Virchows Arch. A Path. Anat. and Histol. 363, 333—342 (1974)

Experimental Parathyroiditis in the Rat by Passive Immunisation*

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Received April 22, 1974

Summary. By immunisation of rabbits with rat parathyroid-(PTG)-homogenates or bovine PTG-homogenates antisera with species- and organ-specific antibodies against rat PTG or bovine PTG, respectively, have been produced. After passive immunisation of rats with the antiserum against rat PTG a marked immunoparathyroiditis developed in three out of 15 rats. Hypoparathyroidism, however, was not observed.

An autoimmune etiology is discussed for the idiopathic hypoparathyroidism (Blizzard *et al.*, 1966a, 1966b; Mitschke *et al.*, 1973; Van de Casseye and Gepts, 1973) and circulating tissue antibodies against parathyroid glands (PTG) were detected in 38% of those patients. The autoimmune etiology seems to be obvious particularly in the syndrome of hypoparathyroidism, chronic adrenal insufficiency, and moniliasis. In some rare cases of this syndrome a lymphocytic parathyroiditis was found at autopsy (Craig *et al.*, 1955; Kössling and Emmrich, 1971; Mitschke *et al.*, 1973). Moreover, Van de Casseye and Gepts (1973) report on a lymphocytic parathyroiditis in a patient with idiopathic hypoparathyroidism and Paget's disease. Seemann (1967) observed a distinct lymphocytic parathyroiditis in a patient without anamnestic data of altered calcium metabolism. The significance of the sparse to moderate lymphocytic infiltrations of PTG which were found in 10–16% of unselected autopsy series by Reiner *et al.* (1962) and Seemann (1967) is not clear.

To acknowledge the autoimmune etiology of a disease Witebsky and Rose (1957) also require active and passive immunisation for its experimental reproducibility. Jancovic *et al.* (1965) induced an experimental parathyroiditis by *active* isologous immunisation in chickens. And Lupulescu *et al.* (1965, 1968a, 1968b) succeeded in challenging an experimental parathyroiditis with hypoparathyroidism by *active* immunisation of dogs and rats.—In our present study we investigated the formation of precipitating antibodies against PTG tissue and the possibility of *passive* immunisation of rats.

Materials and Methods

For immunisation two and two rabbits were injected subcutaneously with the following tissue homogenates and tissue extracts in 1 ml Freund's adjuvant (Difco Lab.) once a week for 8 weeks:

Group A: Rat PTG homogenate of 20 PTG each,
Group B: Rat thyroid (TG) homogenate of 6 TG each,
Group C: Bovine PTG homogenate of 2 PTG each,
Group D: 1 ml bovine PTG extract PARA-THOR-MONE® (Lilly),
Group E: 1 ml bovine PTG extract PARATHORM® (Hormonchemie).

* Supported by DFG, Sonderforschungsbereich 34 "Endokrinologie".

Table 1. Design of immunisations and results

Immunisation of rabbits				Passive immunisation of rats			
2 rabbits per group	Immunisation once per week with 1 ml Freund's adjuvant and....	Rabbit serum	Antibodies found against	15 rats per group	Parathyroiditis[a]	Thyroiditis[a]	Hypoparathyroidism[a]
A	Rat parathyroid homogenate of 20 rPTG	Antiserum *A*	1. rat PTG (specific) 2. rat tissue (not specific)	A′	+++ (3) + (12)	0	0
B	Rat thyroid homogenate of 6 rTG	Antiserum *B*	rat tissue (not specific)	B′	0	+ (5)	0
C	Bovine parathyroid homogenate of 2 bPTG	Antiserum *C*	1. bovine PTG (specific) 2. bovine tissue (not specific)	C′	0	0	0
D	Bovine parathyroid extract (1 ml PARA-THOR-MONE®)[b]	Antiserum *D*	bovine tissue (not specific)	D′	0	0	0
E	Bovine parathyroid extract (1 ml PARATHORM®)[b]	Serum *E*	—				
		Normal rabbit serum (Behring)		X	0	0	0

[a] +++ = marked infiltration, + = discrete infiltration, 0 = negative.
[b] PARA-THOR-MONE® was kindly provided by Eli Lilly GmbH, Gießen, PARATHORM® was kindly provided by Hormonchemie, München.

