Histological and Histochemical Changes of the Parathyroid Gland by the Injection of Parathormone in the Rat

By

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Introduction

Regarding experimental works on the effect of parathyroid hormone injection, the majority of investigators have exclusively studied the changes in bone, kidney and calcium and inorganic phosphorus in serum of some laboratory animals under the condition of hypoparathyroidism. Some of the reports, however, have noted functional changes of the parathyroid glands under a similar condition. Bodansky and Jaffe (1931) reported involution and atrophy of the parathyroid glands of guinea-pigs; Wider, Higgins and Sheard (1934) found functional involution of the hen parathyroid gland; Burrows (1938) observed that the injection of parathyroid extract into rats led to hypoplasia of the glands; De Robertis (1940) showed a diminution of the cellular volume of the glands in rats, while Zawistowski (1963) histochemically noticed inhibition of the glandular function by the injection of parathyroid hormone into rats. All these reports indicated that parathyroid extract caused hypofunction of the parathyroid glands.

In this study the morphological changes of the rat parathyroid glands under a hypofunctional state caused by the injection of parathormone were examined by the aid of a series of histochemical methods.

In the course of previous investigations at our laboratory (Hara and Yamada 1963 and 1964; Hara, Yamada and Hotta 1963; Hotta 1965 and 1966; and Yamada 1963 and 1964), it has been assumed that certain types of protein granules may be closely associated with the physiological function of the parathyroid cell. Such an assumption was supported by the findings that certain types
of protein granules increase in amount and stainability in the hyper-functional state of the rat parathyroid glands caused by bilateral nephrectomy (Hara, Yamada and Hotta 1963) and pregnancy and lactation (Hotta 1965), while on the contrary, these granules decrease in amount and stainability in the hypofunctional state of the rat glands caused by experimental weaning (Hotta 1966).

In the course of this experiment, cytoplasmic RNA and cytoplasmic glycogen in the parathyroid glands were simultaneously examined. These intracellular properties have been considered to be an important indicator of cytophysiological function of the parathyroid cells (Petersen 1903; Sundberg 1924; Baker 1945; Wymouth 1957; Lever 1958; Hara, Furuta, Murata and Yang 1959; Isono and Isono 1959; Fujii 1960; Hara, Yamada and Hotta 1963; Zawistowski 1963; and Hotta 1965 and 1966).

In this study the authors attempted to investigate more fully the morphological changes and true cytophysiological significance of these intracellular properties in the parathyroid cells under the hypofunctional state caused by injection of parathormone.

Materials and Methods

Twenty-eight non-pregnant adult female rats of the Wistar strain were employed in this study, and ten non-pregnant adult female rats of the same strain were used as control animals. They were bred with Oriental (NMF) Chow and water, and kept in an air-conditioned breeding chamber maintained at a temperature of 20 ± 2°C. Parathormone (Eli Lilly and Co.) was injected subcutaneously twice daily, in a dose of 150 U. S. P. parathyroid units (total daily dose, 300 units) on 2, 3 and 4 successive days. On each day the treated animals were killed under ether anesthesia 3 hours after the final injection of parathormone. Accordingly, five groups were prepared for the experiments; namely, a control and four groups injected with total doses of 300, 600, 900 and 1200 units of parathormone respectively. In the last group, unfortunately, the treated animals died, excepting 2, with overdosage phenomena such as vomiting, diarrhea and extensive metastatic calcification in the soft tissues.

