

## A Biochemical and Histochemical Study on the Activity of Acid Peptide Hydrolase in the Hypothalamus at Some Periods of Starvation and Refeeding after Starvation

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This study was carried out to determine acid peptide hydrolyse activity in the homogenate of the lateral (feeding centre) and ventro-medial (satiety centre) nuclei of hypothalamus and fractions of the cells (mitochondria, nucleus, cystocoele) in those centres of rats fed after being starved for a five day period. One hundred and sixty Wistar Albino rats were used in this study. The rats were starved for periods of 1, 3, 5 and 7 d. The rats starved for 5 d were then allowed to eat for periods of 5, 15 and 30 d. The activity of acid peptide hydrolyse was biochemically determined in the feeding and satiety centres. In addition, histochemical study was also done in these centres. It was observed that the activity of acid peptide hydrolyse became closer to the levels of the control group in the satiety centre on the 30th d of access to food after a 5 d starvation. It was concluded that the feeding centre was more sensitive to starvation than the satiety centre and the satiety centre was restored faster than the feeding centre.

**Key Words:** Starvation, Acid peptide hydrolyse, Hypothalamus.

### INTRODUCTION

Ballard *et al.*<sup>1</sup> state that 80% of amino acids needed for protein synthesis in an organism are constituted by the hydrolysis of proteins. Peptidases are involved in the hydrolytic processes and regulator function of proteins. They provide physiologically the activity or inactivity of peptides, hormones and enzymes in the organism. The organism is fed endogenously at the starvation<sup>2,3</sup>. So, it is important to know the level of acid peptide hydrolyses in the hypothalamus during the starvation because it is the place of all metabolic centres<sup>4,5</sup>.

This study was carried out to determine acid peptide hydrolyse (APH) activity in homogenate of the lateral (feeding centre) and ventro-medial (satiety centre) nuclei of hypothalamus and fractions of the cells (mitochondria, nucleus, cystocoele) in those centres in rats that were fed after being starved for a five day period.

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## EXPERIMENTAL

A total of 160 adult, healthy male Wistar Albino rats were used in this study. Twenty rats were randomly allotted into each treatment group. Ten rats from each treatment group were utilized for histochemical and biochemical studies.

**Control animals:** 20 rats were used in the control group. These animals were fed with commercial diet (Van-Yem A.S., Turkey) and watered *ad libitum*.

**Starved animals:** In this group 80 rats were used. They were divided into 4 groups. These groups were starved for periods of 1, 3, 5 and 7d, respectively. They were watered *ad libitum*.

**Animals fed after starvation:** In this group 60 rats starved for 5 d were used. The rats were then divided into 3 groups and fed with commercial diet and watered *ad libitum* for periods of 5, 15 and 30 d, respectively.

**Biochemical methods:** The rats were killed by cervical dislocation at the end of the treatment. The activity of the acid peptide hydrolases was observed by establishing the tyrosine levels in the homogenate of the hypothalamus and the fractions of the hypothalamic cells<sup>6</sup>. The findings in these periods of the starvation and the restoration were compared with those of the control group.

**Histochemical methods:** To determine the nucleoproteides in the neurones, histological sections of the Carnoy's solution-fixed and paraffin embedded in the feeding and satiety centres were prepared<sup>7</sup> and stained with O-dianizidin modified by Gerstein and Svetkova<sup>8</sup> from Bernet-Zelikmann (1960). Photographs of the sections were taken with the Nikon AFX-DX Optiphot-2 (Nikon, Japan).

**Statistical analysis:** All data were subjected to analysis of variance using general linear model Procedure of SAS<sup>9</sup>. Mean Treatment differences were determined by Duncan's t-test with a level of statistical significance of 5%.

## RESULTS AND DISCUSSION

**Biochemical results:** The tyrosine levels in homogenate of the hypothalamus and fractions of the hypothalamic cells in the control group and different periods of the starvation groups are shown in Table-1.

