THE USE OF DYNAMIC FUNCTIONAL TESTS IN THE CLINICAL EVALUATION OF PARATHYROID DISORDERS

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The remarkable diagnostic difficulty in the parathyroid disorders has induced many authors to study tests of dynamic investigation of this gland in order to detect functional changes.

The most widely employed dynamic investigation tests of the parathyroid may be schematically considered as divided into: stimulation tests, inhibition tests and calcium turnover determination tests. The prednisone test (De Sèze et al., 1961), the mannitol test (Fourman et al., 1960), the calciferol test and the parathormone test (Ellsworth and Howard, 1934) are not widely used in the chemical practice, because of the limited value of the information which can be obtained through them. The assay of parathormone is of high importance, but its application in the clinical practice has been restricted because of the technical difficulties involved (Davies, 1958).

The authors have, therefore, limited this study to the investigation of parathyroid stimulation and inhibition tests, as well as of the test based on the determination of calcium turnover. The authors will refer their considerations on the above tests, based on their clinical experience resulting from the study of over 40 cases (Ghiringhelli et al., 1963a; Ghiringhelli et al., 1963b; Ghiringhelli and Respighi, 1963c). As typical example, the authors will report the results obtained with 3 patients, affected alterations of parathyroid: 2 of these patients, affected by hypoparathyroidism, were characterized by a tetanic syndrome, which, in one of the cases, was generated after a thyroidectomy. The hypoparathyroid patient was affected by parathyroid adenoma.

I. Parathyroid stimulation test

The stimulation is accomplished either decreasing the calcium level in the blood or increasing the inorganic phosphorus level. With the tests using the first
way of stimulation, it is possible to check the calcemia variations; while with the tests using the latter one, it is possible to follow the variations of phosphate-mia and tubular reabsorption of phosphorus.

The hypocalcemia is generally determined either with infusion of calcium-chelating agents or providing a low phosphorus intake, in association with agents to prevent its intestinal absorption.

The sodium versenate (Disodium-ethylendiamin-tetracetate) is universally used as a calcium-chelating agent because of its low toxicity, of its marked activity in producing hypocalcemia and in consideration of its rapid elimination through the kidney (Kaiser and Pensold, 1959; Albach, 1960).

Versenate is given intravenously, diluted in a 5 % glucose in water solution over a 2-hour period. The calcium serum level is evaluated before, and at different intervals of time from the moment the intravenous injection is over.

As far as the dosage of versenate is concerned, there is disagreement among the various authors. The amount of 70 mg/kg recommended by Kaiser and Pensold (1959) is believed to be too high according to Klotz and Clément (1961) who feel that such a dosage may induce, even in normal subjects, a decreasing of the calcemia similar to that induced in hypoparathyroid patients. According to Albach (1960), the quantity of versenate and glucose solution to be given should be calculated, in each single case, in proportion to the volume of the circulating blood and to the total quantity of calcium ion.

Regarding the interpretation of the results, Kaiser and Pensold (1959) and Albach (1960) believe that the evaluation of the calcemia after 12 hrs. is a certain index of the grade of the function of the parathyroid. Klotz and Clément (1961), on the other hand, think that the serum level of the calcium obtained 1 hr. after the infusion is more important; according to these two authors, a value lower than 92 % of the initial figure stands for a hypofunction of the parathyroid.

The present observation, performed through the original technique by Kaiser and Pensold (1959), allow to consider as significant the entire proceeding of the calcemic curve during the Kaiser-Pensold test. In Figure 1, where the results in 2 hypoparathyroid and 1 hyperparathyroid case compared with the results in 6 normal controls, it is observed that the entire calcemic curve in the hypoparathyroid patient is definitely lower than that of the controls. Of a particularly remarkable interest is the calcemic value of 7.4 mg observed at the end of the test in this case. Such an observation makes it possible to consider the parathyroid function in the patient as seriously impaired. According to Copp's experiments in parathyroidectomized animals (Copp, 1957), the rise of the
calcium serum level, after a sodium versenate intravenous infusion, does not exceed the value of 7 mg. The action of the parathormone, which is responsible for the ultimate raising of the calcemia in normal controls, according to Copp would be detectable only above this value. The rise of the serum calcium level up to 7 mg would be due, in effect, solely to the establishment of an equilibrium in the calcium exchange between the labile fraction of the bone and the extracellular fluids. This rise would depend mainly on the exchangeable calcium available in bone and on the bone blood-flow (Copp, 1957).

Checking the calcemia during the Kaiser-Pensold test seems to be of a great help in the hyperfunction of the parathyroid too. In a female patient (Fig. 1) not only a lesser decrease of the calcemia compared to the controls, but also a more rapid calcemia recovery were observed to the point that after 12 hrs. the calcemia was sensibly above normal.