Parathyroid glands with some neighboring organs such as thyroid and trachea were excised and fixed in Carnoy’s fluid and trichloroacetic acid alcohol (1 per cent trichloroacetic acid in 80 per cent ethanol) at room temperature for periods varying from 1 to 24 hours.
The tissues were blocked in paraffin and sections were cut at a thickness of 6 to 8 μ. Histological and histochemical staining methods employed were as follows: hematoxylin-eosin for the observation of general structure, coupled tetrazonium (Pearse 1960) for the demonstration of proteins in general, 2, 2'-dihydroxy-6, 6'-dinaphthyl disulfide (DDD) diazo blue B (Barrett and Seligman 1952) for the detection of proteins and amino acids with reactive sulfhydryl groups, 2-hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B (Barrett and Seligman 1958) for the visualization of proteins and amino acids with reactive carboxyl groups, periodic acid-Schiff (PAS) (McManus 1948) with and without prior amylase (preparation of Ueda Chemical Works, Osaka, Japan; 2 mg/ml in citric buffer at 37°C for 1 hour) digestion for recognition of glycogen, and methyl-green pyronin (modification of Brachet's method 1940) with and without previous ribonuclease (preparation of Sigma Chemical Works, St Louis, U.S.A.; 0.01 mg/ml in citric buffer at 65°C for 1 hour) treatment for discernment of cytoplasmic RNA. In some sections stained with DDD diazo blue B prior reduction of protein bound disulfide groups with thioglycolic acid (5.0 M thioglycolic acid adjusted to pH 8.0 by addition of 0.1 N NaOH at 50°C for 1 hour) was also conducted as described by Barrett and Seligman (1954).

Observations

I. Histological observation

In the first group injected with 300 units of parathormone, the morphological changes in volume and stainability of the cytoplasm and nucleus of the parenchymal cells are hardly seen as compared with the control group. The parenchymal cells with cytoplasm rather faintly stained with eosin are polygonal or cuboidal in shape and usually uninucleate (Fig. 1). In the second group injected with 600 units of parathormone, the morphological appearances of the parenchymal cells are generally similar to those of the control or the first group. In the parenchyma, however, cells with faintly stained cytoplasm and a diminished, intensely stained nucleus are occasionally observed. In the third group injected with 900 units of parathormone, cells with diminished, faintly or moderately stained cytoplasm and intensely stained nucleus are more numerous than in the second group (Fig. 2). In the fourth group injected with 1200 units of parathormone, the above-mentioned morphological changes are marked
in the greater part of the parenchymal cells. Sometimes, vacuolation or hydropic appearances are observed, and rarely the nucleus is not differentiated from the cytoplasm.

Throughout all experimental stages, however, noticeable multiplication or reduction of the interstitial connective tissue is not recognized.

II. Histochemical observation

1. Coupled tetrazonium reactive proteins and amino acids:

In the first group injected with 300 units of parathormone, the cytoplasm of the parenchymal cells contains numerous tetrazonium reactive fine granules. The amount, stainability and distribution pattern of the granules are similar to those of the controls. The distribution pattern is generally diffuse throughout the cytoplasm and appearance of granules in the extracellular spaces hardly occurs (Fig. 3). In the second group injected with 600 units of parathormone, parenchymal cells which show a decrease in amount of reactive granules are recognized here and there. In some cells the granules are hardly seen, and others contain only a small amount of the faintly stained granules. In the majority of parenchymal cells, however, moderately stained granules are abundantly noted in the cytoplasm. In the third group injected with 900 units of parathormone, such decrease in amount and stainability of the granules is intensified throughout the majority of cells (Fig. 4). Within the diminished cytoplasm the granules are remarkably decreased in amount and stainability or disappear completely. These granules are sparsely scattered throughout the narrow cytoplasm of the parenchymal cell. When 1200 units of parathormone are injected into rats, those granules are more markedly decreased. But the tetrazonium reaction of the nucleus does not show any significant changes by the injection of parathormone.

