CHANGES IN THE BASE COMPOSITION OF NUCLEAR RIBONUCLEIC ACID OF NEURONS DURING A SHORT PERIOD OF ENHANCED PROTEIN PRODUCTION

HOLGER HYDÉN, M.D., and ENDRE EGYHÁZI

From the Institute of Neurobiology, University of Göteborg, Göteborg, Sweden

ABSTRACT

Nuclei from isolated nerve cells were sampled by microdissection. The content and composition of the nuclear RNA was studied and compared with that of the cytoplasmic RNA of Deiters' nerve cells of rabbits. Analyses were made of control nerve cells and of cells in which an enhanced RNA and protein production had been induced by chemical means, tricyano-amino-propene, for 60 minutes. The nuclear RNA content of the control nerve cells was 56 $\mu\mu$g, *i.e.* 3 per cent of the total RNA content of the nerve cell. The base ratios were: adenine 21.3, guanine 26.6, cytosine 30.8, uracil 21.3. Purine-pyrimidine analyses showed that the nuclear RNA differed significantly from the cytoplasmic RNA in having higher adenine and uracil values. The guanine and cytosine values were high, however, and the ratio G/C was 0.86 as compared with 1.16 for the cytoplasmic RNA. The composition of the nuclear RNA was interpreted as reflecting the extraordinarily strong development of the nucleolus in these neurons. During the 60 minutes of enhanced neuronal RNA production (+25 per cent) the guanine value increased and the uracil value decreased significantly in the *nuclear RNA*. In the *cytoplasmic RNA* the guanine value also increased although not so much as the nuclear guanine. The cytoplasmic cytosine value decreased. The result indicated that the production of the characteristic cytoplasmic RNA had been influenced by the change in the nuclear RNA.

BACKGROUND

Among somatic cells, the neurons are outstanding as producers of ribonucleic acid (RNA). Determined per cell body, including the thick part of the dendrites, the amount of RNA varies from about 30 $\mu\mu$g to 1600 $\mu\mu$g, depending on the cell size (Hydén, 14).

The amount of RNA per nerve cell has been shown to increase as a function of stimulation (Hydén, 12, 14; Hydén and Pigon, 18). The glia surrounding the nerve cell also contains RNA. Determined on the same volume basis, and thereby the same dry weight, the neuronal glial cell contains one-tenth as much RNA as does its adjacent neuron. The base composition of the nerve cell RNA is characterized by higher guanine and cytosine than adenine and uracil values (Egyházi and Hydén, 9). This RNA does not, therefore, differ in this respect from other types of cytoplasmic RNA. When the base ratios of neuronal RNA are compared with those of glial RNA, a complementariness can be noted with respect to guanine and cytosine. The functional implication of this as an expression of mutual interrelation has been discussed. The neuronal glia responds

with quantitative RNA changes as a function of stimulation as does the nerve cell, although these changes are of inverse direction relative to those of the nerve cell (Hydén and Pigon, 18). Studies of enzymic activities and kinetic analyses have shown that the nerve cell and its glia constitute an energetically coupled system (Hydén and Lange, 16).

RNA production in the nervous tissue can be induced by chemical substances. Tricyano-aminopropene (triap) given intravenously in doses of 20 mg/kg of body weight in rabbits has been shown to be an effective inducer of neuronal RNA production (Egyházi and Hydén, 9). *Quantitatively*, in 60 minutes the amount of RNA per Deiters' nerve cell increased from 1500 $\mu\mu$g to 2100 $\mu\mu$g (27 per cent). The protein content per cell increased by 25 per cent, and the respiratory enzyme activities by 100 per cent. The glial RNA decreased by 45 per cent, from 125 $\mu\mu$g to 70 $\mu\mu$g. *Qualitatively*, the adenine and uracil values remained unchanged in both neuronal and glial RNA. In the nerve cell, the guanine increased and the cytosine fell. In the glial RNA, the guanine decreased and the cytosine increased. The glial changes were more pronounced than the nerve cell changes. Part of the neuronal and glial RNA consisted of RNA synthesized during 60 minutes. The inversely changed base ratios may be a reflection of the production of a RNA fraction(s) having a base composition the characteristics of which are such as to reveal themselves in an electrophoretic analysis even of the total amount of the nerve cell RNA.