After this immunisation the rabbit sera *A*, *B*, *C*, *D*, and *E* were analysed with the Ouchterlony immunodiffusion test (1958) for precipitating antibodies in order to investigate the reaction

1. against the antigen containing tissue homogenate or extract used for immunisation,
2. against other organ tissues of the respective animal species, and
3. for cross reacting antibodies between rat PTG and bovine PTG.

The organ specificity of the antisera was tested by comparing Ouchterlony tests and also with immunoabsorption by other organs of the respective animal species. Furthermore, immune electrophoretic studies were done.

A parathyroiditis was induced by means of passive immunisation. Test groups of 15 rats each were daily injected intraperitoneally with 0,3 ml rabbit antiserum for 50 days.

Rats of test group A′ were injected with rabbit antiserum *A*;
rats of test group B′ were injected with rabbit antiserum *B*;
rats of test group C′ were injected with rabbit antiserum *C*;
rats of test group D′ were injected with rabbit antiserum *D*;
rats of test group X were injected with rabbit control serum.

Serum calcium (flame photometry) and serum phosphorus (method according to Zilversmit and Davis, 1950; C.F. Boehringer, GmbH, Mannheim) were measured repeatedly in the rats during the experimental period. After 50 days the rats were sacrificed and both lobes of the TG together with the PTGs were dissected. The left TG and PTG were fixed in formaldehyde and embedded in paraffine for light microscopical histology. The right TG and PTG were fixed in glutaraldehyde, postfixed in osmic acid and embedded in epoxy resin for electron microscopy. In addition, liver, pancreas, kidney and adrenals were examined histologically.

Results

After injection of the different tissue homogenates and tissue extracts in rabbits for immunisation the following *antisera* could be characterized:

Antiserum A: contained a) a species specific antibody against rat tissue and b) a species and organ specific antibody against rat PTG (Fig. 1a, c, d, e).

Antiserum B: contained a species specific antibody against rat tissue without organ specificity against TG or PTG.

Antiserum C: contained a) a species specific antibody against bovine tissue and b) a species and organ specific antibody against bovine PTG (Fig. 1b).

Antiserum D: contained a species specific antibody against bovine tissue without organ specificity against PTG.

Serum E: no antibodies against bovine tissue or PTG could be found by the methods used.

A cross reaction of antiserum *A* with bovine PTG or of antiserum *C* with rat PTG was not present: no precipitation lines were developed in the Ouchterlony test.

A marked *parathyroiditis* was found in three rats of the group A′ by passive immunisation after injections of antiserum *A*. The other rats of group A′ showed only discrete lymphocytic infiltrations and a slight increase of connective tissue stroma. In parathyroiditis the inflammatory infiltrate was developed within the gland and along the capsular zone (Fig. 2a–c). There were broad infiltrations along the perivascular interstices and dissociating the parenchymal cords. The infiltrate contained predominantly lymphocytes, moreover macrophages as well as eosinophilic and neutrophil leukocytes. The lymphocytes frequently had pseudopod-like processes, which extended both to the interstitial conective tissue and to the parenchymal cells (Fig. 3c, d). In the infiltrated regions some epithelial cells showed degenerative changes, i.e., dilatation of the rough endoplasmic reticulum and of

