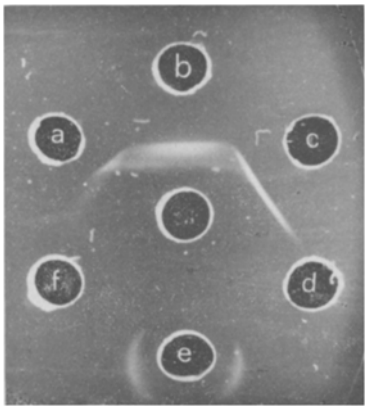
difference in antibody content between the two antisera, in addition to the location of the precipitin bands in relation to the wells, enables one to distinguish between AFP and AMFP (Figure). Rats, which were on or about the 18th day of gestation, were placed in metabolism cages on alternate days and 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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with the technical assistance of G. GANEM

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[11] This investigation was supported by a grant from David Nessim Gaon of Geneva.

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.

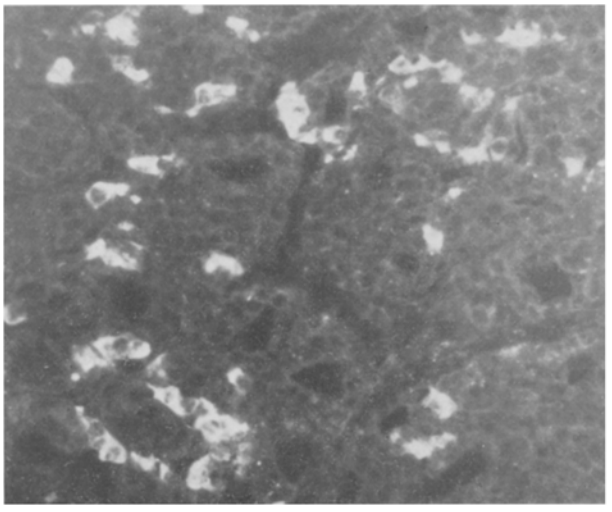

Fig. 1. Specific staining of C-cells with anti-human calcitonin antiserum in rat thyroïd.

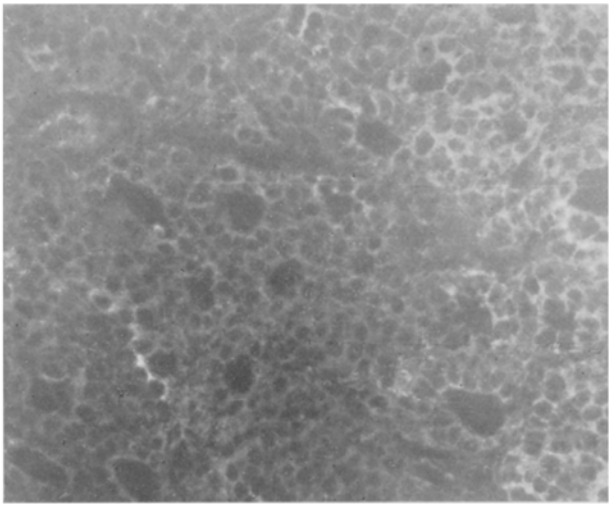

Fig. 2. Adjacent slide showing absence of specific staining in the presence of normal rat serum.

In the present study, these conclusions were further tested by using synthetic fragments of human calcitonin to characterize the antigenic sites towards which the two antibodies were directed. The antibody cross-reacting best with rat calcitonin (type I) was then used for fluorescent staining of C-cells in the rat thyroïd, and to determine which fragments would selectively block such staining.

Material and methods. Antibody production. Male Wistar CF rats and male albino guinea-pigs were immunized with partially purified human calcitonin (100 mU MRC/100 g body weight) emulsified in complete Freund adjuvant. Injections were repeated every 2 weeks in rats and once a month in guinea-pigs. Terminal bleedings were performed after $1\,^1/_2$ months in the rat and 6 months in the guinea-pig.

Radioimmunoassays. Antibodies at a final dilution of 1/2000 (type I) and 1/8000 (type II) were incubated with 50 pg of I^{125} human synthetic calcitonin[3], serial dilutions of synthetic human calcitonin, cold 0.2 *N* HCl extracts of rat thyroïds, and the following fragments of human calcitonin: 1–10, 11–32, 17–32, 24–32. The synthetic human calcitonin as well as the rat extracts were bioassayed against the MRC research standard for human calcitonin in the young male rat. All reagents were diluted in phosphate buffer 0.1 *M*, containing 0.2% human albumin and the incubations were carried out for 18 h at 4 °C. Bound and free hormone were separated by a modification of the dextran coated charcoal method[4]. Appropriate controls with normal serum were performed for each experiment.