TABLE-1  
THE ACTIVITY OF ACID-PEPTIDE HYDROLASE IN HYPOTHALAMUS  
AT DIFFERENT PERIODS OF DEPRIVATION (TYROSINE CHANGE AS  
μg/mg PROTEIN IN 1 h)

Group (n = 10)	Homogenate (μg)	Cell fractions		
		Nucleus (μg)	Mitochondria (μg)	Cystocoele (μg)
Control	1.88 ± 0.19	1.76 ± 0.07	3.45 ± 0.03	18.16 ± 0.05
First day of deprivation	4.46 ± 0.32‡	7.14 ± 0.04‡	10.54 ± 0.05‡	9.79 ± 0.05‡
Third day of deprivation	3.55 ± 0.27‡	10.34 ± 0.03‡	16.22 ± 0.07‡	14.79 ± 0.12‡
Fifth day of deprivation	2.52 ± 0.17*	2.40 ± 0.12†	5.00 ± 0.19‡	4.10 ± 0.10‡
Seventh day of deprivation	2.35 ± 0.16‡	1.74 ± 0.09‡	8.05 ± 0.13‡	12.79 ± 0.22‡

The difference between control group and individuals under experimentation is statistically important.

\* P < 0.05, †P < 0.01, ‡P < 0.001.

While the APH activities increased 2 to 4 folds in the homogenate compared with the control group on the 1st d of starvation, they decreased gradually at the later periods of starvation. The APH activities in the nucleus increased 4 folds on the 1st d of starvation and 5 to 9 folds on the 3rd d of starvation. However the activities started to decrease after the 5th d of starvation. They increased on the 1st and 3rd d of starvation and decreased on the 5th and 7th d of starvation in the mitochondria as in the nucleus. It was established that the APH activities in cystocoele were low during the starvation periods compared with the control group.

The levels of tyrosine in the homogenate of the hypothalamus and fractions of the hypothalamic cells in the control group and in the group fed after 5 d starvation groups are shown in Table-2.

TABLE-2  
THE ACTIVITY OF ACID-PEPTIDE HYDROLASE IN HYPOTHALAMUS AT  
DIFFERENT PERIODS OF FOOD DIET (5, 15 AND 30th DAY) AFTER FIVE DAYS  
OF FOOD DEPRIVATION (TYROSINE CHANGE AS  $\mu\text{g}/\text{mg}$  PROTEIN IN 1 h)

Group (n = 10)	Homogenate ( $\mu\text{g}$ )	Cell fractions		
		Nucleus ( $\mu\text{g}$ )	Mitochondria ( $\mu\text{g}$ )	Cystocoele ( $\mu\text{g}$ )
Control	1.88 $\pm$ 0.19	1.76 $\pm$ 0.07	3.45 $\pm$ 0.03	18.16 $\pm$ 0.05
Fifth day of deprivation	2.26 $\pm$ 0.31‡	7.05 $\pm$ 0.43‡	12.64 $\pm$ 1.05‡	8.30 $\pm$ 0.75‡
Tenth day of deprivation	2.05 $\pm$ 0.26*	6.53 $\pm$ 0.59‡	11.90 $\pm$ 1.09‡	9.81 $\pm$ 0.79‡
Thirtieth day of deprivation	1.49 $\pm$ 0.15†	3.93 $\pm$ 0.28‡	12.67 $\pm$ 1.20‡	5.84 $\pm$ 0.27‡

The difference between control group and individuals under experimentation is statistically important.

\*P < 0.05, †P < 0.01, ‡P < 0.001.

It was noted that while the APH activities (tyrosine levels) were high in the nucleus and mitochondria in all periods fed after starvation, they were low in cystocoele compared with the control group. In addition, it was observed that the APH activities in homogenate and fractions did not reach the same levels in the control group on the 30th d of being fed after the starvation period.

**Histochemical findings:** The acid proteins in the neurones of the feeding centre, especially pyramidal neurones in the control group, had dark staining in the glial cells and apical dendrites zones (Fig. 1). It was determined that the acid proteins were abundant in the large neurones of the satiety centre and glial cells (Fig. 2).