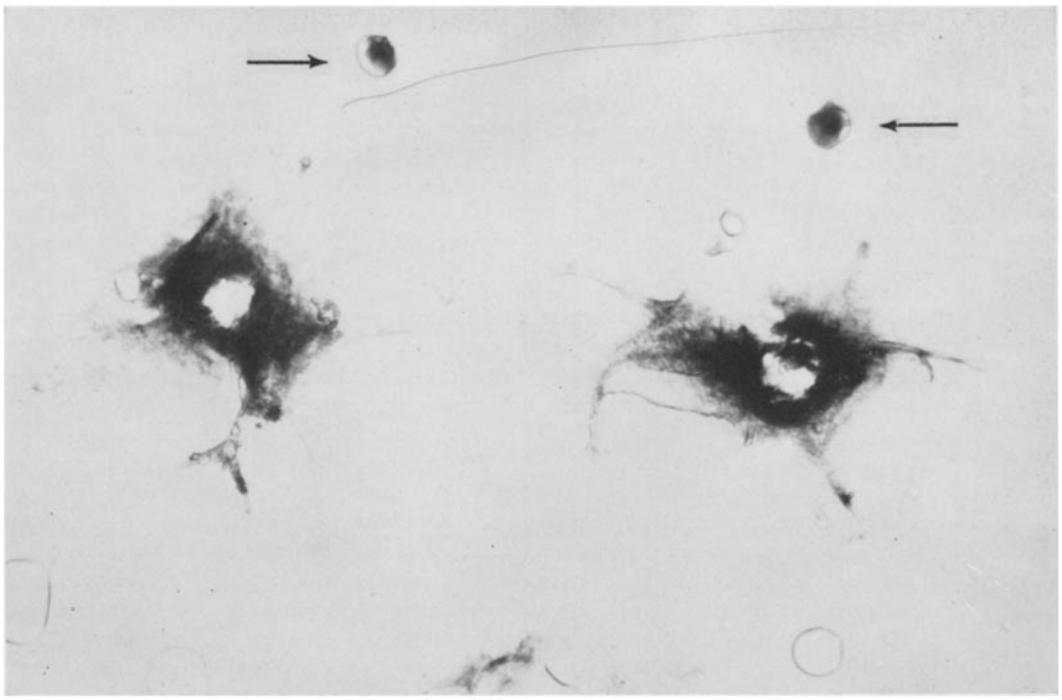

FIGURE 1

Two isolated Deiters' nerve cells, rabbit, photographed in ultraviolet at 2570 A. The nuclei of the two cells have been removed by micromanipulation and are seen above the nerve cells at arrows. × 460.

In view of this finding, the question arises whether the process leading to the changed base composition of the nerve cell RNA can be traced to nuclear activity. This paper reports analyses of nuclear RNA in neurons from control animals and from animals in which an enhanced neuronal RNA production has been induced. A method is described by which individual nerve cell nuclei can be collected by microdissection and the nuclear

TABLE I

Comparison of RNA Determination after Precipitation with Cold Perchloric Acid and with Phenol-Saturated Water

	Amt of RNA per nerve cell
	μμg
Perchloric acid	1560 ± 31
Phenol-water	1565 ± 44

RNA precipitated for subsequent analysis by microelectrophoresis.

EXPERIMENTAL

Six white rabbits, weighing 1.5 to 1.7 kg, were used for controls and eight for experimental purposes. Triap was given intravenously, 20 mg/kg. The animals were killed 60 minutes later. After being given an air embolus (20 ml of air injected into the ear vein), the animal became unconscious within a few seconds and its carotid arteries were cut to permit the remaining heartbeats to drain the blood from the brain and body. The brain was removed rapidly; a slice was cut through the lateral vestibular nucleus and placed in sterile 0.25 M sucrose solution. The big Deiters' nerve cells were collected by free-hand dissection with a stainless steel instrument and a stereomicroscope as described earlier (Hydén, 13; Hydén and Pigon, 18). The isolation and cleaning of 10 nerve cells from the surrounding glia did not take more than 5 minutes. The isolated cells were placed on a slide and the small amount of sucrose solution around the cells was removed.

In order to precipitate the nuclear RNA and at the same time allow the nerve cell nuclei to be taken out by microdissection, a modification of Harris' procedure (11) was used. The isolated nerve cells, placed on a glass slide, were briefly rinsed in cold isotonic NaCl solution. They were then treated with cold phenol-saturated water for 15 minutes followed by cold absolute ethanol for 10 minutes and covered with paraffin oil (*pro analysi*). The effect of this treatment is to cause a slight contraction of the nuclei, hardly noticeable at a magnification of 600 times. The nucleus from each nerve cell was then removed with the aid of a de Fonbrune micromanipulator, using a glass instrument (Fig. 1). For each RNA analysis 15 nuclei were used.

