(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.

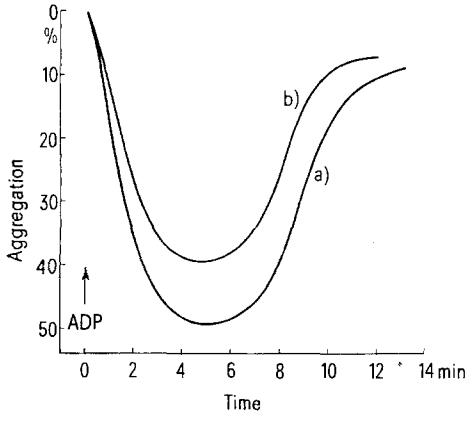
Discussion. From the results reported it appears that hyperventilation increases the responsiveness of the platelets to ADP in the arterial PRP.

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

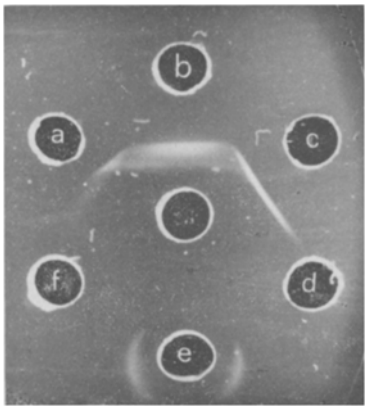
Urinary excretion of antigens not pertaining to the blood plasma proteins proper has been the subject of a recent review[1]. We have previously reported that, whereas α-fetoprotein (AFP) is present in the urine of normal pregnant rats, α-M-fetoprotein (AMFP) cannot be detected[2]. These findings are 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

difference in antibody content between the two antisera, in addition to the location of the precipitin bands in relation to the wells, enables one to distinguish between AFP and AMFP (Figure). Rats, which were on or about the 18th day of gestation, were placed in metabolism cages on alternate days and urine was collected for 24 hours. The urine samples were lyophilized; 10–20 mg of the total non-dialyzable material were suspended in 1 ml of phosphate buffered saline and reacted in immunodiffusion against AAF and AEB.

150 urine samples obtained from 50 virgin female rats did not react with either antiserum. 40 urine specimens collected from 20 normal pregnant rats contained AFP only. Masugi nephritis was induced in 6 pregnant rats by injecting i.p. nephrotoxic serum (NTS) on the 13th and 15th day of gestation[4]. The urine obtained on the 18th to 20th day of gestation was shown to contain AMFP in addition to AFP in all 6 instances. Histological examination revealed mixed membranuos and proliferative glomerulonephritis.

α-M-fetoprotein is found in the serum of rats with acute toxic liver injury[5] and following a variety of experimental procedures[6]. Both AFP and AMFP are present in the blood of pregnant rats and hepatoma bearing animals[2,3,7], but only AFP is excreted in their urine[2]. The appearance of AMFP in the urine, as far as we are aware, has not been described previously. It is suggested that in pregnant rats with Masugi nephritis, urinary excretion of AMFP represents a manifestation of non-selective proteinuria. GANG et al.[8] have shown a loosening of the molecular structure of the glomerular basement membrane in the kidneys of rats with NTS induced nephritis. Excretion of AMFP can be ascribed to such a lesion of the glomerular basement membrane, which permits the passage of macromolecules into the urine. We, therefore, propose that the detection of AMFP in the urine is an indicator of glomerular damage of a magnitude resulting in non-selective proteinura. Because of its high molecular weight, excretion of AMFP appears to be limited to those renal disorders in which glomerular permeability is increased. On the other hand, impairment of tubular functions is apparently not associated with the appearance of AMFP in the urine. In 3-month-old rats, during CCl_4 intoxication, in which functional changes of the tubular apparatus are prominent[9], the urine contains AFP in a certain proportion of cases but never AMFP[10].



Double immunodiffusion test of antiserum to rat embryonic blood reacted with urine specimens of a) hepatoma bearing animal; b) NTS-injected pregnant rat; c) normal pregnant rat; d) virgin rat; e) male rat and f) raline. Note that 2 precipitin bands have developed only with the urine of the nephritic rat (b), indicating the passage of both fetoproteins into the urine.

Zusammenfassung. Nachweis von α-M-Fetoprotein im Harn trächtiger Ratten, die an Masugi-Nephritis litten. Da dieses hochmolekulare Eiweiss im Harn normaler trächtiger Tiere nicht vorkommt, beweist sein Auftreten bei den Nephritis-Ratten eine nicht selektive Proteinurie.

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Immunological Similarity of Human and Rat Calcitonin Confirmed by Immunofluorescent Methods

The amino acid sequence of 5 calcitonins is known[1]: porcine, ovine, bovine, salmon and human. Nine amino acids out of 32 have common positions in all the 5 calcitonins, 7 of which are found in the N terminal portion of the molecule.

We have shown elsewhere that antibodies against human calcitonin raised in the rat (type I) cross react extensively with rat calcitonin (extractive), but not with porcine or salmon calcitonin[2]. Furthermore, antibodies against human calcitonin raised in the guinea-pig (type II) cross react to a low degree with rat calcitonin. These results can only be explained if there are differences in the antigenic sites towards which the two types of antibodies are directed, and if rat and human calcitonins have structural similarities circumscribed to certain regions of the molecule.