A METHOD FOR EVALUATING AN EXPERIMENTAL ATHYREOSIS

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ABSTRACT

A micro-$^{131}$I-test is presented which allows an easy separation of completely surgically thyroidectomised experimental animals from animals with aberrant or residual thyroid tissue.

The application of the method is easy and not time-consuming: the total time spent on one animal is approximately 3 min. The method is based on the principle that the $^{131}$I storage is measured over the cervical region.

The application of this method gives the following advantages:

1) When performing studies with proven athyroid rats it is not necessary subsequently to demonstrate athyreosis (histologically, BMR, or PBI).

2) Time-consuming experiments with animals which are not definitely athyroid can be avoided.

3) The additional fractionated radio-iodine resection after surgical thyroidectomy, used by many authors to destroy residual thyroid tissue, becomes superfluous. Such a procedure takes 4 to 8 weeks.

4) The animals may be used after a 5-day-period. This excludes secondary changes like weight loss and disturbed development which have to be taken into consideration when using a radio-iodine resection.

Surgical thyroidectomy of experimental animals does not always lead to a complete athyreosis since sometimes as much as 3–5% of the thyroid tissue

This work was supported by grants from the Deutsche Forschungsgemeinschaft given to the Sonderforschungsbereich 29, »Embryonale Entwicklung und Differenzierung (Embryonal-Pharmakologie)«.

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may remain within the operation area which at least in part is able to compensate the hypothyreosis. Moreover, it has to be taken into account that some of the animals show aberrant thyroid tissue which cannot be removed by surgical means. Therefore, we developed a method which allows the separation of completely thyroidectomised animals from those which still preserve approximately 3–5% of the overall thyroid tissue after surgery. Such a method should be easy enough to be performed on a large number of animals and should be performed without taking any blood samples or affecting the animal before the experiment.

**METHODS**

**Animals**

Experiments were done with Wistar rats SW 69, weighing 200 ± 20 g and 60 ± 10 g. They were divided into four groups:

1) Control animals (partly sham operated, thyroid glands remaining intact, n = 11.

2) Completely thyroidectomised animals, n = 25.

3) Incompletely thyroidectomised animals: These included removal of the lobes, the isthmus of the thyroid remaining intact. Since the isthmus of the thyroid makes up about 3–5% of the total thyroid tissue, this group represents incompletely thyroidectomised animals, n = 12.

4) »Lugol« group: These animals received 1 ml Lugol’s solution twice a day 48 and 24 h before the test. This group represents animals the thyroid glands of which do not accept any ¹³¹I in the radio-iodine test thus supplying values of the »background«, n = 13.

We investigated whether the animals of group 3 can be differentiated from the completely operated group 2, i.e. whether the micro-radio-iodine test developed by us helps select incompletely operated from completely thyroidectomised animals.

**Thyroidectomy**

The animals were anaesthetised with 50 mg/kg hexobarbital given intraperitoneally 10 min before surgery and 25 mg/kg given iv immediately before surgery. Thyroidectomy was performed under the stereomicroscope. The thyroid gland can often be extirpated together with the capsule without causing much haemorrhage. Care has to be taken when separating the recurrent laryngeal nerve from the lobe. The best method available to us is to use two pairs of Du Mont-watchmaker’s forceps.

Animals which are to preserve 5% of their thyroid tissue (group 3) retain their isthmus with a width corresponding to the diameter of the trachea. – When completely thyroidectomised, 10–20% of the animals die after surgery, ½ of them following a bilateral paresis of the nervus recurrents. In the case of juvenile animals the survival rate is somewhat better. These animals are given 0.2% Ca++ dissolved in the drinking water.

**Micro-radio-iodine test**

One day after surgery the animals were given 25 µCi/kg Na¹³¹I iv. The measurement within the energy region of the 364 keV-photopeak was carried out for 1 min over the cervical region 2 min after the injection, the maximum counting rate being
looked for between tongue and sternum. It was performed with a NaI (I)-cryst.; its collimator was in direct contact with the cervical region (diameter of the collimator: 20 mm, dimension of the cryst.: 2" × 2"). The counting rate 2 min after the injection of $^{131}$I was taken as the initial value. At that time the impulse rate per $\mu$Ci did not vary significantly, either in the case of non-operated controls (522 ± 50), or in that of the completely operated animals (507 ± 97), the isthmus group (495 ± 72) or the Lugol group (602 ± 180). In order to be as far as possible independent of the injected activity and of the differing weights of the individual animals, the counting rates at different times after the injection of $^{131}$I are given as the ratio between the absolute counting rate obtained after a fixed time (e.g. 96 h) and of the values of
the absolute counting rates obtained 2 min after the injection of $^{131}$I. This ratio is called »relative counting rate« (RCR). The relative counting rate was measured over a 120 h period and a regression analysis was then performed*.

In some animals the overall retention of $^{131}$I was determined in a whole body counter.

RESULTS

Fig. 1 shows the time course of the relative counting rates (RCR) measured and the regression curves obtained with the animals of group 1 to 3. The following results have been obtained:

1. Controls: After 15 to 20 h the animals show a maximal $^{131}$I uptake in the thyroid gland. The time course of the RCR may be expressed as the difference of 2 exponential functions.

2. When compared with control animals the initial increase of radioactivity measured over the thyroid gland cannot be observed in all the treated groups. In both groups, the completely thyroidectomised (2) and the incompletely thyroidectomised group (3), the decrease in the RCR differs in the following 120 h. This difference becomes significant after 72 h ($P < 0.02$) or 96 h ($P < 0.001$).