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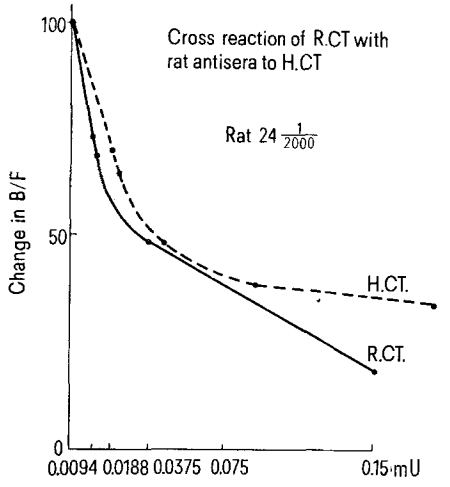

Fig. 3. Displacement ol labelled human calcitonin by rat (R. CT–) and human (H. CT ---) calcitonins. Antiserum type I.

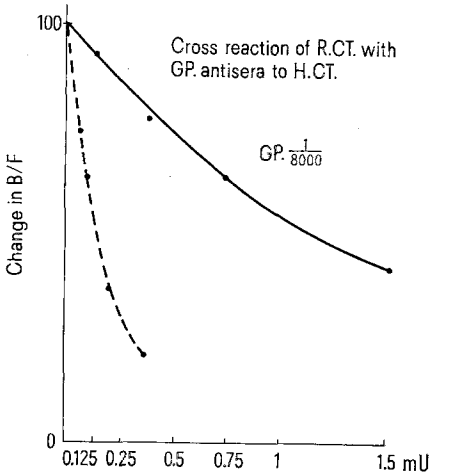

Fig. 4. Displacement of labelled human calcitonin by rat (R. CT–) and human (H. CT---) calcitonin. Antiserum type II.

Blocking of the specific staining of C-cells by porcine (P.CT), human calcitonin (H.CT) and fragments of the human hormone

Controls				Results (fluorescence of *C*-cells)
A.B.a.H.CT.			A.B.a. γ G.R.FITC	+++
Normal serum			A.B.a. γ G.R.FITC	–
A.B.a.H.CT	P.C.T.	2,5 n.	A.B.a. γ G.R.FITC	+++
A.B.a.H.CT	H.C.T	2,5 n.	A.B.a. γ G.R.FITC	–
A.B.a.H.CT	Fr. 1–10	2,5 n.	A.B.a. γ G.R.FITC	+++
A.B.a.H.CT	11–32	2,5 n.	A.B.a. γ G.R.FITC	–
A.B.a.H.CT	17–32	2,5 n.	A.B.a. γ G.R.FITC	–
A.B.a.H.CT	24–32	2,5 n.	A.B.a. γ G.R.FITC	++

M

A.B.a.H.CT, antiserum Type I; A.B.a. γ G.R.FITC, fluorescent antiserum to rat γ-globulin.

Fluorescent localization of rat C-cells. Rat antiserum used in the radioimmunoassay was diluted 1/10 and spread over sections of rat thyroïd fixed in cold formol for 5 min and washed 3 times with phosphate buffered saline. After overnight incubation at 4 °C, the slides were washed 3 times in saline phosphate buffer and incubated for 4 h with fluorescent anti γ-globulins.

After further washings, the slides were examined in a fluorescence microscope. Adjacent sections were always included as controls for non-specific staining. Saturation of the antisera with porcine and human calcitonin and with synthetic fragments of the human molecule listed above was carried out in the blocking studies before spreading on the sections.

Results. The specific antibodies stained certain cells in the thyroïd gland which were parafollicular or interstitial in position (Figure 1) and were never present in the lower poles of the thyroïd gland or the isthmus. The cells increased in number in the middle and upper thirds of the thyroïd lobes and were found either isolated or in clusters. No staining of these cells was observed in sections incubated with normal rat serum (Figure 2). Sections in which the antiserum was saturated with either porcine calcitonin or human fragment 1–10 showed no decrease in fluorescence, while with fragment 24–32 a slight decrease in the intensity of the fluorescence was noted. Complete inhibition of staining was obtained when the antibody was saturated with human calcitonin, or when fragments 11–32 or 17–32 were used (Table).