To check the effect of phenolic precipitation of the total amount of RNA in nerve cells, the following experiments were carried out. The determination of RNA in μμg per nerve cell nucleus and cell body was performed according to Edström's method (4–6). RNA was extracted from the cells with a buffered solution of ribonuclease. The RNA in the extracts was determined in micro drops by a photographic-photometric method using ultraviolet radiation at 2570 A. It has been demonstrated that treatment with Carnoy's fixation fluid followed by absolute ethanol and benzene or treatment with cold 1 N perchloric acid is an effective precipitation method for RNA of nerve cells (Edström, 4, 6; Edström *et al.*, 8). Deiters' nerve cells from a rabbit were isolated from the left and from the right side of the vestibular nucleus. The nerve cells from one side were precipitated by cold 1 N perchloric acid followed by cold absolute ethanol and chloroform, and from the other by cold phenol-saturated water followed by cold absolute ethanol. The RNA of each cell was extracted with a buffered ribonuclease solution and the amount was determined as described above. Table I gives the results. The phenol procedure thus precipitates all RNA in nerve cells.

For purine-pyrimidine analysis (Edström, 6), the pooled RNA extracts from 30 nerve cell nuclei were hydrolyzed in a micropipette with 4 N HCl at 100°C for 30 minutes. Each hydrolysate, containing approximately 700 μμg of RNA, was placed on an alkali-treated cellulose fiber containing a buffer solution of high viscosity and high acidity. The fiber on a quartz slide was transferred to a constant humidity chamber of 42 per cent relative humidity. The RNA constituents were separated by high voltage electrophoresis (2000 to 3000 v/cm) for 10 minutes. After the separation, the bands were photographed at 2570 A and their optical densities recorded with a

TABLE II

Amount of RNA in μμg per Nucleus of Deiters' Nerve Cells from Rabbits Injected with 20 mg/kg of Tricyano-amino-propene and Killed after 1 Hour

	RNA per nucleus	No. of animals	No. of nuclei	Variation coefficient	P*
	μμg				
Controls	56 ± 2.7	6	27	12	$0.05 \gg P > 0.01$
Tricyano-amino-propene	47 ± 1.9	6	28	10	

* P = Probability after t-test.

microdensitometer. From the curves recorded, the purines and pyrimidines were calculated as molar proportions in percentages of the sum. For 500 μμg of RNA the average values of the determinations showed a coefficient of variation of 5 per cent.

RESULTS

The number of nuclei analyzed was 602, 250 of which were control nuclei. The total number of analyses was 36.

Table II gives the amount of RNA per nerve cell nucleus. In the control material the nucleus contained 56 ± 2.7 μμg of RNA, *i.e.* 3 per cent of the total amount of RNA in the nerve cell body, 1550 μμg. The variation coefficient was satisfactory. In the nuclei from animals with increased RNA values in the nerve cells induced by triap there was a significant decrease of the RNA content. This result will be discussed below.

Table III demonstrates that the base composition of the nuclear RNA differs characteristically from that of the cytoplasmic RNA. The adenine and uracil values were higher than corresponding values for the cytoplasmic RNA. The nuclear guanine and cytosine values were also high, and the ratio G/C was 0.86 as compared with 1.16 for the cytoplasmic RNA. The purine/pyrimidine ratio was 0.92 for the nuclear RNA, and the (A + U)/(G + C) ratio was 0.74.

Thus, the nuclear RNA of the neuron had a base composition more similar to that of DNA than to that of cytoplasmic RNA and showed a good complementariness. It can be noted that the nuclear cytosine value was higher than the guanine value, whereas the converse was found in the cytoplasmic RNA.

