamic equilibrium of the lungs is changed, also the time at disposal for the passage of blood through pulmonary vessels may be varied, with the result that the activity of the plasmatic factor affecting the platelet aggregation may be modified.

In addition, the same changes of the pulmonary circulation during the hyperventilation could influence directly the platelet behaviour, according to the results of KIEN WHITE, SHEPRO and HECHTMAN[16], which suggest the occurrence of an interaction between the pulmonary endothelium and the platelets, during their passage through the lungs. The present findings provide one more piece of evidence of the important role of the lungs in regulating the platelet behaviour.



Changes in O.D. induced by ADP (final concentration $9.2 \times 10^{-6} M$) in PRP (700.000 platelets/μl obtained from the arterial blood of a) hyperventilated and of b) normally ventilated rats. Each curve represents the mean value obtained in 8 and 8 experiments respectively.

Riassunto. È stata studiata con aggregometro l'aggregazione piastrinica indotta da ADP in PRP ottenuto da sangue arterioso di ratto normalmente ventilato o iperventilato. È risultato che dopo la iperventilazione l'aggregazione piastrinica è aumentata.

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Excretion of α-M-Fetoprotein in the Urine of Rats

Urinary excretion of antigens not pertaining to the blood plasma proteins proper has been the subject of a recent review[1]. We have previously reported that, whereas α-fetoprotein (AFP) is present in the urine of normal pregnant rats, α-M-fetoprotein (AMFP) cannot be detected[2]. These findings are not surprizing in view of the estimated molecular weights of AFP and AMFP, being 70,000 and 570,000, respectively[3]. The purpose of the present communication is to describe the excretion of AMFP in the urine of pregnant rats with induced injury to the glomerular capillary wall.

The production of rabbit antisera against rat amniotic fluid (AAF) and embryonic blood (AEB) was described in detail elsewhere[2]. The AAF detects the AFP only, while the AEB detects the AFP as well as the AMFP. Using Ouchterlony's double immunodiffusion test, the