Pharmacology Biochemistry and Behavior, Vol. 2, pp. 579–584. ANKHO International Inc., 1974. Printed in the U.S.A.

Avoidance Training and Incorporation of ^{3}H-Uridine into RNA, UMP and UDP-Sugars in Mouse Brain[1]

DAN ENTINGH AND TERRI DAMSTRA-ENTINGH

Division of Chemical Neurobiology, Department of Biochemistry, School of Medicine
University of North Carolina, Chapel Hill, North Carolina 27514

(Received 15 February 1974)

ENTINGH, D. AND T. DAMSTRA-ENTINGH. *Avoidance training and incorporation of ^{3}H-uridine into RNA, UMP and UDP-sugars in mouse brain.* PHARMAC. BIOCHEM. BEHAV. 2(5) 579–584, 1974. – The incorporation of ^{3}H-uridine into RNA and selected nucleotides of mouse brain during avoidance training was assayed using intracranial injections of precursor. The net incorporation of uridine into total brain RNA, as assayed by three methods, was not detectably affected by training. Training induced a decrease in the amount of radioactivity recovered from the brain as uridine monophosphate, while concomitantly increasing the amount of radioactivity in substances that chromatographed as uridine diphosphate sugars. The results suggest that changes in the cerebral metabolism of uridine observed during this form of avoidance training may reflect changes in the cellular uptake of uridine, or the metabolism of carbohydrate compounds, rather than increases in the rate of cerebral RNA synthesis.

Learning Neurochemistry Mouse Active avoidance Nucleotides Uridine RNA Nucleotide sugars Intracranial injections Radioactivity

VARIOUS forms of behavioral training have been reported to change the rates at which pyrimidine nucleosides (uridine and cytidine) are incorporated into cerebral RNA in rodents [1, 2, 6, 7, 8, 11, 22, 23]. Although these neurochemical changes have been interpreted as evidence for increased rates of RNA synthesis, changes in metabolism of the radioactive nucleotide precursor used in each experiment could account for such changes [14].

The idea that some of these RNA changes may be only indirectly related to RNA synthesis has recently been underscored by reports that the metabolism of pyrimidine nucleotides in brain is altered by influences such as anesthesia and convulsions [5] and hypercapnia [3]. Moreover, active avoidance training was found to reduce the proportion of subcutaneously injected ^{3}H-uridine that was recovered from mouse brain as uridine monophosphate (UMP) [12]. This neurochemical change was greater in the subcortical forebrain than in either the neocortex or in combined cerebellum and posterior brain stem. The absence of this change in the brains of mice that were yoked in stimuli to trained mice suggests that presentation of the stimuli associated with training is not sufficient to induce this chemical change.

It was important to study this neurochemical effect of avoidance training when the precursor compound was injected directly into the brain. First, earlier experiments in which avoidance training was found to increase the radioactivity in RNA relative to radioactivity in UMP employed intracranial injections of precursor [1, 2, 7, 8, 22, 23]. Second, at least one behaviorally-induced change in the metabolism of pyrimidine nucleotides in brain, an increased incorporation of cytidine into RNA during reversal learning, was apparent after peripheral injections but not after intracranial injections of the precursor [16].

The present experiments also investigated whether the apparent loss of radioactivity from brain UMP could be related to alteration in the metabolism of other uridine nucleotides, particularly UTP, UDP and uridine diphosphate sugars.

METHOD

Animals

Male C57BL/6J mice (Jackson Laboratories) between 6 and 8 weeks old were used. Mice were kept in the laboratory for 2 weeks before use, housed 6 to a cage, with food and water available ad lib.

[1] This research was supported by grants from the University (VF334), the U.S. Public Health Service (MH18136, NS07457) and the U.S. National Science Foundation (GB18551, GB35634X). The authors are indebted to S. Diane Davis, Edward Glassman and John E. Wilson for research support and suggestions.