TABLE III

Composition of RNA in Deiters' Nerve Cells from Control Rabbits: Whole Nerve Cells and Their Nuclei

	Whole nerve cells		Nuclei		
	Molar proportions	Variatiom coefficient	Molar proportions	Variation coefficient	*P*
Adenine	19.7 ± 0.37	4.2	21.3 ± 0.42	4.9	0.02
Guanine	33.5 ± 0.39	2.6	26.6 ± 0.27	2.5	0.001
Cytosine	28.8 ± 0.36	2.8	30.8 ± 0.38	3.0	0.01
Uracil	18.0 ± 0.18	2.3	21.3 ± 0.43	5.0	0.001

TABLE IV

Composition of RNA in Deiters' Nerve Cell Nuclei from Rabbits Injected with 20 mg/kg Tricyano-amino-propene and Killed after 1-Hour

	Controls		Tricyano-amino-propene	
	Molar proportions	Variation coefficient	Molar proportions	Variation coefficient
Adenine	21.3 ± 0.42	4.9	21.5 ± 0.54	7.0
Guanine	26.6 ± 0.27	2.5	30.0 ± 0.45	4.3
Cytosine	30.8 ± 0.38	3.0	30.4 ± 0.49	4.6
Uracil	21.3 ± 0.43	5.0	18.1 ± 0.44	6.9

In Table IV are listed the base ratios of the nuclear RNA from neurons in which the RNA content had increased from 1550 μμg to 2120 μμg per cell during 60 minutes after injection of triap. It can be noted that the guanine value had increased significantly (Table V) and the uracil value had decreased (Table VI). The purine/pyrimidine ratio had thus shifted from 0.92 to 1.06. The complementariness is pronounced.

In Table VII a comparison is made between the significant changes occurring in the nuclear and the cytoplasmic RNA of the neuron during this brief period of intense RNA and protein production induced by triap.

EVALUATION OF RESULTS

Quantitatively, the nuclear RNA constitutes only 3 per cent of the total RNA in the nerve cell, 50 $\mu\mu$g out of 1550 $\mu\mu$g. The base composition of the nuclear RNA shows a good complementariness; nuclear RNA has higher adenine and uracil values than cytoplasmic RNA, but has also high guanine and cytosine values. The composition of the cytoplasmic RNA seems characteristic for the cell body and exhibits a guanine/cytosine ratio that is the reciprocal of that of the nuclear RNA.

The aim of the present work was, first, to determine whether the base composition of the nuclear RNA in the nerve cell changed during a period of increased RNA and protein production; and, second, to observe whether changes found in the synthesized cytoplasmic RNA could be related to changes in the nuclear RNA.

TABLE V

Guanine Content in Nuclear RNA of Deiters' Nerve Cells from Rabbits

	Mean	No. of animals	No. of analyses	Variation coefficient	*P*
Controls	26.6 ± 0.27	6	15	2.5	0.001
Tricyano-amino-propene	30.0 ± 0.45	8	20	4.3	

TABLE VI

Uracil Content in Nuclear RNA of Deiters' Nerve Cells from Rabbits

	Mean	No. of animals	No. of analyses	Variation coefficient	*P*
Controls	21.3 ± 0.43	6	15	5.0	0.001
Tricyano-amino-propene	18.1 ± 0.44	8	20	6.9	

TABLE VII

Changes in the Nuclear and Cytoplasmic RNA in Neurons after Increased RNA and Protein Production for 60 Minutes

	Nuclear RNA			Cytoplasmic RNA*		
	Controls	Induced synthesis	*P*	Controls	Induced synthesis	*P*
Adenine	21.3 ± 0.42	21.5 ± 0.54		19.7 ± 0.37	20.5 ± 0.31	
Guanine	**26.6 ± 0.27**	**30.0 ± 0.45**	0.001	**33.5 ± 0.39**	**34.6 ± 0.28**	0.05
Cytosine	**30.8 ± 0.38**	**30.4 ± 0.49**		**28.8 ± 0.36**	**26.7 ± 0.24**	0.001
Uracil	**21.3 ± 0.43**	**18.1 ± 0.44**	0.001	18.0 ± 0.18	18.2 ± 0.20	

* These values are taken from Egyházi and Hydén (9).

Nuclear RNA ratio $\frac{A + G}{C + U}$: controls, 0.92; induced synthesis, 1.06

Cytoplasmic RNA ratio $\frac{A + G}{C + U}$: controls, 1.14; induced synthesis, 1.23

Nuclear RNA ratio $\frac{A + U}{G + C}$: controls, 0.74; induced synthesis, 0.65

Cytoplasmic RNA ratio $\frac{A + U}{G + C}$: controls, 0.61; induced synthesis, 0.63

A 25 per cent increase, within 60 minutes, in the total amount of RNA and proteins of the nerve cell had been shown to be the effect of triap when given intravenously in small doses (9). The composition of the cell body RNA changed significantly and showed higher guanine and lower cytosine values. The conclusion was drawn that a fraction(s) of RNA was produced of such a base composition as to be able to express its characteristics in an electrophoretic analysis despite the preponderance of cytoplasmic nerve cell RNA.