2. 2,2'-Dihydroxy-6, 6'-dinaphthyl disulfide (DDD) diazo blue B reactive proteins and amino acids:

In the first experimental group the amount of DDD diazo blue B reactive granules is decreased in the cytoplasm of some parenchymal cells. In the control group the cytoplasm of the parenchymal cells contains abundant reactive granules with moderate staining intensity and these granules often show peripheral accumulation in the cytoplasm (Fig. 5). These granules are also scattered in the extracellular spaces. In the parathyroid glands of the first experimental group, the parenchymal cells with these peripheral accumulation of
granules are decreased in number and the coloration of granules is also less intense. In the second experimental group, the amount and stainability of DDD diazo blue B reactive granules are decreased more noticeably than in the former group, and therefore the cytoplasm of the numerous parenchymal cells is poorly stained in this experimental group, the less intensely stained granules are merely scattered throughout the narrow cytoplasm (Fig. 6). The accumulation of the granules at the peripheral region of the cytoplasm is decreased, and the presence of granules in the extracellular spaces is hardly seen. In the group injected with 1200 units of parathormone, the fourth experimental group, the above-mentioned changes of the granules in the cytoplasm are more and more intensified. In all experimental animals the nuclear DDD diazo blue B reaction of the parenchymal cells is similar to that in the controls.

3. 2-Hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B reactive proteins and amino acids:

The property of the HNAH diazo blue B reactive granules in the cytoplasm of the parenchymal cells of the first experimental group is in general similar to that of the control animals. The parenchymal cells loaded with abundant intensely reactive granules are seen throughout the parenchyma of the control animals (Fig. 7). In the parenchyma peripheral accumulations of the granules are also seen here and there. In some parts of the parenchyma of the parathyroid of this experimental group, however, a tendency for reduction in amount and stainability of the HNAH diazo blue B reactive granules is recognized. In the second experimental group, the reduction of reactive granules in amount and staining intensity in the cytoplasm is more intensified than in the former experimental group. In the third experimental group, weakly reactive granules are seen scattered throughout the narrow cytoplasm in the majority of parathyroid cells (Fig. 8). Peripheral accumulation of the granules in the cytoplasm is hardly seen. In the fourth experimental group, the above-mentioned changes in HNAH diazo blue B reactive granules are more frequently observed. In all experimental animals the nuclear HNAH diazo blue B reaction of the parenchymal cells is essentially similar to that in the controls.

4. Glycogen:

PAS reaction, with prior digestion by β-amylase, in the parenchymal cells of the control animals differs from cell to cell. In general the majority of parenchymal cells either lack the glycogen granules or contain only a small amount (Fig. 9). In the first ex-
Experimental group, the polysaccharide in the cytoplasm of parenchymal cells generally shows a similar tendency as in the control animals. When 600 units of parathormone are injected into rats the glycogen granules differ in amount from cell to cell. In the third experimental group, parenchymal cells which contain abundant glycogen granules in the cytoplasm are more increased in number than in the former group (Fig. 10). But cells which contain no glycogen granules are occasionally found. In the fourth experimental group, the majority of the parenchymal cells contain a large amount of glycogen in the cytoplasm. In some parts, however, the parenchymal cells devoid of polysaccharide granules are seen.

5. Ribonucleic acid (RNA):

In the control animals a RN-ase digestible pyroninophilic substance, RNA, is seen abundantly in the cytoplasm of the parathyroid cells. The substance in the cytoplasm exhibits either a granular or diffuse appearance with moderate or intense coloration (Fig. 11). In the first experimental group, the chemocytological appearance of RNA in the cytoplasm is essentially similar to that in the controls. In the second experimental group, the parenchymal cells which contain faintly stained diffuse pyroninophilic substance in the cytoplasm are increased in number. Moreover, parenchymal cells devoid of pyroninophilia are recognized here and there. In the third experimental group, the majority of parenchymal cells contain faintly stained diffuse pyroninophilic substance throughout the narrow cytoplasm (Fig. 12). The parenchymal cells devoid of pyroninophilic substance in the cytoplasm are more increased in number than in the former group. In the fourth experimental group injected with 1200 units of parathormone, parenchymal cells with cytoplasm showing faint pyroninophilia are remarkably increased in number.