Isotopes

5-^{3}H-uridine (specific activity 25 to 29 Ci/mmole, 1 μCi/μl, in water, radiochemical purity greater than 95% by our tests) and 4-^{14}C-uridine-5′-monophosphate (specific activity 30 mCi/mole) were purchased from Amersham/Searle.

Injections

Each mouse was anesthetized by exposure to ether at room temperature for 25 sec and then the scalp was split at the midline. Five μCi of ^{3}H-uridine was injected into both sides of the brain through a 27 ga needle inserted vertically 2.5 mm deep at the point 0.5 mm anterior to the coronal suture and 1.0 mm lateral to the sagittal suture. These coordinates placed the needle tip in the lateral ventricles just posterior the the septal region. Methylene blue solution injected in this manner was rapidly distributed to all ventricular spaces in test mice. All mice injected in this way were actively walking about their cages within 5 min after the injection.

Behavioral Procedure

General plan. Quiet mice were injected, and held in individual cages with no food or water for 45 min before sacrifice. Trained mice were injected, placed in individual cages with no food or water for 28 min, allowed to explore the training apparatus for 1 min, and then subjected to avoidance training for 15 min. They were sacrificed 1 min after training finished, resulting in a total isotope pulse of 45 min for all animals.

Avoidance training. The apparatus and trial sequence were those used by Zemp *et al.* [23]. The training apparatus was a wood box 18 cm × 18 cm × 18 cm with a shock-grid floor and a removable Plexiglas lid. A 2.5 cm wide shelf ran completely around the inside wall, 8.5 cm above the floor.

Each trained mouse received 40 training trials. At the start of each trial the conditional stimulus (CS), a 100 dB SPL buzzer and 100 W overhead lamp, was turned on for 3 sec. If the mouse had not jumped to the shelf in 3 sec, the CS was turned off and footshock, 0.25 mA from a Lafayette A-615C shocker, was applied until the mouse jumped onto the shelf. After a 12–16 sec intertrial interval, the mouse was removed from the shelf by its tail and placed on the grid floor, and 3–6 sec later, the next trial was begun.

Biochemical Procedures

Experiment 1. Six mice were trained and 6 served as quiet controls. Forty-five min after intracranial injections of 5-^{3}H-uridine each mouse was decapitated and the brain, minus the olfactory bulbs, was removed. Each brain was homogenized in 5.0 ml of 1% hexadecyltrimethyl ammonium bromide (CTAB) which contained 0.100 μCi of ^{14}C-UMP for 10 strokes in a close-clearance Teflon/glass homogenizer turning at 800 rpm.

Triplicate aliquots of 0.100 ml of homogenate were treated by the methods used in the previous study [12] to give samples of total homogenate ^{3}H (less tritiated water driven off by drying) and ^{3}H in CTAB-precipitated RNA. These samples were dissolved in 1.0 ml of Soluene-100 (Packard Instrument Co.) and assayed for ^{3}H and ^{14}C in a Packard 3375 scintillation counter in 10 ml/sample of scintillation fluid consisting of 4 g/l 2,5-diphenyl-oxazole and 0.1 g/l 1,4-bis [2-(4 methyl-5-phenyloxazolyl)]-benzene in toluene. Counts were converted to disintegrations per min (dpm) using external standards. Soluble radioactivity was calculated as the difference between dpm and RNA dpm.

The ^{3}H in nucleotides was measured by a variation of the methods used previously [1, 2, 7, 8, 12, 22, 23]. Duplicate aliquots of 1.00 ml of homogenate were made 1 N in perchloric acid (PCA). Then 1 ml of 10% acid-washed charcoal was added to the supernatant, shaken thoroughly, and allowed to stand 10 min. The charcoal was pelleted and washed 3 times with 1 ml of 0.05 M sodium acetate buffer, pH 4.5. The nucleotides absorbed to the charcoal were eluted into 1 ml of 10% pyridine in 50% ethanol at 37°C for 2 hr. The eluates were separated from the charcoal by centrifugation, dried for 8 hr at 80°C and resuspended in 100 μl of water.