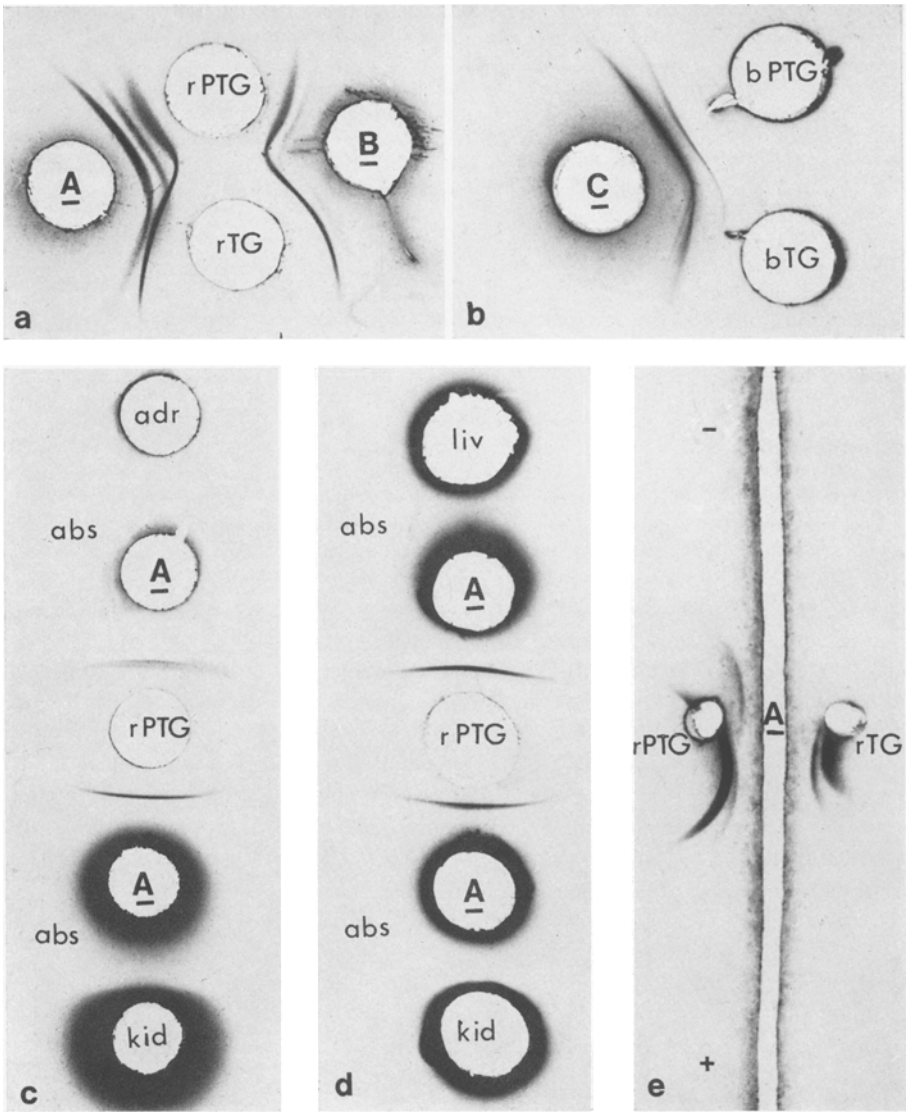

Fig. 1a—e. Immunologic characteristics of antisera: a Comparative agar-gel diffusion test: antiserum *A* and *B* response to rat parathyroid (*rPTG*) and rat thyroid (*rTG*) homogenates. Yet an additional precipitation line appears between antiserum *A* and rPTG which is indicative for an organ specific antibody against rat PTG tissue. b Comparative agar-gel diffusion test: antiserum *C* reacts with bovine parathyroid (*bPTG*) and bovine thyroid (*bTG*) homogenate. An additional precipitation line, however, appears against PTG homogenate which is indicative for an organ specific antibody against bovine PTG tissue. c and d Agar-gel diffusion test after absorption: antiserum *A* was absorbed by homogenates of rat adrenal (*adr*), rat kidney (*kid*) and rat liver (*liv*). The absorption is complete because the antiserum does no longer response to the homogenates. Another precipitation line with rat PTG homogenate appears after this absorption. e Immuno-electrophoresis of antiserum *A* with rat PTG and rat TG homogenates: an additional organ specific precipitation line appears migrating to the cathode