In the radioimmunoassay displacement studies, antibodies of type I cross reacted extensively with rat calcitonin (Figure 3) in contrast to the type II antibodies (Figure 4). Neither type of antibody crossed significantly with fragment 1 to 10. Both antibodies reacted with sequence 11 to 32, but only type I antibodies reacted extensively with fragment 17 to 32 and to a slight extent with fragment 24 to 32 (Figures 5 and 6).

Discussion. As expected from the fact that the antibodies used cross react extensively in vitro with rat extractive calcitonin, the immunofluorescent staining of the rat thyroïd is specific for C-cells. No staining is observed in sections incubated with normal rat serum or with antibody saturated with human synthetic calcitonin.

The two types of antibodies differ in the extent of their cross reaction with rat calcitonin and are directed towards different regions of the human molecule. Type I antibodies cross react completely with fragment 17 to 32 of the human molecule, and extensively with rat calcitonin, while type II cross slightly with fragment 17 to 32, 24 to 32 and have a low degree of cross reaction with the rat hormone. The experiments involving the use of type I antibodies saturated with the different fragments of the human molecule show that saturation of the antibody with sequence 17 to 32 is sufficient to inhibit totally the binding of the antibodies to the rat C-cells. Thus this high cross reaction between antibodies of type I and rat calcitonin is likely to be due to the presence in this molecule of amino acids either identical in sequence with fragment

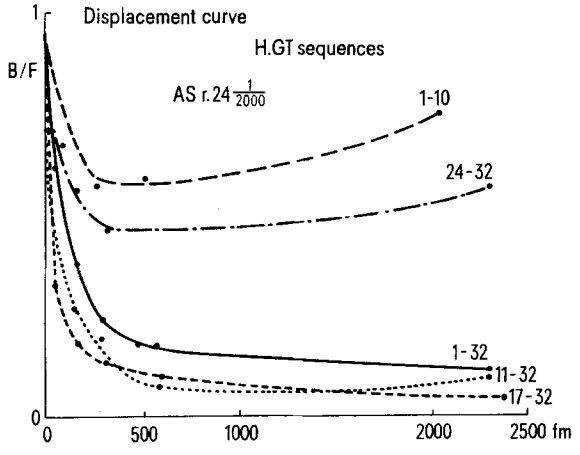

Fig. 5. Displacement of labelled human calcitonin (H. CT) by fragments of the human hormone. Antiserum type 1.

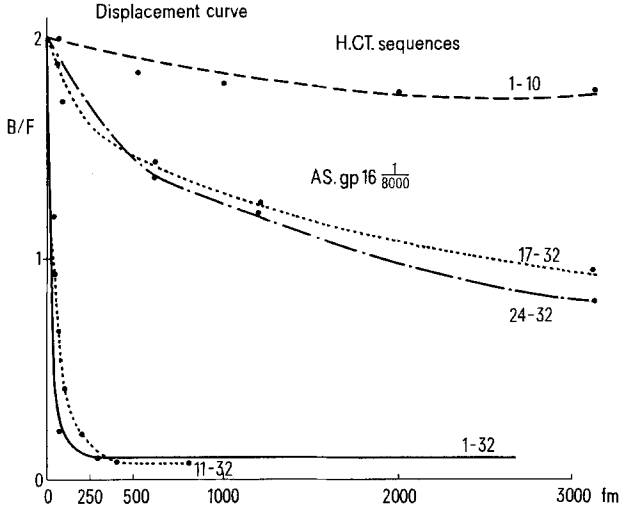

Fig. 6. Displacement of labelled human calcitonin (H. CT) by fragments of the human hormone. Antiserum type II.

17–32 of the human molecule or having electrical charges and conformational properties quite similar to this fragment[5].

Résumé. Les cellules C de la thyroide de rat ont été localisées par un anticorps anticalcitonine humaine (immunofluorescence indirecte). L'inhibition de la réaction par la calcitonine humaine et ses fragments a été étudié.

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[5] Fragments 1–10, 11–32, 17–32, 24–32 were kindly supplied by Merck, Sharp and Dohme and synthetic human calcitonin was a generous gift of Ciba Basel.

Effect of Manganese on the ADP-Induced Platelet Aggregation

In a previous work it was found that Mn^{2+} inhibits clot retraction and that it induces a very quick and complete relaxation of a fully contracted clot, as well as preventing clot retraction[1]. As substances known to influence the clot retraction could also modify the adhesion-aggregation reaction [2–4], it seemed interesting to see whether the aggregation of platelets by ADP is dependent on some similar connections to comparably Mn^{2+}-sensitive mechanisms. The present paper shows that the responsiveness to ADP of the rat platelets is effectively modified by adding $MnCl_2$.