Aliquots (20 μl) of this nucleotide solution were spotted on thin layer polyethyleneimine-cellulose plastic plates (Brinkman, CEL 300 PEI/UV254), along with 5 μg each of uridine, cytidine-5′-monophosphate (CMP), UMP, uridine-5′-diphosphate (UDP), uridine-5′-triphosphate (UTP), UDP-glucose, and UDP-galactose as marker compounds. Chromatograms were developed in 1N acetic acid: 3M LiCl (9:1, v/v) for 12 cm, ascending [20]. The marker compounds have distinct R_f values in this system, except for UDP-glucose and UDP-galactose. The region marked by UMP was identified under ultraviolet light and cut from the dried chromatogram. UMP was eluted in 1 ml of 1N HCl, and assayed for radioactivity in the above scintillation fluid to which 33% by volume Triton X-100 (M & H Distributors, Winston-Salem, N. C.) had been added.

The ^{3}H-radioactivity in UMP per 0.100 ml of brain homogenate was determined by measuring the dilution of ^{14}C-UMP in the original homogenate during the isolation procedure and chromatography, using the formula: ^{3}H-UMP in 0.100 ml of homogenate = ^{3}H in UMP spot × (^{14}C in 0.100 ml of homogenate)/(^{14}C in UMP spot).

Experiment 2. Trained mice and quiet mice (8 per group) were given intracranial injections of ^{3}H-uridine. After the appropriate behavioral treatment and elapsed time, each mouse was sacrificed by immersion in liquid Freon (−155°C). The brain was chiseled out and pulverized under liquid nitrogen. The brain powder was placed in 5 ml of ice-cold 0.6 N PCA containing 0.100 μCi of 4-^{14}C-UMP and homogenized as described above.

The ^{3}H incorporated from uridine into RNA in the brains of 5 of these trained and 5 of these quiet mice was measured by two different methods. The first method employed PCA as the precipitating agent for RNA. For determining total ^{3}H, 3 samples of 100 μl of the brain homogenate was pipetted directly into a scintillation vial. For each of 3 RNA_F samples, 100 μl of homogenate was pipetted into a test tube containing 1 ml of ice-cold 0.2 N PCA, vortexed, and then filtered through a 2.4 cm Whatman GF/C glass filter disc. Each test tube was washed twice with 10 ml of cold 0.2 N PCA and these washings were filtered through the glass filter. RNA_F and total homogenate samples were dried at room temperature for 18 hr and further treated and assayed for radioactivity as described for similar samples in Experiment 1.

The second method for measuring ^{3}H in RNA was adapted from the alkaline hydrolysis procedure of Munro and Fleck [18]. A 1.00 ml aliquot (two replicates) of each

brain homogenate was centrifuged at 1000 x g for 10 min at 4°C. The pellet was washed twice with 1 ml of 0.2 N PCA, and the supernatants from the three centrifugations were combined and saved for nucleotide analysis, as described below. The pellet was dissolved in 2 ml 0.3 N NaOH and incubated at 37°C for 1 hr. After cooling to 4°C, DNA and protein were precipitated from the samples by adding 2 ml of 0.6 N PCA. After standing for 20 min in the cold, the samples were centrifuged at 1000 g for 10 min, and the RNA hydrolysate fraction was decanted and saved. The pellet was washed twice with 2 ml of 0.2 N PCA, centrifuged each time, and the supernatants were added to the RNA hydrolysate fraction. Aliquots (0.4 ml) of the RNA hydrolysate fraction (RNA_H) were added to 10 ml of Triton-containing scintillation fluid and assayed for radioactivity.

Charcoal was added to the PCA supernatant, and the nucleotides and nucleosides were purified and chromatographed as described for Experiment 1. Each chromatogram was cut in its entirety into six pieces, each containing one of the spots marked by the nonradioactive carrier compounds uridine, CMP, UMP, UDP-Sugars, UDP and UTP. The spots were eluted in 1 N HCl and the eluate was assayed for radioactivity as described for Experiment 1. The ^3H in UMP per unit volume of homogenate was determined by the isotope dilution procedure described in Experiment 1. This procedure allowed ^3H-UMP to be compared to total ^3H and ^3H-RNA in the brain homogenate. The relative proportion of the ^3H in each of the nucleotide fractions was calculated as: (^3H-dpm in spot)/(total of ^3H-dpm in all six regions of the chromatogram).