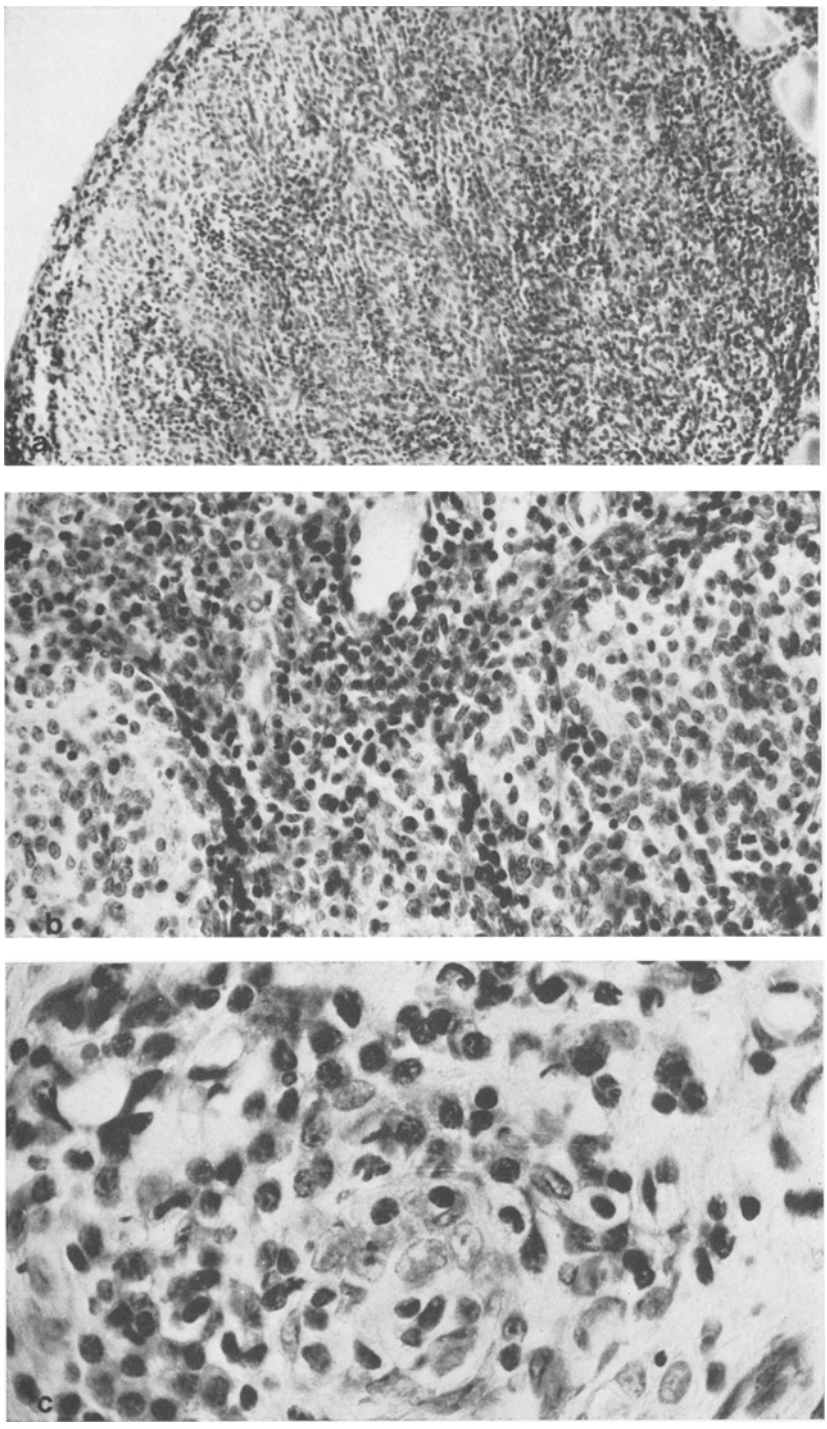

Fig. 2a—c. Parathyroiditis of the rat after passive immunisation with antiserum *A*: Marked inflammatory infiltration of the PTG and its capsule with lymphocytes, plasmacytes and single leucocytes. Light microscopy a × 150, b × 375, c × 800

