GROUNDS FOR USING PIERRE'S METHOD OF ACTH ESTIMATION IN HUMAN BLOOD PLASMA

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Rat adrenal glands were immersed in Ringer-Locke solution (control) and, in parallel tests, in human blood plasma. After incubation for 2 h the corticosterone concentration in the extracts was determined. Activity of the pituitary adrenocorticotropic function of the patients tested was judged from the difference between the corticosterone values in the experimental and control extracts. To determine the sensitivity of the method ACTH was added to blood plasma in doses of 1, 5, and 10 μg. A significant and regular increase in the corticosterone concentration was found. The modification of Pierre's method is perfectly suitable for the estimation of ACTH in man.

KEY WORDS: ACTH; Pierre's method; pituitary gland.

Most methods suggested for the estimation of adrenocorticotropic hormone (ACTH) have proved to be very laborious or even unsuitable under clinical conditions. Only one of these methods [1] attracted our attention because of its convenience. Pierre showed that if rat pituitary glands are placed for 2 h in Krebs-Ringer solution, and the adrenals of intact rats are then immersed in the same medium, under the influence of the ACTH secreted by the pituitary the adrenals secrete corticosteroids into the solution, and on this basis the quantity of ACTH secreted can be judged.

Considering the species-nonspecificity of action of ACTH, the writers decided to modify this method for clinical use.

EXPERIMENTAL METHOD

Blood was taken from the cubital vein of fasting patients in a volume of 10 ml in order to obtain 5 ml plasma. The plasma was poured into 20-ml weighing bottles. Rat adrenal glands were then immersed in the plasma (by Pierre's method). Another weighing bottle of the same capacity was filled with 5 ml Ringer-Locke solution, in which the same number of rat adrenal glands were immersed. The adrenals in both bottles were incubated for 2 h at 37°C. The concentration of corticosterone secreted by the adrenals in the first bottle under the influence of the ACTH contained in the plasma, and in the second bottle without the influence of ACTH, was then determined. The difference reflected activity of the pituitary adrenocorticotropic function in the patients tested.

Corticosterone was determined quantitatively by the color reaction adapted for the fluorometric method of determining small quantities of corticosterone [2]. Crystalline corticosterone in a dose of 1 μg was used as the standard. The result was expressed in micrograms per gram rat adrenal tissue and calculated by the equation:

$$x = a \cdot b \cdot c/d \cdot e$$

where x is the corticosterone content per gram rat adrenal tissue (in μg), a) the difference between the fluorometer readings in the experimental series and those obtained when testing the purity of the reagents,

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TABLE 1. Corticosterone Content in Blood Plasma from Women with Carcinoma of the Cervix Uteri before and after Addition of ACTH, Calculated as Corticosterone in μg/g Weight of Rat Adrenal Tissue

Test No.	Rat adrenals immersed in Ringer – Locke solution	Rat adrenals immersed in plasma	Quantity of ACTH added to plasma (μg)		
			1	5	10
1	47,6	115,4	122,4	162,5	180,0
2	50,4	103,5	145,1	157,1	188,1
3	50,0	107,1	135,5	153,0	180,0
4	50,7	108,7	130,4	156,6	164,8
5	48,3	101,8	121,8	145,4	195,0
6	40,0	81,3	137,1	163,3	232,9
7	50,0	90,0	130,5	158,2	191,6
8	50,6	96,0	115,4	141,7	204,1
9	50,6	121,4	142,8	176,4	235,0
10	55,5	111,1	133,3	170,3	222,2
Mean	49,4±1,23	103,6±3,80	131,4±2,99	158,4±3,33	199,4±7,52

b the quantity of crystalline corticosterone acting as the standard (1 μg), c the quantity of adrenal tissue against which the corticosterone content was calculated (1 g); d the difference between the fluorometer readings for the standard and for purity of the reagents, and e the weight of the adrenals.

To test the reliability of the modified method of determining ACTH a series of additional tests was carried out to determine different but known quantities of ACTH added to the plasma. Plasma (5 ml) obtained from patients with carcinoma of the cervix uteri was poured into four weighing bottles. One bottle acted as the control and ACTH was added to the other three in different amounts (1, 5, and 10 μg). The same number of rat adrenals was then added to all the bottles and, after incubation for 2 h, the plasma was tested for its corticosterone content.

The results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

As was expected, in the first experiment (in which the adrenals were incubated in Ringer–Locke solution) the corticosterone content was much less (49.4 ± 1.23 μg) than in the second experiment in which the adrenals were incubated in plasma containing ACTH (103.6 ± 3.71 μg; P = 0.0000). The results also showed that the tested plasma contained ACTH, which stimulated corticosterone synthesis (Table 1).

The question naturally arose of the significance of the relationship between this increase in corticosterone synthesis and the presence of ACTH in the plasma. Accordingly, another experiment was carried out, the results of which are also included in Table 1. The mean corticosterone content in plasma without the addition of ACTH was 103.6 ± 3.71 μg. In plasma with the addition of ACTH in doses of 1, 5, and 10 μg, a perfectly regular and significant increase in the corticosterone content was found. For example, after the addition of 1 μg ACTH the mean corticosterone content increased to 131.4 ± 2.99 μg (P = 0.0000), with 5 μg ACTH it rose to 158.4 ± 3.33 μg (P = 0.0000), and with 10 μg to 199.4 ± 7.52 μg (P = 0.0000).

Our modification of Pierre's method is thus perfectly suitable for the estimation of ACTH in man, whether in health or in disease.

LITERATURE CITED

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