Significance levels of differences between means were determined by *t*-tests for populations with unequal variances.

RESULTS

Experiment 1

The 6 trained mice made a mean of 24 ± 1 (SEM) avoidance responses during the 40 training trials.

The radioactivity in UMP was combined with that in CMP in these results (Table 1) because the chromatography plates did not consistently resolve these two compounds. The relatively large standard deviations associated with the means of the direct radioactivity measures (RNA, UMP + CMP, soluble) reflect variability in the injection procedure, and prevent any of the simple radioactivity differences from reaching statistical significance.

As found previously, avoidance training did not alter the ratio of radioactivity in RNA to the radioactivity in soluble compounds. Training did, however, lower the amount of radioactivity in compounds that chromatographed as UMP plus CMP, relative to the soluble radioactivity, by 11%. This apparent loss of radioactivity from UMP plus CMP algebraically elevated the calculated ratio of dpm in RNA to dpm in nucleotide monophosphates in trained mice. These results are similar to those seen using subcutaneous injections of precursor [12].

Experiment 2

The 8 trained mice made a mean of 19 ± 4 (SEM) avoidance responses during the 40 training trials.

The radioactivity recovered from the alkaline hydrolysis (RNA_H, Table 2) was consistently about 35% greater than the radioactivity retained on the glass fiber filters (RNA_F), indicating that the filters do not retain all of the RNA.

TABLE 1

EFFECTS OF AVOIDANCE TRAINING ON INCORPORATION OF URIDINE INTO RNA AND NUCLEOTIDE POOLS IN BRAIN. RESULTS ARE MEAN ± S.D. FOR 6 MICE IN EACH GROUP. (dpm, PER 100 μl HOMOGENATE)

	Trained	Quiet	% Difference
RNA dpm	12,400 ±4,600	14,000 ±6,500	−11.4
UMP + CMP dpm	19,600 ±7,400	24,500 ±10,900	−20.0
Soluble dpm*	64,900 ±20,100	72,600 ±31,000	−10.6
RNA/(UMP + CMP)	0.631 ±0.056	0.568 ±0.045	+11.1†
RNA/Soluble	0.233 ±0.023	0.235 ±0.018	− 0.9
(UMP + CMP)/Soluble	0.370 ±0.043	0.414 ±0.013	−10.6†

*Soluble dpm = Total dpm − RNA dpm.
†$p<0.05$, one-tailed *t*-test.

Avoidance training had no effect on the ratio of the radioactivity in RNA, determined by either method, to the radioactivity in the total homogenate. Training lowered the proportion of homogenate radioactivity that was recovered as UMP, in this case by 17% on the average.

This experiment also assayed radioactivity in the major classes of uridine-containing compounds. The low temperature methods did not protect the nucleotide triphosphates from hydrolysis. The data of Piccoli *et al.* [19] suggest that the ratio of net radioactivity for UTP to UMP in the resting state at 45 min after injection of ^3H-uridine is on the order of 5. The present methods resulted in a ratio of about 0.04 (Table 3). The failure of this extraction method to preserve UTP was subsequently traced to hydrolysis during the purification steps of the nucleotide analysis. Control experiments showed that less than 5% of the radioactivity in samples of commercially prepared 5-^3H-UTP survived the purification steps to chromatograph as UTP, even when there was no brain tissue present in the initial 0.6N PCA solution. Thus, the radioactivity that is isolated as UMP using these procedures [1, 2, 7, 8, 12, 22, 23] is derived in large part from the degradation of UTP and UDP.