Discussion

I. Histological appearances

That the administration of parathyroid extract, parathormone, causes hypofunction of the parathyroid gland has clearly been demonstrated by Bodansky and Jaffe (1931), Wider, Higgins and Sheard (1934), Burrows (1938), Derobertis (1940), Weymouth (1957) and Zawistowski (1963). On the other hand, hypofunction of the parathyroid cells caused by a high calcium concentration in the diet or in tissue culture-fluid has been found to occur by Engfeldt, Hjertquist and Strandth (1954) and
Au and Raisz (1965) or Raisz (1963) and Roth and Raisz (1964 and 1966).

In this study the authors also observed that a hypofunctional state of the parathyroid gland was caused by the injection of parathormone in adult rats. In the experimental rats the parenchymal cells were generally decreased in size, and the nuclei often suggested existence of pyknosis. These findings are in accord with those of the above-mentioned investigators. However, these findings are contrary to the results obtained in the parathyroid glands of rats during pregnancy and lactation (Hotta 1965), of nephrectomized rats (Hara, Yamada and Hotta (1963), of animals fed on low calcium diet (Sinclair 1941; Stoerk and Carnes 1945; Engfeldt, Hjertquist and Strandth 1954 and Au and Raisz 1965) and in parathyroid cells cultured in low calcium medium (Raisz 1963; Roth 1965; and Roth and Raisz 1964 and 1966).

De Robertis (1940) has reported that the mitochondria in the rat parathyroid cells were reduced or spread and broken by the administration of parathyroid extract, and Roth and Raisz (1964 and 1966) have recognized that in rat parathyroid cells cultured in high calcium medium the cell organelles became smaller and more indistinct, the secretory granules decreased in number and distributed dispersedly in the cytoplasm and the nucleus showed pyknotic change or vacuolation.

According to De Robertis (1940) wider connective tissue septa were seen in the parenchyma of the parathyroid glands of rats injected with a single dose of parathormone. However, the authors did not recognize a difference to occur in the interstitial connective tissue of the experimental groups and the controls.

II. Histochemical appearances.

1. Coupled tetrazonium reactive proteins and amino acids:

In previous histochemical studies of the parathyroid glands one of the authors, Hotta (1965 and 1966), commented, with reference to the works of Hara and Yamada (1964) and Trier (1958), that the tetrazonium reactive granules within the parathyroid cells should present mostly a pattern of mitochondrial protein.

In this experimental study the reactive granules in the parathyroid cells were decreased in amount and stainability or disappeared completely in accordance with the increase in dose of parathormone. In a previous study on experimental weaning, one of the authors, Hotta (1966), observed a tendency for decrease in quantity and
stainability of these granules in the cytoplasm with the progress of experimental weaning, and commented that this fact might be a reflection of the decline in cellular secretory activity caused by the weaning. The above-mentioned findings in these granules obtained in this study may support this and be in accord with the results obtained by De Robertis (1940) in the rat parathyroid cells injected with parathormone and by Roth and Raisz (1964 and 1966) in rat parathyroid cells cultured in high calcium medium.

The authors recognized that the tetrazonium reactive granules in the rat parathyroid cells were decreased in amount and stainability when the secretory activity was suppressed by the injection of parathormone.

2. 2,2'-Dihydroxy-6,6'-dinaphthyl disulfide (DDD) diazo blue B reactive proteins and amino acids:

The correlation existing between the biological activity of parathyroid hormone and cysteine, an amino acid with the sulphydryl group, pointed out by Rasmussen (1958 and 1961), was initially considered by Hara and Yamada (1962) and Yamada (1963 and 1964) to suggest that DDD diazo blue B reactive granules in the parathyroid cells are in entity closely associated with the cellular reaction of the active principles. The appearance of reactive granules in normal parathyroid cells of several species of animals has been previously described in detail by Hara and Yamada (1962 and 1964) and Yamada (1963 and 1964). In our laboratory the increase in amount and reactivity of the DDD diazo blue B reactive granules in the parathyroid cells was recognized in the hyperfunctional state of the rat parathyroid gland (Hara, Yamada and Hotta 1963; Hotta 1965).