The low-calcium diet associated with agents inhibiting the intestinal reabsorption of this electrolyte has been less used in diagnosing parathyroid disorders. However, from the data by Smith et al. (1960), it is apparent that such a diet, associated to sodium phytate which is decreasing the calcium intestinal absorption (Mellanby), may cause in hypoparathyroidism a fall on the blood level of this electrolyte in comparison with normal controls.
The stimulation of the parathyroid through the increase of the inorganic phosphorus level is obtained by supplying intravenously, or preferably per os, a load of inorganic phosphorus salts. The technique of performance of this test is not well established, but varies according to different authors (Crawford et al., 1950; Harrison and Harrison, 1941; Klein and Gow, 1953; Harrison and Klein, 1953).

The authors usually give a subject kept on a constant intake of calcium (200 mg per day) and phosphorus (700 mg per day), a progressive load of this latter electrolyte as a solution of monobasic potassium phosphate and dibasic sodium phosphate which, after 6 days from the beginning of the test, reaches the maximum figure of 2 g per day.

Whatever technique is used to accomplish a phosphorus overload, the various results agree, in that they point out, in the hypofunction of the parathyroid, the following factors: 1) Hyperphosphatemia not depending on the variations of the calcemia, 2) A persistently high figure of the tubular reabsorption and glomerular filtration of phosphorus (TRP/GEP) ratio, that is, on the contrary, decreased in normal controls.

This is clearly shown in Figure 2 where are reported the results in a case of hypoparathyroidism and in a normal control, who have been subjected to a phosphorus overload per os.

II. Parathyroid inhibition test

This inhibition is accomplished through a calcium overload per os, or through a low phosphorus diet. The techniques used by the various authors are different as to the quantity of this electrolyte to be given (from 180 mg recommended by Goldsmith et al., 1962), up to about 1100 mg recommended by Klotz and Clément (1961), and as to the duration of the infusion period, the type of diet, the time for the urine and blood connection and, finally, for the parameters taken into consideration (Klotz and Clément, 1961; Goldsmith et al., 1962; Chambers et al., 1956; Spencer-Laszlo et al., 1961; Moore and Smith, 1963).

The authors supply to the subject, who should be fasting all through the duration of the test, a medium dose (500~600 mg of calcium diluted in 500 cc of 5% glucose in water solution over a 2-hour period) and they believe that the disagreement in the results report in the literature is due to either an insufficient or an excessive dosage. This test is used exclusively in diagnosing hypoparathyroidism; therefore, in agreement with Chambers et al. (1956), Courvoisier (1959) and other authors, the authors are of the opinion that only the study of the calciuria
Fig. 2. Calcium and inorganic phosphorus serum concentrations and TRP/GFP ratio during phosphorus overloading in a normal subject and in a hypoparathyroid patient.

The dark area is for the quantity of phosphorus supplied per os besides the amount already contained in the diet.
variation is significant, and check it before the test and at 2, 6 and 8 hrs. since its starting. The calciuria in parathyroid insufficiently shows a lesser increase than in normal controls. Such a course is clearly shown in the Figure 3 in which the values of the calciuria, reported from 2 hypoparathyroid patients, are compared with the results found in normal subjects. Howard et al. (1953), Courvoisier (1959), Pronove and Bartter (1961), Spencer-Laszlo et al. (1961), who used this test also in the diagnosis of hyperparathyroidism, have found a lesser increase of the phosphatemia and a lesser decrease of the phosphaturia in comparison to normal controls.

Fig. 3. Calciuria data during the calcium overloading test in controls (average of 5 cases) and in 2 hypoparathyroid (average of 2 cases)

The calcium infusion ends after 2 hrs.

It is believed that the use of such a test in subjects affected by possible hyperparathyroidism is dangerous because such patient have an elevated calciuria and they are, therefore, predisposed to a precipitation of calcium salts in the urinary tract.

Also, the techniques of the parathyroid inhibition through a low-phosphorus diet, used by several investigators, are remarkably different because 1) of the calcium and phosphorus contained in the diet, 2) of the duration of the test, 3) of the different time in checking the results, and 4) of the diet of substances pre-
venting the phosphorus intestinal absorption (Chambers et al., 1956; Pronove et al., 1961). The most studied parameters, during the performance of the test, are: phosphatemia, calcemia, calciuria and the phosphorus tubular reabsorption; the variation of the phosphaturia has been shown to be of little value.

To the controls a daily diet containing 500 mg phosphorus and 200 mg calcium is supplied; the phosphorus absorption is reduced by 1.5 g of aluminum hydroxide daily. The total calciuria and phosphaturia are checked daily and the calcemia, phosphatemia and the TRP/GFP ratio every other day. The phosphorus deprivation test is used only to diagnose hyperparathyroidism; in this ailment, it is found that: 1) Phosphatemia, which in controls decrease to levels lower than normal, does not show a significant variation in hyperparathyroid patients with low phosphatemia, in those having a normal phosphatemia, decreases insteads of definitely pathological levels (Chambers et al., 1956); 2) Calcemia, which is significantly modified in normal subjects, is slightly increased in hyperparathyroidism (Chambers et al., 1956; Pronove et al., 1961); 3) The TRP/GFP relation increases progressively in hyperparathyroid patients up to the end of the test, as opposed to normal cases in which the increase is limited to the first days of the test. According to Chambers et al., (1956), the particular difference between normal and hyperparathyroidic subjects would be most evident from the 3rd to the 5th day from the beginning of the test. 4) Calciuria increases more evidently (above 250 mg/day according to Pronove et al., 1961) in hyperparathyroidism than in normal condition.