A 20% decrease of radioactivity in UMP in the brains of the trained mice was apparent against the baseline of radioactivity in all nucleotides (Table 3). This was accompanied by a relative gain of about 60% of radioactivity that chromatographed as UDP-sugars. The proportion of radioactivity that chromatographed as uridine, UDP, UTP, and CMP were not significantly affected by training.

Figure 1 indicated that there were no peaks of radioactivity in the chromatograms other than those that were

TABLE 2

EFFECTS OF AVOIDANCE TRAINING ON INCORPORATION OF URIDINE INTO RNA AND NUCLEOTIDE POOLS IN BRAIN. RESULTS ARE MEAN ± S.D. FOR 5 MICE IN EACH GROUP. (dpm, PER 100 μl OF HOMOGENATE)

	Trained	Quiet	% Difference
RNA_F dpm*	33,400 ±12,100	36,400 ±11,100	− 8.2
RNA_H dpm*	45,300 ±22,800	49,500 ±14,900	− 8.5
Total dpm	166,800 ±73,000	179,100 ±48,400	− 6.9
UMP dpm	50,800 ±17,700	67,700 ±17,600	−25.0
RNA_H/RNA_F	1.350 ±0.176	1.360 ±0.130	− 0.7
RNA_F/UMP	0.653 ±0.025	0.534 ±0.048	+22.3§
RNA_H/UMP	0.884 ±0.130	0.726 ±0.056	+21.8†
RNA_F/Total	0.204 ±0.051	0.202 ±0.012	+ 1.0
RNA_H/Total	0.273 ±0.018	0.274 ±0.015	− 0.4
UMP/Total	0.314 ±0.032	0.380 ±0.031	−17.4‡

*RNA_F samples were prepared by filtering PCA homogenates through glass fiber discs. RNA_H samples were prepared by alkaline hydrolysis of the PCA-insoluble fraction of the homogenates.

†$p<0.05$; ‡$p<0.01$; §$p<0.001$; two-tailed t-tests.

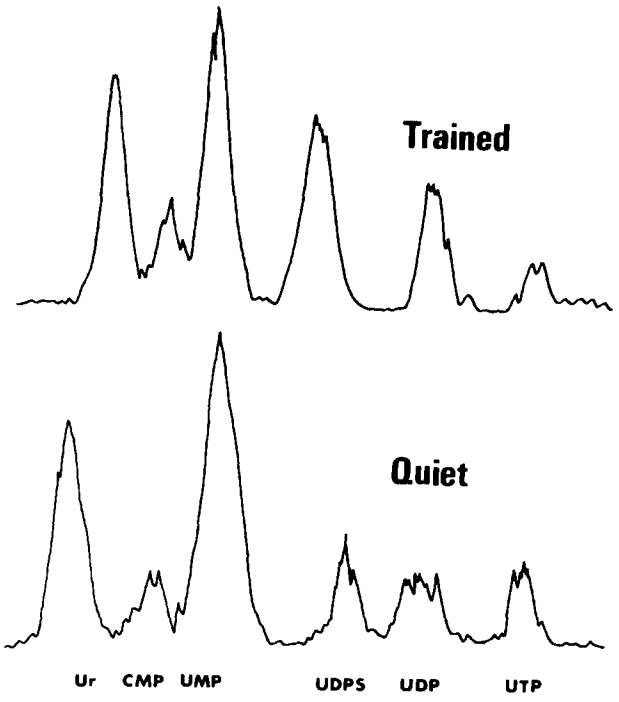

FIG. 1. Radioactivity in uridine compounds isolated from the brain of one trained mouse and one quiet mouse. Chromatograms prepared as described in *Method* from the brains of two trained and two quiet mice from Experiment 2 were assayed for radioactivity in a Packard 7201 Radiochromatogram Scanner (Gain - 3×10^3, TC = 3 sec, linear count scaling, 5 mm window, scanned at 2 cm/min). One set of chromatograms is shown. The second set also displayed the relative increase in the radioactivity in uridine-diphosphate sugars in the brain of the trained mouse that is apparent above.

cut out for scintillation counting. The scanned chromatograms also gave substantiating evidence for a relative shift of radioactivity from the UMP region to the UDP-sugar region of the chromatograms from the brains of trained mice.