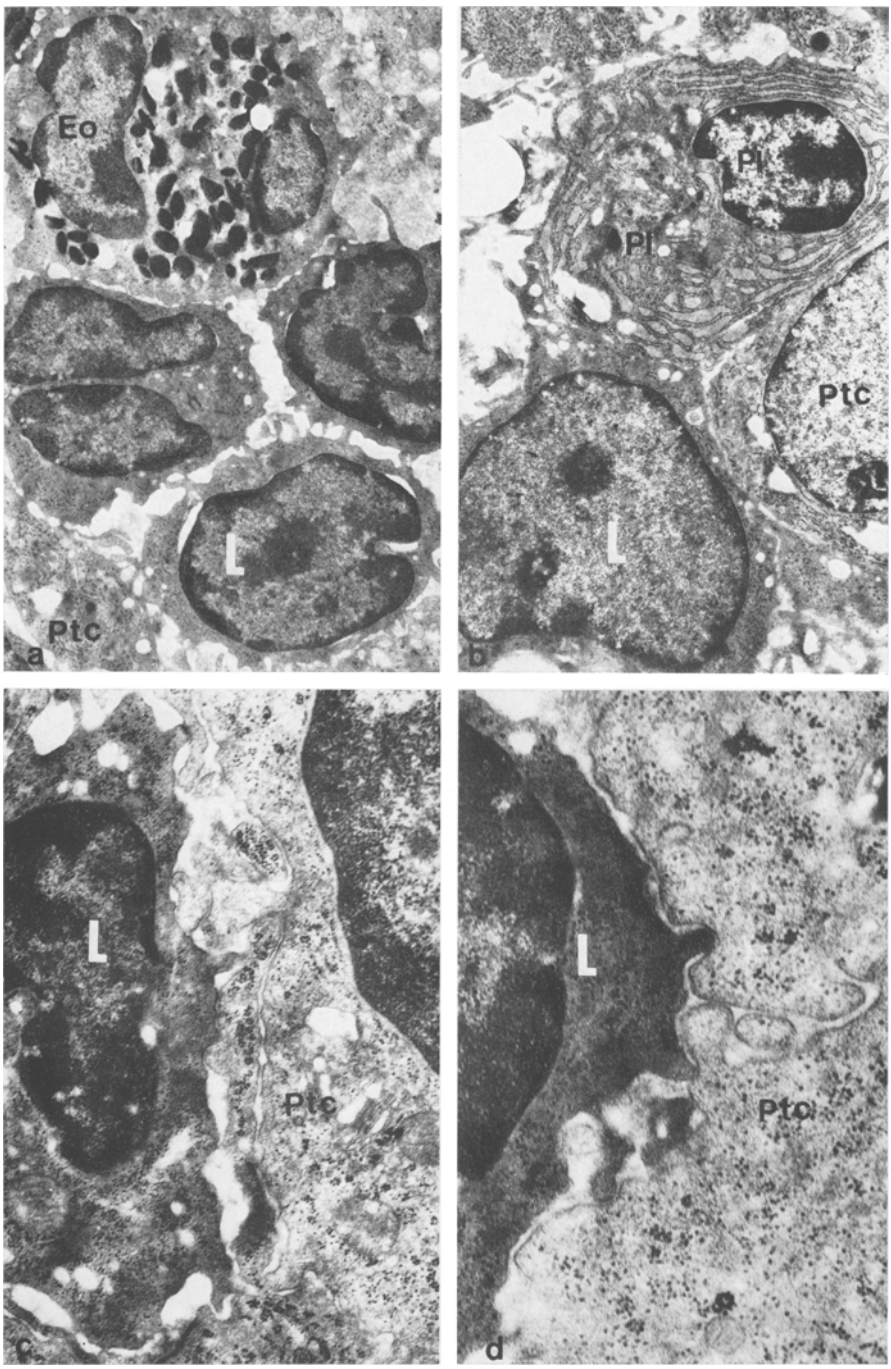

Fig. 3a—d. Parathyroiditis of the rat after passive immunisation with antiserum *A*: a and b Different types of inflammatory cells: lymphocytes (*L*), eosinophilic leucocytes (*Eo*) and plasmacytes (*Pl*) in close vicinity to parathyroid cells (*Ptc*). c and d Lymphocytes with villous and pseudopod-like processes extending toward the parathyroid cells (*Ptc*). Electron microscopy. a $\times 5500$, b $\times 5500$, c $\times 17000$, d $\times 28000$