It was observed that the staining intensity of the acid proteins in the glial cells increased the acid proteins in the peripheral regions of the nucleus bringing it to the border of cells and the protein levels in the nucleus were decreased in the feeding centre on the 1st d of starvation. It was not shown that biochemical changes were any different in the neurones of the satiety centre compared to the control group.

Acid proteins had homogeneity in the cytoplasm of the astrocytes in the feeding centre on the 3rd d of starvation. These proteins in the pyramidal neurones were located in the cortical area of the cell and had decreased in the nucleus of neurones.

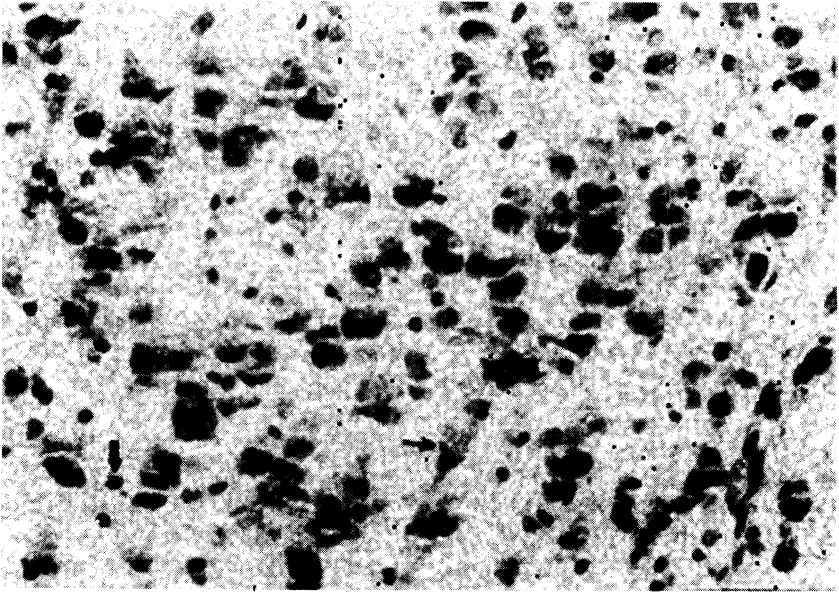


Fig. 1. The appearance of hunger centre of control group in light microscope, Dark stained apical dendrites of large neurones (arrow) and glial cells. O-dianizidin staining X560

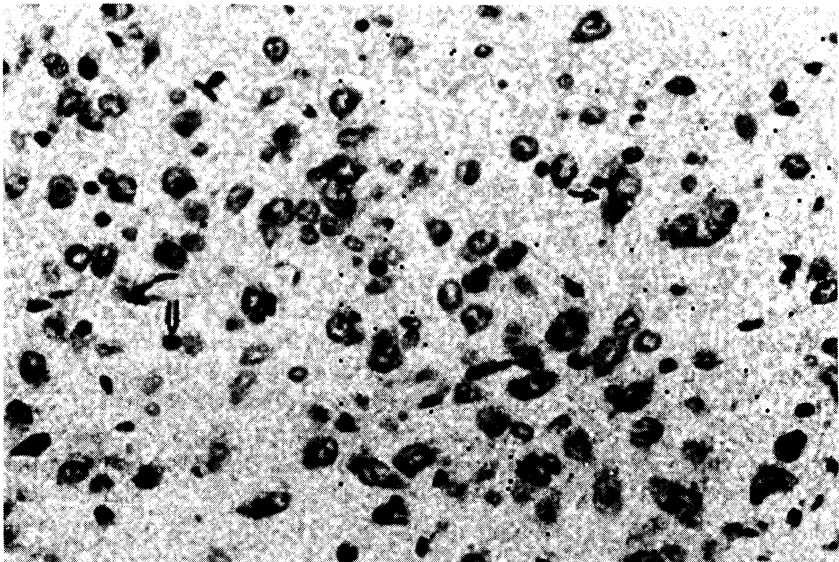


Fig. 2. The appearance of satiety centre of control group in light microscope. Dark stained apical dendrites of large neurones (arrow) and glial cells. O-dianizidin staining X560

In addition, the neurone-glia cell complexes started to occur (Fig. 3). Acid protein levels decreased in the neurones of the satiety centre, especially in the small neurones. It was observed that the pyramidal and bipolar neurones formed the neurone groups, and the neurone-glia cell complexes started to form in the satiety centre (Fig 4).