DISCUSSION

The identity of the compounds that have been chromatographed in these experiments is only partially specified at this time. Other compounds that are present in brain and chromatograph into the various regions in this system (S.D. Davis, unpublished) are listed in Table 3. The brain possesses enzymes that convert UTP to cytidine triphosphate [10]. It is estimated from the values for CMP and UMP in Table 3 that cytidine compounds contribute about 10% of the radioactivity in the UTP, UDP and UDP-sugar spots.

The present results indicate that avoidance training reduces the amount of ^{3}H-uridine that is incorporated into cerebral compounds that chromatograph as UMP, while elevating the amount of ^{3}H-uridine in compounds that chromatograph as UDP-sugars. Most of the radioactivity that chromatographed as UMP was probably derived from degraded UTP and UDP, but it is possible that some of the radioactivity in the UMP was derived from UDP-sugars. Although the distribution of radioactivity amongst the nucleotides given by the data in Table 3 is not an accurate picture of physiological states, some firm conclusions can be drawn.

Radioactivity in UMP in the brains of trained mice was decreased by about the same percentage magnitude when compared to the three baselines of soluble radioactivity (−17%), RNA (−18%), and radioactivity in all chromatographed nucleotides (−20%). Thus any of these three measures forms a stable background against which training-induced changes in the distribution of radioactivity amongst the nucleotides may be analyzed. This relative stability of the baseline measurements enhances the likelihood that the changes depicted in Table 3 represent a physiological shift of radioactive uridine from the UMP-UDP-UTP pool to the UDP-sugar pool in the brains of trained mice.

These data contribute to indications that cerebral pyrimidine metabolism is altered by various forms of stimulation [3,5]. Thus, the use of radioactive pyrimidines to measure effects of stimulation on RNA synthesis is a complex matter. As a minimum, repeated measurements of

TABLE 3

EFFECTS OF AVOIDANCE TRAINING ON THE DISTRIBUTION OF [^{3}H] URIDINE INTO MOUSE BRAIN NUCLEOTIDES. THE METHODS USED FOR FREEZING THE BRAINS AND ISOLATING THE NUCLEOTIDES ARE DESCRIBED IN THE TEXT. RESULTS ARE MEAN ± S.D. FOR 8 ANIMALS IN EACH GROUP

R_f value of spot from center	Primary Radioactive Compound in Spot (Marker Compound)	Other Radioactive Compounds in Spot	Percentage of Nucleotide Radioactivity in Spot		% Increase in Brains of Trained mice
			Quiet	Trained	
96	Uridine	Cytidine, Uracil Cytosine	17.1 ± 3.0	17.6 ± 3.5	+ 2.9
84	CMP	Unidentified Nucleotide Sugars	3.8 ± 1.0	4.1 ± 1.4	+ 7.9
68	UMP	UDP-N-acetylglucosamine	51.9 ± 1.6	41.3 ± 3.7	−20.4*
44	UDP-glucose and UDP-galactose	CDP	14.3 ± 2.6	23.2 ± 2.6	+62.2*
24	UDP	CTP	10.5 ± 2.1	11.6 ± 1.5	+10.5
12	UTP	CTP, UDP-glucuronic acid	2.4 ± 1.3	2.8 ± 1.8	+16.7
	TOTAL:		100.0	100.6	

*$p<0.001$, two-tailed t-test.

the specific radioactivities of the precursor at various times within the period of interest must be made before inferences about rates of RNA synthesis can be drawn from such studies [17]. When studies of this sort rely on a single measurement of precursor radioactivity [1, 2, 6, 7, 8, 11, 12, 16, 21, 22, 23], changes of radioactivity in RNA indicate that the metabolic fate of uridine or cytidine is altered by stimulation, but give little concrete information about the metabolic step at which the change occurred.