In this study a tendency to decrease in amount and intensity of staining of the intra- and extracellular DDD diazo blue B reactive granules was found in accordance with the increase in dose of parathormone. In the fourth experimental group injected with 1200 units of parathormone the above tendency was accelerated.

Zawistowski (1963) has observed histochemically that the function of the parathyroid gland of the rat injected with parathyroid hormone was depressed and the activity of SH groups dropped almost to zero. By the aid of electron microscopic observation Roth and Raisz (1964 and 1966) reported that in parathyroid cells cultured in high calcium medium the secretory granules become rare but in cells cultured in low calcium medium the granules are abundant.
In this study the authors confirmed that the DDD diazo blue B reactive granules in the parathyroid cells were decreased in amount and stainability when the function of the parathyroid gland was depressed by the injection of parathormone. This finding is not inconsistent with the above-mentioned results reported by Zawistowski (1963) and Roth and Raiz (1964 and 1966). This fact may be supported further by the following observations of the morphological changes in cytoplasmic glycogen and ribonucleic acid within the parathyroid cells in the hypofunctional state.

3. 2-Hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B reactive proteins and amino acids:

The presence of HNAH diazo blue B reactive granules in the rat parathyroid cells has been initially demonstrated by Hara and Yamada (1962) and it was assumed that the granules may be a morphological reflection of mitochondrial proteins in the cells from the chemocytological observations on the parathyroid glands of the bilaterally nephrectomized rats (Hara, Yamada and Hotta 1963) and on the oxyphil cells of the monkey parathyroid glands (Hara and Yamada 1964). This assumption may be valid, because these reactive granules with intense stainability abundantly appeared in the parenchymal cells when the mitochondria were increased in number by bilateral nephrectomy (Baker 1945) and in oxyphil cell in which abundant mitochondria were electron-microscopically recognized (Trier 1958).

In this experimental study the authors recognized that the HNAH diazo blue B reactive granules in the parathyroid cells showed a tendency to decrease in amount and stainability in accordance with increase in dose of parathormone. In rats injected with 900 units of parathormone the reactive granules with faint coloration were only scattered throughout the cytoplasm of almost all parathyroid cells, and peripheral accumulation of the granules was hardly seen.

The authors confirmed that the HNAH diazo blue B reactive granules may reflect cellular activity of the parathyroid gland, because the reactive granules were increased in amount and stainability in the hyperfunctional state of the gland caused by bilateral nephrectomy (Hara, Yamada and Hotta 1963), pregnancy and lactation (Hotta 1965), while on the other hand, the granules were decreased in amount and stainability in the hypofunctional state of the gland caused by injection of parathormone.

4. Glycogen:

Hara, Yamada and Hotta (1963), Zawistowski (1963)
confirmed the loss of glycogen in the rat parathyroid cells after experimental stimulation and concluded that intracellular glycogen is possibly utilized as energy source for the maintenance of secretory activity of the parathyroid cells.

In the present study, the authors recognized that the glycogen content in the parathyroid cells was increased in quantity in parallel with increase in dose of parathormone.

In the hyperfunctional state of rat parathyroid glands caused by the bilateral nephrectomy (Hara, Yamada and Hotta 1963) and pregnancy and lactation (Hotta 1965) or in parathyroid cells cultured in low calcium medium the intracellular glycogen content showed an appreciable tendency to decline (Roth and Raisz 1964 and 1966). On the contrary, in the hypofunctional state of rat parathyroid glands caused by the injection of parathormone and experimental weaning (Hotta 1966), the glycogen content in the parenchymal cells showed a remarkable tendency to increase, and this tendency was electron-microscopically recognized in parathyroid cells cultured in high calcium medium (Roth and Raisz 1964 and 1966).