After five days of starvation, acid proteins at the feeding centre of hypothalamus

got darker. It is observed that the cluster of glia cells around some pyramidal neurones occurs.

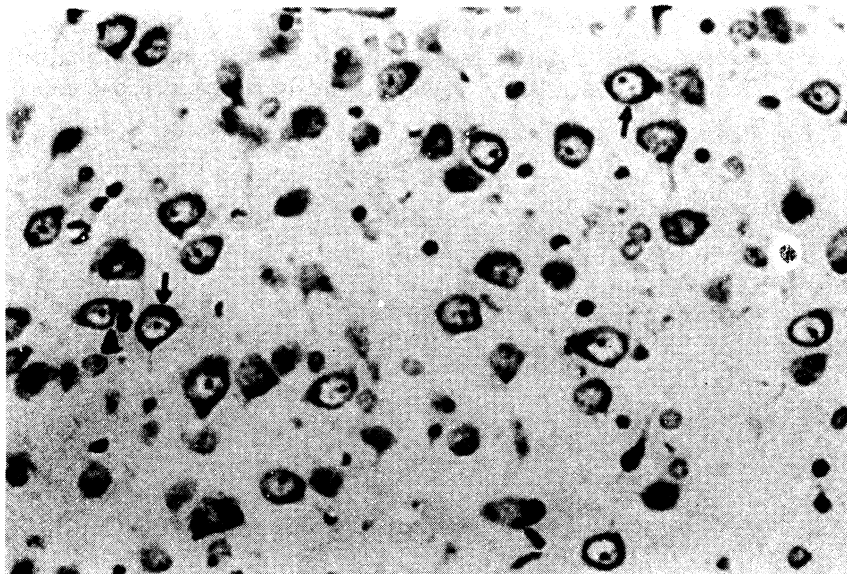


Fig. 3. The appearance of hunger centre in the third day of deprivation. Arrow: The movement of acid proteins in pyramidal neurones toward the cortical area. Arrowhead: Neuron-glia complex

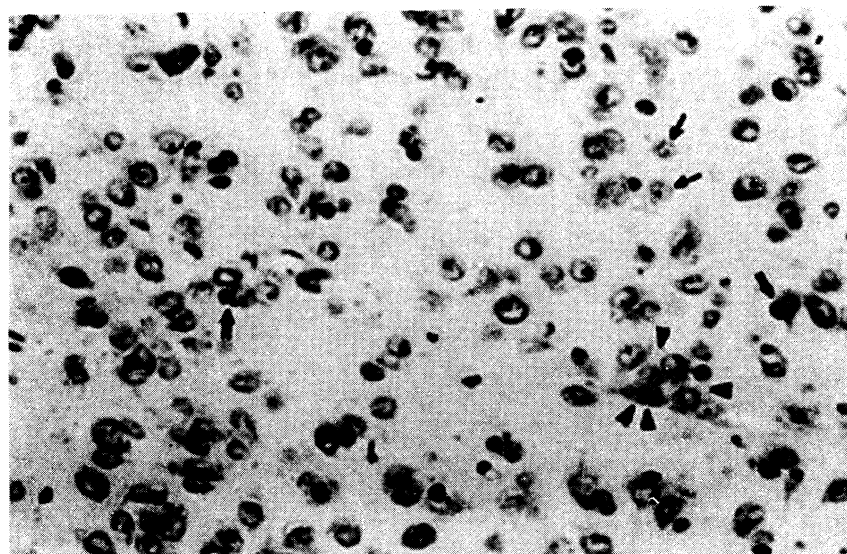


Fig. 4. The appearance of satiety centre in the third day of deprivation. Arrow: The decrease of acid protein amounts in small neurones. Double arrow: Neuron-glia complex. Arrowhead: Cluster of neurones

The border between the nucleus and cytoplasm disappears in some neurones (ectopia). In some other neurones the state of hydropia takes place (Fig. 5). In this study some additional changes were observed such as acid proteins of neurones

getting darker at satiety and feeding centres, ectopia and neurone-glia cell clusters (Fig. 6).