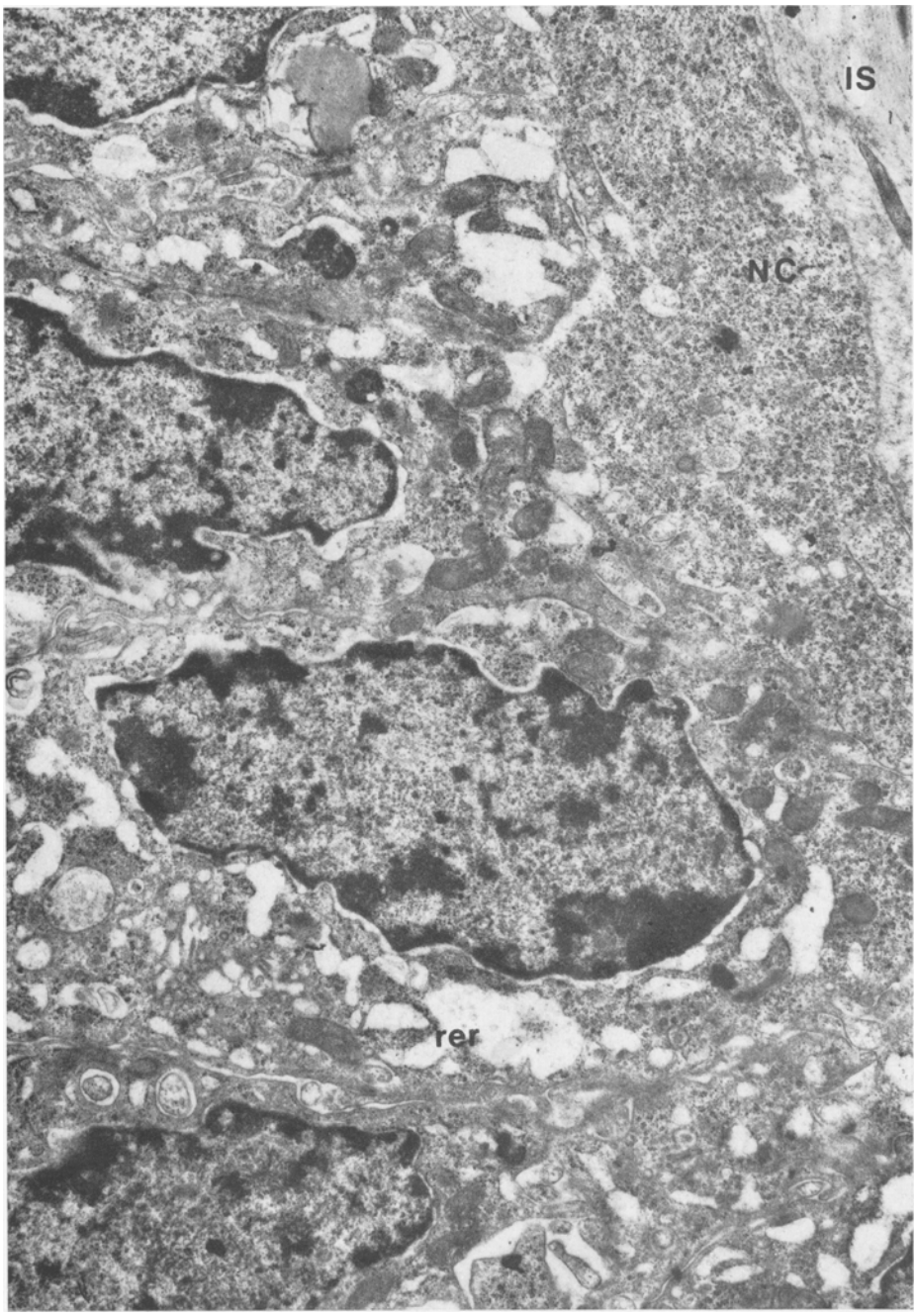

Fig. 4. Degenerative lesions of rat parathyroid cells in parathyroiditis: dilatation of the rough endoplasmic reticulum (*rer*) and the perinuclear spaces. Partial loss of distinct plasmamembrane. In the upper right corner the part of a necrotic cell (*NC*). *IS* interstitial space. Electron microscopy. × 12000