Control nuclear RNA might have been expected to bear a similarity to DNA with respect to base ratio. Our analyses show that this is not the case. If anything, its similarity to nucleolar RNA is more pronounced. Edström *et al.* (7,8) found almost the same base composition in the nucleolar as in the cytoplasmic RNA from starfish oocytes. One might ask whether the nucleolar RNA of neurons can be quantitatively so dominating as to mask the composition of a smaller DNA-similar RNA fraction. In view of the extraordinary development of the nucleolus and its associated parts with respect to concentration of RNA (Hydén, 12, 14, 15) this may very well be the case.

A gradient in RNA concentration has been found extending from the nucleolus to the nuclear membrane, and from the nuclear membrane to the periphery of the nerve cell cytoplasm (Hydén, 12; Hydén and Larsson, 17). RNA precursors are rapidly incorporated in the nerve cell nucleus (Koenig, 19). Fig. 2 shows an isolated nerve cell from a rabbit which had previously been given $P^{32}O_4$. Free nucleotides and lipids were extracted prior to placing the cell against a photographic emulsion. As can be seen, the most intense blackening occurs over the nucleus, fading away in the cytoplasm. Even if such findings do not provide direct proof, they are suggestive; and, in addition to the electrophoretic results of the nuclear RNA (composing 3 per cent of the RNA total of the cell), they support the view that the *nuclear RNA of the nerve cell seems to be rapidly produced and can vary in base composition depending on the functional situation.*

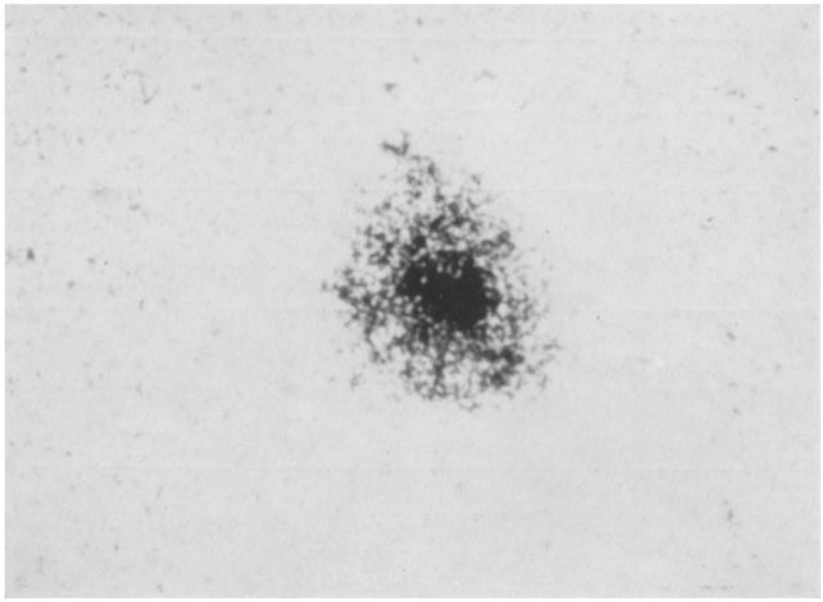

FIGURE 2

Autoradiograph of nerve cell from rabbit 1 hour after the injection of $P^{32}O_4$ intracisternally. Lipids and acid-soluble nucleotides were removed. Highest blackening over the nucleus. × 600.

Although less likely, an alternative interpretation cannot be excluded. It seems that the nuclear RNA constitutes a mixture of d-RNA high in adenine and uracil, with other RNA high in guanine and cytosine. Sibatani *et al.* (22) found in thymus cells a nuclear RNA with high guanine and cytosine values, but also another nucleolar fraction

with base ratios closely resembling those of DNA. The nuclear RNA changes observed in the neurons might also be explained by assuming a selective loss of RNA rich in adenine and uracil into the cytoplasm. The nuclear RNA content decreased from 56 to 47 $\mu\mu$g per nucleus. This could explain the relative increase in guanine content and the decrease in uracil content of the RNA remaining in the nucleus.