Seven days after starvation, the amount of acid proteins of neurones at satiety and feeding centres is increased. It is especially noticed that the amount of glial cells around pyramidal neurones in the feeding centre is increased and norofagia

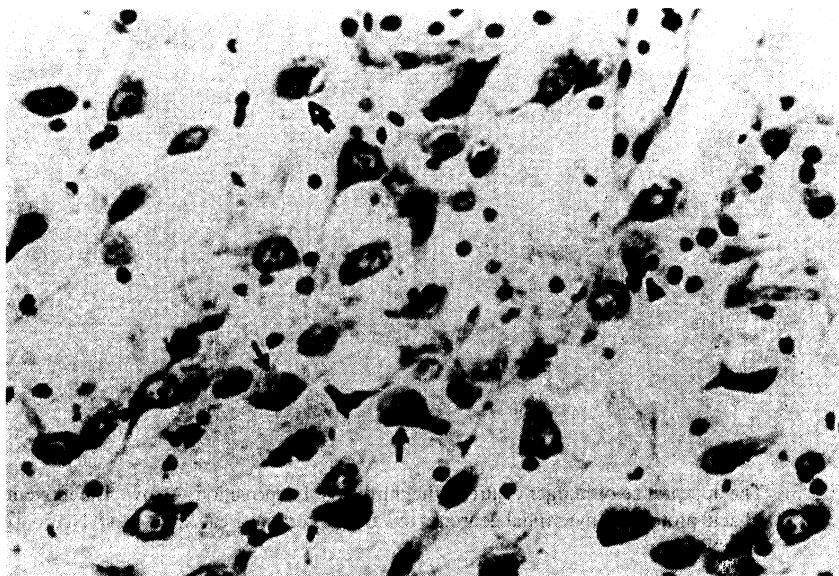


Fig. 5. The appearance of hunger centre in the fifth day of deprivation. Arrow: Ectopia. Double arrow: Hydrophia. Arrowhead: Neurones surrounded by cluster of glia cells

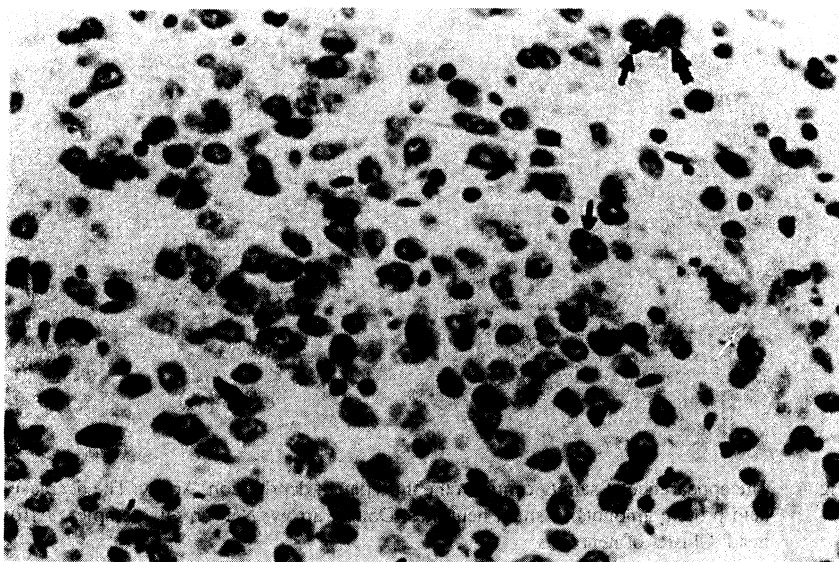


Fig. 6. The appearance of satiety centre in the fifth day of deprivation. Arrow: Neuron-glia complex. Double arrow: Ectopia

becomes frequent. While ectopia and hydrophia was seen at the neurones of satiety centre, norofagia at the satiety centre is less compared to the feeding centre.