The present results can be compared most directly to studies of cerebral RNA metabolism made by Zemp, Adair, and Coleman and their associates [1, 2, 7, 8, 22, 23]. Those studies used the radioactivity in UMP, purified and chromatographed by methods nearly identical to those used here as the baseline correction factor for the uptake of radioactivity into brain cells. The present data lend additional support to the view [12] that substantial portions of those earlier effects, particularly those reported for nuclear RNA [22,23], can be accounted for on the basis of training-induced changes in radioactivity in UMP rather than by absolute increases in the incorporation of uridine into RNA. Because incorporation was measured at only one time point the present data are insufficient to determine whether or not changes in nucleotide metabolism account for the changes detected in the incorporation of uridine into polyribosome-associated RNA during avoidance training [1, 2, 7, 21, 23].

The route by which precursor compounds are introduced into the brain is an important factor in the use of radiochemicals to study cerebral metabolism. For example, alterations in the matabolism of cytidine and/or RNA in rat brain during reversal learning are apparent after intravenous injections of ^{3}H-cytidine, but not after intracranial injections of the same precursor [16]. The changes in radioactivity in UMP discussed here are apparent after either intracranial or subcutaneous [12] injections. Thus, it seems likely that these changes occurred within the cells of the analysed tissue, or in the uptake of precursor into those cells. Until otherwise demonstrated, changes that are detectable after peripheral, but not after central, injections of precursor must be suspected to be related to changes in cerebral blood flow, or in the penetration of the blood-brain barrier.

The present results can be viewed as evidence of a stimulation-induced change in the metabolism of cerebral uridine-diphosphate sugars. The changed distribution of radioactivity in UDP-sugars could have resulted from changes in the rate of their synthesis, or degradation, or both. These compounds serve as cofactors in the synthesis of glycogen, glycoproteins, gangliosides and lipids [17]. Thus it is striking that behavioral stimulation has been reported to alter the cerebral metabolism of both glycoproteins [4,9] and gangliosides [15]. A failure to find effects of stimulation on glycoproteins has also been reported [13]. Further work is needed to determine if the chemical changes reported here are the products of interactions of nucleotides with glycosidic compounds.

These biochemical changes may be related either to some fundamental aspect of avoidance learning or to stresses or forms of exercise peculiar to avoidance learning. The training paradigm induced effective avoidance responding. Such training induces long-term memory storage, since

mice of the same strain, age, sex, supplier, etc. show good savings for the avoidance response when retested 1, 2, 3 or 9 days after one 15-min training session [1; Entingh, unpublished]. Yoked mice (paired in stimuli to mice that were being trained, but given no shelf to which to make effective avoidance responses) tended not to show decreased incorporation of ^{3}H-uridine into UMP [12] or increased RNA/UMP radioactivity ratios [1,12]. This suggests that the general stimulatory effects of the training situation are not sufficient to trigger these changes. The question of whether particular stresses contribute to these chemical changes will remain open until stress and its effects can be defined and measured much more precisely than present ideas and techniques allow [14].