If the RCR after 96 h is taken as 100% in controls, the corresponding values for the treated animals are: Incompletely thyroidectomised animals (group 3) 27 ± 11%; completely operated animals (group 2) 8 ± 1.5%.

Blocking the thyroid with Lugol's solution before the micro-radio-iodine test, gives a relative counting rate after 96 h of 3 ± 1.5%. These data could indicate that completely thyroidectomised animals still contain some residual thyroid tissue, since their RCR is above that of the Lugol-group. The next experiment (3), however, does not support this interpretation. Hence, it may be assumed that not only the thyroid but also the cervical region in the Lugol-group are occupied by unlabelled iodine thus diminishing the $^{131}$I kinetics in this region.

* We are indebted to Prof. Dr. P. Koepp for performing the regression analysis with a digital computer.
Time course of radioactivity measured over the cervical region in rats.
After an injection of 25 μCi/kg $^{131}$I the relative counting rate (RCR) decreases over the thyroid region faster in completely thyroidectomised rats than in those with remnants of thyroid tissue (isthmus animals). The difference becomes significant after 72 h and 96 h. The RCR is obtained by the counting rate (CR) measured after $t$ hours divided by the CR obtained after 2 min (RCR$_t$ = CR of $t$ hours / CR of 2 min). Controls are euthyroid animals.

Mathematically the time course of the RCR for both the completely operated animals and the incompletely operated animals is expressed by the sum of two exponential functions. The graphical illustration of the course of the regression functions makes it possible to determine at any time from 72 h after the injection of $^{131}$I onwards whether an experimental animal is completely operated or not.

3. Further evidence of an optimal thyroidectomy is obtained by the results of the following experiment: When comparing the counting rate in the cervical region of a sacrificed animal with the measurement after removal of the whole operation area including the trachea (in order to remove any possible thyroid remnants), the quotient of the counting rates has to be 1 when measuring completely operated animals (group 2) i.e. $1.02 \pm 0.35$ ($n = 14$). The animals
of group 3, the isthmus of which was not removed, showed a significantly
\( P = 0.001 \) higher quotient of the counting rates: \( 3.1 \pm 1.7 \) \( (n = 11) \). In
the case of non-operated control animals it is even higher, i.e. \( 26.6 \pm 3.8 \)
\( (n = 6) \).

4. In order to investigate the problem whether thyroid tissue can be re-
generated from remnant thyroid cells within 3 weeks, a micro-radio-iodine
test was performed with the same group, first 3 days and again 24 days after
thyroidectomy. The first 96 h RCR-value was about 0.13 (ideal RCR-value
0.12). Three weeks later this value had not changed significantly (0.15).

5. Group 2 cannot be differentiated from animals of group 3 by means
of whole body counting. This is because the \( ^{131} \)I activity of the whole body
highly exceeds the activity of the incompletely removed tissue in group 3.

6. A further criterion for a hypothyreosis of completely operated animals
was the weight gain. The weight of completely operated animals \( (n = 16) \)
increased in 8 weeks from 200 \pm 20 g to 205 \pm 23 g, in the controls \( (n = 16) \)
to 260 \pm 17 g \( (P < 0.001) \). The weight of thyroidectomised young – 60 g –
animals \( (n = 11) \) increased in 2 weeks to 65.8 \pm 9.7 g but in the controls
this was 89.3 \pm 10.1 g \( (P < 0.001) \).

**DISCUSSION**

Surgical thyroidectomy does not always guarantee a complete removal of the
thyroid tissue. It is, therefore, a generally accepted practice to perform a
subsequent fractionated radio-iodine resection (*Tata & Widnell 1966; Rudolph
et al. 1969; De Leo et al. 1969*).

This procedure has the disadvantage that during the radio-iodine resection
the healthy control animals still continue to develop. A correlation of the
experimental results of the two groups raises a number of problems, i.e. the
“control values” result from a population which is not comparable with regard
to body weight and development.

In order to make possible comparative studies with animals which differ only
with regard to their function but have, on the other hand, an identical develop-
mental level, we developed a method which allows us to separate completely
thyroidectomised from incompletely operated animals within 4 days of thyroi-
dectomy. For each animal about 3 min (30 seconds for the iv injection of
the activity and 2 \( \times 1 \) min for the measurements) are needed, thus time con-
suming procedures like the estimation of the basal metabolic rate are not
necessary.

The additional fractionated radio-iodine resection to guarantee athyreosis
also becomes superfluous. Secondary changes in hypothyroid animals are
avoided.

The principle of the method is based on the fact that a temporal change
in the relative counting rate of $^{131}$I in the thyroid region depends on the amount of residual thyroid tissue (Fig. 1). Animals which have preserved 3 to 5% of their thyroid tissue (the isthmus of the thyroid was not removed in group 3) can be shown to store more $^{131}$I than the totally resected animals (group 2). The difference between the relative counting rates of the two groups becomes significant 72 h after the administration of $^{131}$I ($P < 0.02$; 96 h: $P < 0.001$).

In order to find out whether a surgically thyroidectomised animal still shows residual thyroid function, the relative counting rate – as described above – in the thyroid region is determined 72 h or 96 h after the administration of $^{131}$I. – If the value is higher than 0.15 the animal cannot be considered as completely thyroidectomised. – It may be added that the blanks are not influenced by 25 $\mu$Ci/kg $^{131}$I when studying nuclei acid metabolism in cell fractions of the animals with radioactively labelled precursors.

REFERENCES


Received on July 9th, 1971.