What is said above agrees fully with some of the data reported in Figure 4 where the values of calcemia, phosphatemia and TRP/GFP ratio in a hyperparathyroid patient are compared with a normal subject during the phosphorus deprivation test. To complement the data shown in Figure 4, it may be added that, according to the observations of Pronove and co-workers (1961), the calciuria in this same patient has been fluctuating, during the performance of the test, around values of 250 mg per day, while in the controls it has never exceeded the figure of 170 mg per day. The values obtained in the present cases are different from the data recorded in the literature only because of the action of the calcemia, which, in fact, underwent a limited decrease in the first days of the test; at the present status of the present investigation, the reason for such a reaction is unknown.

III. Calcium turnover determination tests
It is performed supplying a strontium salt or a radioactive isotope of it (Har- 
rison et al., 1960; Rich et al., 1961) or radioactive isotopes of calcium (Milhaud 
and Aubert, 1958; Doering, 1963). Using strontium and its isotopes makes it pos-
sible to evaluate the exchangeable calcium pool and its bone fixation rate; using 
radioactive isotopes, the authors can also evaluate the urinary and fecal elimina-
tion rate of this element. Until now the authors have been using for their obser-
vations the Milhaud and Aubert (1958) method, consisting in intravenous supply 
of radioactive calcium. The calcium exchanges is evaluated by dosing in the 6 days 
consecutive to the administration of the 6µc of Ca^{45} instead of Ca^{47} em-

ployed (Doering, 1963; Fraser, 1960), because the former has a half life remarkably longer than the latter (153 days instead of 4.7 days) and avoids, therefore, the demand for a continuous supply; on the other hand, the dose of Ca45 given by us is not significant and, anyway, it is definitely lower that the maximum dose permitted (Fossati et al., 1956).

In Table 1, it was reported that the results of the calcium turnover obtained

<table>
<thead>
<tr>
<th></th>
<th>Pool of the exchangeable Ca (g)</th>
<th>Bone fixation rate (mg/24 hrs.)</th>
<th>Urinary elimination rate (mg/24 hrs.)</th>
<th>Fecal elimination rate (mg/24 hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.P. ♂ 25 normal</td>
<td>3.26</td>
<td>663</td>
<td>86</td>
<td>110</td>
</tr>
<tr>
<td>B.M. ♂ 26 normal</td>
<td>4.24</td>
<td>1310</td>
<td>144</td>
<td>139</td>
</tr>
<tr>
<td>D.A. ♂ 31 hypoparathyroid</td>
<td>2.79</td>
<td>475</td>
<td>228</td>
<td>48</td>
</tr>
<tr>
<td>A.M. ♂ 51 hypoparathyroid</td>
<td>2.35</td>
<td>636</td>
<td>63</td>
<td>41</td>
</tr>
<tr>
<td>P.G. ♂ 24 hyperparathyroid</td>
<td>4.25</td>
<td>1038</td>
<td>107</td>
<td>166</td>
</tr>
</tbody>
</table>

in 2 normal subjects, in 2 with hypoparathyroid and in 1 with hyperparathyroid function. The analysis of the data reported in this table and a comparison to the data reported in the literature, leads to the following remarks: (1) The figures of the present study are comparable to those found by most authors (Bauer et al. 1958; Heaney and Whedon, 1958; Dow et al., 1960; Bluhm et al., 1960) and are different only from the data by Milhaud and Aubert (1958), Milhaud et al. (1959), and by Oeff et al. (1962), particularly as far as the exchangeable calcium pool and the bone fixation rate are concerned. (2) The dispersion of the results is remarkable also in normal subjects. (3) The only phenomenon observed constantly is the decrease of the exchangeable calcium pool and of the bone fixation rate in hypoparathyroid patients in comparison to normal controls. According to these findings and to the fact that the calcium turnover is abnormal in other different diseases (Doering, 1963; Milhaud et al., 1959; Oeff et al., 1962), it is believed that the calcium turnover determination test, at least in this stage of the problem, is of little importance in diagnosing the parathyroid disorder.

SUMMARY

The authors’ observations about the use in the clinical practice of the most important biochemical tests of functional investigation of the parathyroid gland are reported here. In diagnosing hypoparathyroidism, the utility of the parathyroid stimulation test through sodium versenate and through phosphorus over-
load, and of the parathyroid inhibition test through calcium overload, is indicated. In diagnosing hyperparathyroidism, the stimulation test through ver-senate and the inhibition test through a low-phosphorus diet are recommended as well. It is excluded, up to this time, that the investigation with radioactive isotopes of the calcium turnover may be of any practical use in diagnosing parathyroid disorders.

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