REFERENCES

1. Adair, L. B., J. E. Wilson and E. Glassman. Brain function and macromolecules, IV. Uridine incorporation into polysomes of mouse brain during different behavioral experiences. *Proc. natn. Acad. Sci.* **61**: 917–922, 1968.
2. Adair, L. B., J. E. Wilson, J. W. Zemp and E. Glassman. Brain function and macromolecules, III. Uridine incorporation into polysomes of mouse brain during short-term avoidance conditioning. *Proc. natn. Acad. Sci.* **61**: 606–613, 1968.
3. Baskin, F., F. R. Masiarz and B. W. Agranoff. Effect of various stresses on the incorporation of [^{3}H] orotic acid into goldfish brain RNA. *Brain Res.* **39**: 151-162, 1972.
4. Bogoch, S. *The Biochemistry of Memory with an Inquiry into the Function of the Brain Mucoids.* New York: Oxford University Press, 1968.
5. Bonavita, V. and F. Piccoli. Brain nucleotides and excitatory processes. *Adv. exp. Biol. Med.* **13**: 355–380, 1971.
6. Bowman, R. E. and D. A. Strobel. Brain RNA metabolism in the rat during learning. *J. comp. physiol. Psychol.* **67**: 448–456, 1969.
7. Coleman, M. S., B. Pfingst, J. E. Wilson and E. Glassman. Brain function and macromoles, VIII. Uridine incorporation into brain polysomes of hypophysectomized rats and ovariectomized mice during avoidance conditioning. *Brain Res.* **26**: 349–360, 1971.
8. Coleman, M. S., J. E. Wilson and E. Glassman. Incorporation of uridine into polysomes of mouse brain during extinction. *Nature* **229**: 54–55, 1971.
9. Damstra-Entingh, T., D. J. Entingh, J. E. Wilson and E. Glassman. Environmental stimulation and fucose incorporation in brain and liver glycoproteins. *Pharmac. Biochem. Behav.* **2**: 73–78, 1974.
10. Dawson, D. M. Enzymatic conversion of uridine nucleotide to cytidine nucleotide by rat brain. *J. Neurochem.* **15**: 31–34, 1968.
11. Elias, M. F. and B. E. Eleftheriou. Reversal learning and RNA labeling in neurological mutant mice and normal littermates. *Physiol. Behav.* **9**: 27–34, 1972.
12. Entingh, D. J., T. Damstra-Entingh, A. Dunn, J. E. Wilson and E. Glassman. Brain uridine monophosphate: reduced incorporation of uridine during avoidance learning. *Brain Res.* **70**: 131–138, 1974.
13. Holian, O., E. G. Brunngraber and A. Routtenberg. Memory consolidation and glycoprotein metabolism; A failure to find a relationship. *Life Sci.* **10**: 1029–1035, 1971.
14. Horn, G., S. P. R. Rose and P. P. G. Bateson. Experience and plasticity in the central nervous system. *Science* **181**: 506–514, 1973.
15. Irwin, L. N. and F. E. Samson. Content and turnover of gangliosides in rat brain following behavioral stimulation. *J. Neurochem.* **18**: 203–211, 1971.
16. Kottler, P. D., R. E. Bowman and W. D. Haasch. RNA metabolism in the rat brain during learning following intravenous and intraventricular injections of ^{3}H-cytidine. *Physiol. Behav.* **8**: 291–297, 1972.
17. Mahler, H. R. and E. H. Cordes. *Biological Chemistry.* New York: Harper and Row, 1971.
18. Munro, H. N. and A. Fleck. The determination of nucleic acids. In: *Methods of Biochemical Analysis,* edited by D. Glick, Vol. 14. New York: Interscience, 1966, pp. 113–176.
19. Piccoli, F., R. Camarda and V. Bonavita. Purine and pyrimidine nucleotides in the brain of normal and convulsive rats. *J. Neurochem.* **16**: 159–169, 1969.
20. Randerath, K. and E. Randerath. Thin-layer separation methods for nucleic acid derivatives. In: *Methods in Enzymology,* edited by L. Grossman and K. Moldane, Vol. 12. New York: Academic Press, 1967, pp. 323–347.
21. Uphouse, L. L., J. W. MacInnes and K. Schlesinger. Effects of conditioned avoidance training on polyribosomes of mouse brain. *Physiol. Behav.* **8**: 1013–1018, 1972.
22. Zemp, J. W., J. E. Wilson and E. Glassman. Brain function and macromolecules. II. Site of increased labelling of RNA in brains of mice during a short-term training experience. *Proc. natn. Acad. Sci.* **58**: 1120–1125, 1967.
23. Zemp, J. W., J. E. Wilson, K. Schlesinger, W. O. Boggan and E. Glassman. Brain function and macromolecules. I. Incorporation of uridine into RNA of mouse brain during short-term training experiences. *Proc. natn. Acad. Sci.* **55**: 1423–1431, 1966.