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II. Medizinische Klinik der Universität München

AUTOMATIZATION OF SERUM THYROXINE DETERMINATION AND OF T_3-UPTAKE TEST*

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Problem:

Simple, rapid and inexpensive techniques were developed, since in this laboratory the requests for determinations of T_4 an of T_3-uptake exceed the number of 200 per week. This study was aimed at the development and the automatization of a competitive protein binding method for T_4 on the basis of the automatic T_3-uptake test in use.

Methods:

1. *T_3-uptake-test:* 0.2 ml serum and 0.4 ml T_3-^{125}J (0.1 μCi) in 0.5% albumin, 0.05 M sodium phosphate buffer pH 7.4 were equilibrated for 15 min at room temperature. 25 samples were simultaneously aspirated by a 25-channel peristaltic pump and delivered to 25 microcolumns containing 1 g sephadex G-25 fine, placed in a thermoblock (29° C) for separation of protein-bound and so-called free = dextran gel adsorbed T_3-^{125}J. The latter was totally eluted by pooled serum. With this procedure the same columns could be used for weeks. The whole separation was performed automatically within 25 min with a time-controlled apparatus.

2. *Serum-T_4:* 0.1 ml serum and 0.3 ml T_4-^{125}J (0.1 μCi) in 0.5% albumin, 0.05 M sodium phosphate buffer ph 7.4 are incubated for 15 min at room temperature. The incubation mixtures are delivered as above to 1 g sephadex G-25 s. f. columns, which are alkalized with 0.02 N NaOH, resulting in complete retention of cold endogenous and radioactive T_4. The columns are then washed with 0.05 M sodium barbital buffer pH 8.6. Addition of 1.6 ml pregnancy serum (11:00 diluted in barbital buffer) caused competitive protein binding for T_4 on the column and simultaneously eluted so bound T_4. The relative radioactivity in this eluate indicated the amount of endogenous T_4. The remaining hormones were finally totally eluted from the columns with excess serum, so that re-use of the columns was possible. For this CPB-method for serum T_4, the same automatic apparatus as for the T_3-uptake test is used.

Results:

The normal range of T_3-uptake ($x \pm 2\,s$) is 32–42%; quality control is satisfactory with a variation coefficient of 3.5% for a control serum checked for more than 2 years. Clinical data and quality control of the automatic CPB-method for T_4 will be presented.

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