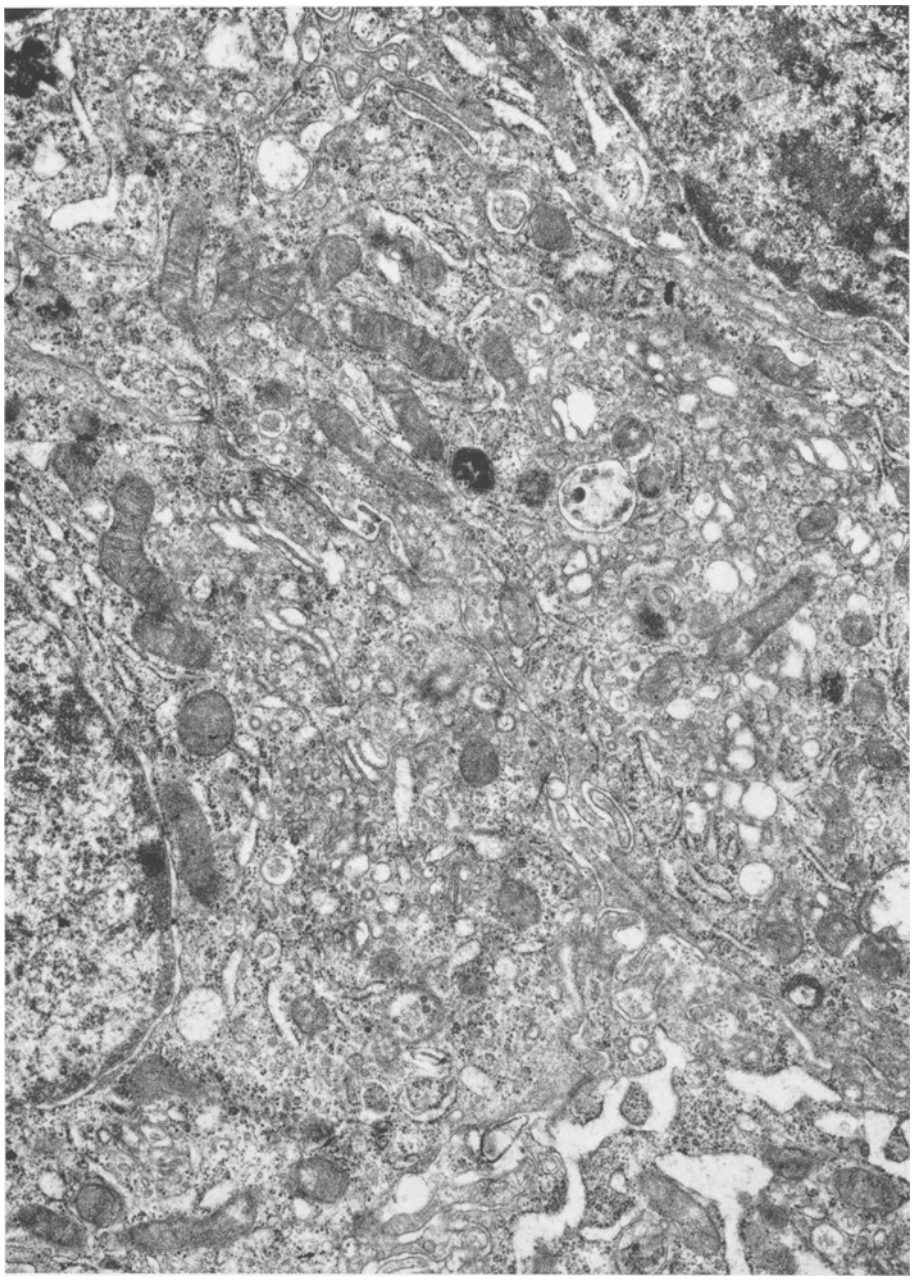

Fig. 5. Compensatory activation of rat parathyroid cells in parathyroiditis: Cytoplasma with a great amount of organelles involved in hormone synthesis and secretion, i.e., many profiles of the rough endoplasmic reticulum, large Golgi fields with many Golgi vacuoles and pro-secretory granules. Electron microscopy. × 17000

the perinuclear space as well as of the Golgi profiles (Fig. 4). Sporadically necrotic cells were found. On the other hand the intact epithelial cells showed signs of elevated endocrine activity, i.e., extended Golgi apparatus and rough endoplasmic reticulum and increased free polyribosomes (Fig. 5).