From the above quoted works and the findings of the present study, it may be concluded that the glycogen content of parenchymal cells is possibly utilized as energy source for the secretory activity of the parathyroid glands.

5. Ribonucleic acid (RNA):

The cytophysiological significance of cytoplasmic RNA in parathyroid cells has been studied by Weymouth (1957), Hara, Yamada and Hotta (1963) and Hotta (1965 and 1966). These authors found that cytoplasmic RNA in parathyroid cells stimulated by bilateral nephrectomy, pregnancy and lactation is much more increased in amount than in normal parathyroid cells. One of the authors, Hotta (1966), also pointed out that the quantity of cytoplasmic RNA in the rat parathyroid cells shows a tendency to decrease in parallel with progress of the experimental weaning period.

In the present study the authors recognized that the pyroninophilic substance in the rat parathyroid cells shows a tendency to decrease in amount and stainability in accordance with increase in dose of parathormone.

Weymouth (1957) and Zawistowski (1963) have also noted the similar appearance of the pyroninophilic substance in parathyroid cells inhibited by injection of parathyroid hormone.
Roth and Raisz (1964 and 1966) have also electron-microscopically proved that in parathyroid cells cultured in high calcium medium the dispersed granular endoplasmic reticulum and the dispersed and aggregated ribonucleoprotein particles can be seen, but in cells cultured in low calcium medium the granular endoplasmic reticulum was largely composed of flattened sacs and ribonucleoprotein particles were largely present as spirals and aggregated.

From the above-mentioned facts that the amount and reactivity of the cytoplasmic RNA in the parathyroid cells were increased in the hyperfunctional state and decreased in the hypofunctional state, it may be concluded that the cytoplasmic RNA probably reflects protein synthesis—parathormone synthesis—in the parathyroid cells.

Summary

Histological and histochemical observations were made of the parathyroid glands of rats injected with 300, 600, 900 and 1200 units of Parathormone (Eli Lilly and Co.) respectively, and the following results were obtained.

1. In parallel with increase in dose of the extract, parathyroid cells showed remarkable regression of the cytoplasm and nucleus.

2. The tetrazonium reactive granules were decreased in amount and reactivity in parallel with increase in dose of the extract.

3. The amount and staining intensity of the DDD diazo blue B reactive granules showed remarkable decrease in amount and stainability in parallel with increase in dose of the extract.

4. The HNAH diazo blue B reactive granules were decreased in amount and stainability in parallel with dose of the extract.

5. The glycogen content of the parathyroid cells were increased in amount in parallel with increase in dose of the extract.

6. Cytoplasmic RNA in parathyroid cells showed conspicuous decrease in amount and stainability in parallel with increase in dose of the extract.

7. From these data obtained in the hypofunctional state caused by the injection of parathormone and previously reported results obtained in the hyperfunctional state caused by bilateral nephrectomy and pregnancy and lactation, it is believed that these three types of protein granules are closely associated with the secretory activity of the parathyroid cells.
References


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Explanation of figures

All micrographs magnified 1500 times.

Fig. 1. Parathyroid gland of a control rat. The parenchymal cells are polygonal in shape and contain a relatively large ovoid nucleus. Carnoy's fixation, Hansen's hematoxylin eosin.

Fig. 2. Parathyroid gland of a rat injected with 900 units of parathormone. The parenchymal cells decrease in size and contain a diminished nucleus with intense stainability. Carnoy's fixation, Hansen's hematoxylin eosin.

Fig. 3. Parathyroid gland of a control rat. The cytoplasm of parenchymal cells contain moderate or intense tetrazonium reactive fine granules. Carnoy's fixation, Coupled tetrazonium.

Fig. 4. Parathyroid gland of a rat injected with 900 units of parathormone. The tetrazonium reactive fine granules in the cytoplasm of parenchymal cells are
Plate 1

J. Hara and T. Hotta