Does the evidence favor the view that the characteristics of the nuclear RNA can be recognized in the cytoplasmic RNA, part of which has been synthesized during 60 minutes? Judging on the guanine value, this base increased by 13 per cent in the nuclear RNA, and by 3 per cent in the cytoplasmic RNA; both of these, however, were significant changes. On the other hand, as regards the cytoplasmic RNA the difference existing between the guanine and cytosine in the control nerve cells increased by 70 per cent in the experimental cells. Thus, the characteristics of the nuclear RNA changes with respect to guanine were found also in the cytoplasmic RNA, the nuclear RNA change being most pronounced. *Production of a small nuclear RNA fraction* may have occurred, therefore, *earlier in time*, only to become noticeable in the cytoplasmic RNA subsequently at the time of analysis.

A final question is, What do these induced base changes of the nuclear RNA in neurons mean from a physiological point of view? We should like to give the following tentative interpretation. It is known that nuclei of amphibians change in activities during development and differentiation (Briggs and King, 2; Fishberg *et al.*, 10). Structural changes in chromosomes of Diptera have been proved to express gene activities (Beerman, 1; Pelling, 20; Rudkin and Woods, 21). The so-called puffs of the giant chromosomes contain RNA, which is thus a primary product of the chromosomes. In a structural analysis of chromosomes it was found that a hormone from the prothoracic gland, injected into the larva, produced formation and disappearance of puffs. The primary effect of the injected hormone thus expressed itself in a change in the activities of gene loci (Clever and Karlson, 3).

In our experiments with neurons, the nuclear RNA base changes occurred during a short period of increased RNA and protein production induced by a chemical substance. The nuclear and the cytoplasmic RNA changes are an expression of the neuron's capacity to synthesize different types of proteins under different conditions. Since the cytoplasmic RNA was changed also, the reaction of the neuron to the chemical may be similar to the bacterial response to antibiotics, or the response of *Euglena* cells to radiation (Pogo *et al.*, 23). In the present studies the base changes of the nuclear RNA probably depend on certain parts of the genome's becoming more active, producing an RNA of a characteristic composition. *This fraction of RNA formed by an activated part of the genome of the neuron will be reflected in changes in the total nuclear RNA.*

These studies have been supported by the United States Air Force Office of Aerospace Research, and by the National Multiple Sclerosis Society.

The skillful assistance of Miss Margareta Pettersson is gratefully acknowledged.

Received for publication, April 19, 1962.

REFERENCES

1. Beerman, W., *in* Developmental Cytology, (D. K. Rudnick, editor), New York, Academic Press, 1959, 83.
2. Briggs, R., and King, T. J., *J. Morphol.*, 1957, **100**, 269.
3. Clever, U., and Karlson, P., *Exp. Cell Research*, 1960, **20**, 623.
4. Edström, J.-E., *Biochim. et Biophysica Acta*, 1953, **12**, 361.
5. Edström, J.-E., *J. Neurochem.*, 1958, **3**, 100.
6. Edström, J.-E., *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 39.
7. Edström, J.-E., to be published.
8. Edström, J.-E., Grampp, W., and Schor, N., *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 549.
9. Egyházi, E., and Hydén, H., *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 403.
10. Fishberg, M., Gurdon, J. B., and Elsdale, T. R., *Exp. Cell Research*, 1959, Suppl. **6**, 161.
11. Harris, H., *Proc. Roy. Soc. London, B*, 1962, **156**, 109.
12. Hydén, H., *Acta Physiol. Scand.*, 1943, Suppl. 171.
13. Hydén, H., *Nature*, 1959, **184**, 433.
14. Hydén, H., *in* The Cell, (J. Brachet and A. Mirsky, editors), New York, Academic Press, 1960, **4**, 215.
15. Hydén, H., Macromolecular Specificity and Biological Memory, Massachusetts Institute of Technology Monographs, (F. O. Schmitt, editor), 1962, 55.

16. HYDÉN, H., and LANGE, P., *in* Fourth International Neurochemical Symposium, New York, Pergamon Press, 1960, 190.
17. HYDÉN, H., and LARSSON, S., *J. Neurochem.*, 1956, **1**, 134.
18. HYDÉN, H., and PIGON, A., *J. Neurochem.*, 1960, **6**, 57.
19. KOENIG, H., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 785.
20. PELLING, C., *Nature*, 1959, **184**, 655.
21. RUDKIN, G. T., and WOODS, P.S., *Proc. Nat. Acad. Sc.*, 1959, **45**, 997.
22. SIBATANI, A., DE KLOET, S. R., ALLFREY, V. G., and MIRSKY, A., *Proc. Nat. Acad. Sc.*, 1962, **48**, 471.
23. Pogo, A. O., Brawerman, G., and Chargaff, E., *Biochemistry*, 1962, **1**, 128.