No inflammatory infiltration of the PTG was developed in the rat test groups B′, C′, D′, and X after the injections of antisera *B*, *C*, *D*, and the normal rabbit control serum. Some rats of group B′ had a discrete focal infiltration of the thyroid gland and a low-grade eosinophilic infiltration of the thyroid capsule.

With regard to the other organs examined histologically, sparse lymphocytes were seen only in the central lobular regions of the livers in all test groups.

Neither the rats of test group A′ nor those of the other groups had developed a hypoparathyroidism. The serum calcium and the serum phosphorus were within the normal range during the whole test period.

Discussion

By immunisation of rabbits with rat and bovine PTG homogenates antisera could be obtained containing species and organ specific antibodies against rat PTG tissue and bovine PTG tissue, respectively. By immunisation with one of the two commercial bovine PTG extracts only species specific antibodies against bovine tissue could be obtained without organ specificity to PTG. According to data made available by the manufacturer this extract has a biologically standardized PTH-concentration of 100 USP/ml equivalent to 0.04 mg/ml pure PTH and, in addition to that, hormonally inactive proteins of about 6.5–10.0 mg/ml. The antigen determinant(s) in the PTG homogenates, which were operative for the formation of the organ specific PTG antibodies in the antisera *A* and *C*, were not determined. Considering the number of possible antigens it cannot be supposed nor postulated that the demonstrated PTG antibodies were antibodies against PTH. The lack of hypoparathyroidism in the rats of group A′ after passive immunisation does not speak for the presence of parathyroid hormone binding antibodies in the antiserum *A*.

The models of experimental immune parathyroiditis so far described in chickens, dogs, and rats were based on active immunisation with isologous tissue homogenates (Jancovic *et al.*, 1965; Lupulescu *et al.*, 1965, 1968). The present study shows that immune parathyroiditis in rats can also be produced by passive immunisation. This was possible only with antiserum *A* according to the species and organ specificity of this antiserum as tested by the Ouchterlony method. However, no more than 3 out of the 15 rats of the test group A′ developed a marked parathyroiditis, whereas the rest showed only a sparse lymphocytic infiltration of PTG. These individual differences may be due to two reasons. On the one hand, it can be assumed that the resorption of the antibodies from the peritoneal cavity after the intraperitoneal injections was irregular and insufficient. On the other hand, the rats themselves could have developed antibodies against the rabbit-γ-globulin, by means of which the PTG antibodies could be absorbed and inactivated.

The inflammatory infiltrate showed the criteria of an immune reaction of the acute type as well as of the delayed type. The neutrophil and eosinophilic leukocytes refer to an immune reaction of the acute type, which was caused by the passive transmission of the humoral antibodies existing in the antiserum. The

lymphocytes, which were predominating after 50 days of experiment, as well as the plasma cells indicate an immune reaction of the delayed type most probably as a secondary effect of antigen-antibodies complexes.

In the experiments of Lupulescu *et al.* (1965, 1968) with active isologous immunisation the animals had a hypoparathyroidism in addition to the parathyroiditis. In our rats, by contrast, serum calcium and serum phosphorus were within the normal range even if a marked parathyroiditis was developed. Obviously, the remaining not degenerated parathyroid cells were able to compensate the loss of parenchyma caused by inflammation. This is supported by their activated cytoplasm rich in organelles. In accordance with this Klöppel *et al.* (1972) suppose that in experimental immune insulitis (after immunisation with insulin) the hyperglycemia is due to the hormone binding antibodies and not due to the degree of the inflammation.

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