Methods and materials. Venous blood was collected by plastic syringe from the right ventricle in open-chest animals (Albino Wistar Rats, body weight 300–400 g) anaesthetized with ether.

Platelet – rich plasma (PRP), Platelet – poor plasma (PPP) and platelet count were performed as previously described[5]. The ADP-induced platelet aggregation was tested in PRP with a standard platelet concentration (700.000/μl) by an aggregometer (169 Platelet Aggregation Meter, Evans Electroselenium Ltd) and recorded by Speedomax XL 690 Series Recorder (Lees and Northrup, North Wales and Philadelphia) as usual[5].

Chemicals used are: Adenosine-5′-diphosphate (ADP) trisodium salt (Boeringher, Mannheim, Germany) dissolved in veronal buffer, pH 7.4, at a concentration of 10^{-3} *M* and stored at −20 °C until use; manganese: $MnCl_2$, crystals, (Mallinckrodt Chemical Works, St. Louis –New York–Montreal).

Statistical analysis: the data obtained were elaborated for their statistical significance by the 2 sample *t*-test, for 0.05 probability.

Results. Figure 1 shows the average O.D. changes of PRP tested in 42 experiments as follows: a) 12 experiments: PRP+ADP (final concentration 10^{-4} *M*); b) 8 experiments: PRP+ADP (as above) + $MnCl_2$ (final concentration 10^{-1} *M*); c) 14 experiments: PRP+ADP (as above) + $MnCl_2$ (final concentration 10^{-2} *M*); d) 8 experiments: PRP+ADP (as above) + $MnCl_2$ (final concentration 10^{-3} *M*).

There is evidence that Mn^{2+}, inhibits both ADP induced platelet aggregation and disaggregation. The inhibitory effect on the aggregation is very marked at higher Mn^{2+} concentrations (until 10^{-2} *M*) at which also the disaggregation is prevented. If the final concentration is less, (10^{-3} *M*) manganese fails to influence the maximal

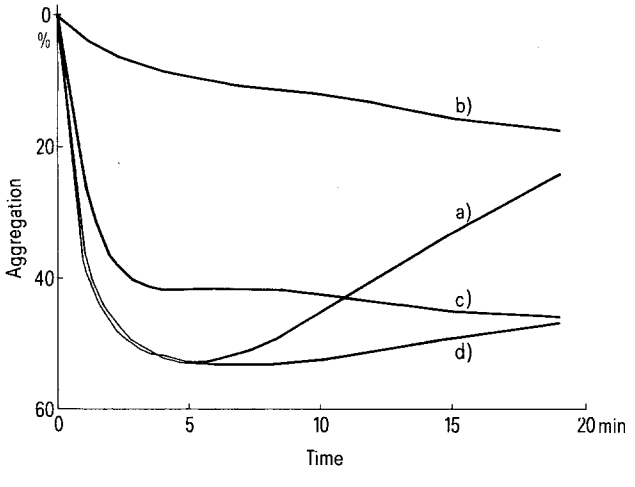

Fig. 1. Average O.D. changes observed in: a) PRP+ADP; b) c) d) PRP+ADP+$MnCl_2$. The final concentrations of ADP was always 10^{-4} *M*; that of $MnCl_2$ was: b) 10^{-1} *M*, c) 10^{-2} *M*, d) 10^{-3} *M*. Number of platelets: 700.000/μl. By the two sample *t*-test computed for the maximal amplitude of the curve, it was found that in respect to a) for b) and c) $p \leq 0.05$ and for d) $p > 0.05$. For the curve d) the values obtained were statistically significant in respect to a) for the beginning and the speed of the disaggregation (disaggregation 10 min after the maximal aggregation).

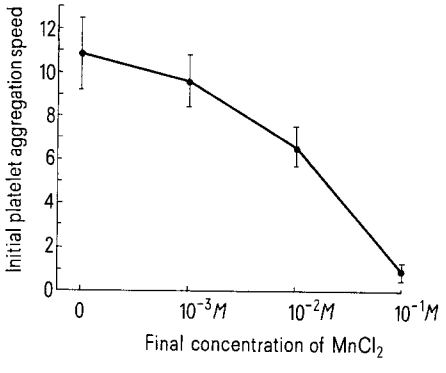

Fig. 2. Influence of manganese on the initial ADP-induced platelet aggregation speed. The speed was calculated through the tangent to the initial part of the curve conventionally expressed in cm. By the two sample *t*-test we found that the inhibition is statistically significant for a final concentration of Mn^{2+} 10^{-1} *M* and 10^{-2} *M*.

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