After 15 d of starvation, while the amount of acid proteins of the neurons and glial cells at the satiety centre was quickly reaching those in the control group, any hint of restoration at the feeding centre was not observed.

After 30 d of starvation, the restoration of the amount of the acid proteins of neurons and the glial cells of the feeding centre is not completely provided, but some hints of the restoration in terms of morphology is observed. During these 30 days of starvation it was seen that the deconstruction (ektopia, hydrophia, chromotolizis and vakuollesme) of the neurons of the satiety centre began to reach the accepted standard.

According to the findings of this research, the activity of the acid proteins of the rats in the control group is higher than the pyramidal neurones. This is the result of increased activity of the neurones of the feeding centre in terms of the function of the neurones because one of the main functions of the feeding centre is to cause protein change. This view matches with previous works<sup>10, 11</sup>.

After 1 and 3 d of starvation the increase of activity of acid proteins at the neurones of the feeding centre means that the feeding centre related to the satiety centre has more functional power to control the behaviour of animals (rats). At the same time the increase of acid peptide hydrolysis of nucleus and mitochondria during the first and third days of the starvation also supports this finding. Askerov<sup>14</sup> found that the amount of astrositer and oligodendrositer glial cells at the feeding centre was more than those in the satiety centre with his biochemical and histochemical study. On the other hand, Schubert *et al.*<sup>12</sup> state that acid peptides at the glial cells are more related to neurones when needed.

In this study, it is observed that the activity of the acid peptide hydrolysis in homogenate nucleus and mitochondria is increased in the first and third days of the starvation. The reason for this is that the activity of the neurones of hypothalamus is increased depending on afferent impulses coming from the stomach-intestine system during starvation. This reflects the demand to synthesize acid protein increases to provide motivational reactions. This demand is provided from the nuclei and mitochondria in the cells. The experiment undertaken to that end<sup>9, 13, 14</sup> supports this approach.

After the fifth and seventh days of starvation, acid proteins got darker in the neurones of the feeding and satiety centre of the hypothalamus. This occurred as a result of high activity of acid peptide hydrolysis as the reserved acid proteins in the nucleus and mitochondria break into pieces and enter cystocoele during the first and third days of starvation. For that reason, the activity of acid peptide hydrolysis in the nucleus and mitochondria is decreased during this starvation period but the mitochondria keep providing acid proteins to the nucleus in this period. Previously, it was stated that the activity of mitochondrial acid proteins in pathological cases is higher<sup>1, 15, 16</sup>.

In all the diet periods after starvation, biochemical measurements indicate an increase in the activity of acid peptide hydrolysis in the nucleus and mitochondria. In this way proper time is created in the cells to renew the proteins and the required proteolytic processes. On the other hand, it is observed during the

histochemical analyses that the feeding centre is restored later than the satiety centre. The reason for this comes from the morphological and anatomic features of the feeding and satiety centre<sup>17</sup> because the cells of the satiety centre are reticular, glucoreceptoric, polyfunctional and polysensor. For that reason, the feeding centre receives sensoric stimulation from the system of the stomach and intestine and sends signals to provide the behaviour of the animal in efferent way during and after all the starvation and diet periods. For this reason, the feeding centre must have more capacity than that of the satiety centre. Some other sources<sup>18-20</sup> support this view.

As a result, the feeding centre is affected by the earlier starvation and intensively compared to the satiety centre; the satiety centre regains the normal state on the 30th day of the diet programme after the starvation, but the restoration activity at the feeding centre progresses. The reason is that the morph functional features of the centres may have different structure. Biochemical measurements indicate that the amount of the acid peptide hydrolysis in the experimental group does not reach that of the control group.

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