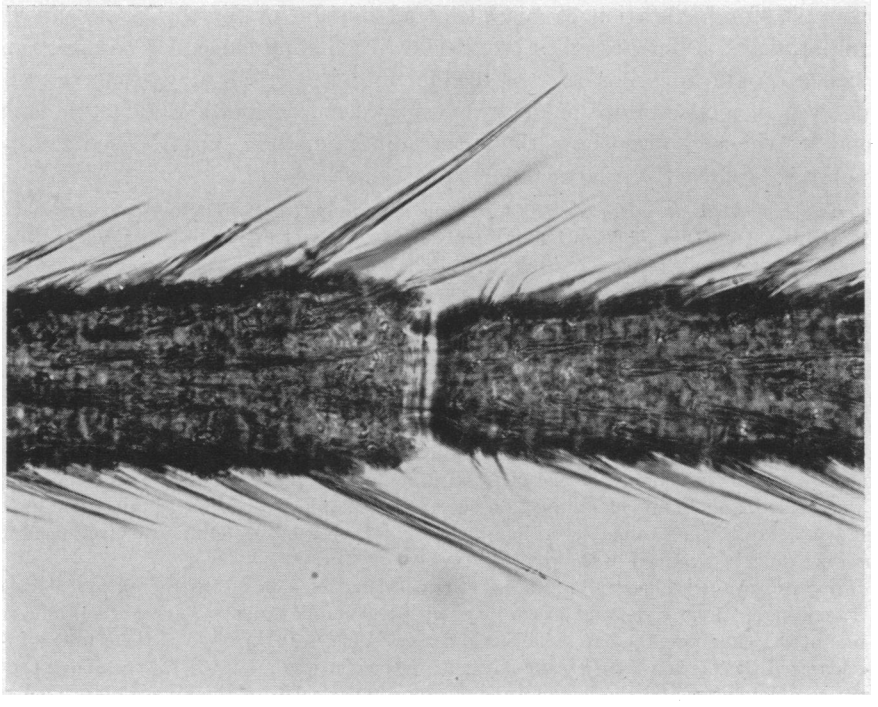

FIG. 3.—An enlarged photograph of portions of two cockroach antenna segments. ×280.

in his brown armor, carries with him a workable version of the olfactory sense. He appears therefore to be employable in our search for the secrets of that sense.

[1] The evaluation and reading of the records, and the statistical computations, were made by qualified technicians.

---

*BRAIN FUNCTION AND MACROMOLECULES, I. INCORPORATION OF URIDINE INTO RNA OF MOUSE BRAIN DURING SHORT-TERM TRAINING EXPERIENCE**

BY JOHN W. ZEMP†, JOHN ERIC WILSON, K. SCHLESINGER, W. O. BOGGAN, AND EDWARD GLASSMAN‡

DEPARTMENTS OF BIOCHEMISTRY AND PSYCHOLOGY, AND THE GENETICS CURRICULUM, UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL

Communicated by J. N. Couch, March 29, 1966

A number of investigations have shown a relation between brain ribonucleic acid (RNA) and learning.[1, 2] Indirect evidence also implicates RNA in memory, since interference with brain protein synthesis has been reported to interfere with memory storage in the mouse[3, 4] and in the goldfish.[5] These experiments have raised the possibility of an RNA memory code, but the results could also be explained by the role of RNA in general protein synthesis which might be necessary,

for example, for facilitation of synaptic transmission or for the establishment of neural pathways. The present study deals with the influence of a 15-min training experience on the incorporation of uridine into the RNA of various subcellular components of mouse brains as determined by use of a double radioactive labeling method.[6] We shall also show the intracellular location and the sedimentation characteristics of the RNA labeled during this experience.

Materials and Methods.—Procedure: For each experiment, a pair of 6- to 8-week-old male C57BL/6J mice from The Jackson Laboratory was used. After light anesthetization with ether, one mouse was given an *intracranial* injection of 2 μc of uridine-2-C^{14} (approximately 40 mc per millimole from Nuclear-Chicago Corp.), and the other was given 10 μc of uridine-5-H^3 (14.3 c per millimole from Nuclear-Chicago) or uridine-5,6-H^3 (2.7 c per millimole from Schwarz Bio-Research). Each mouse received 10 μl on each side of the mid-line into the frontal lobe to a depth of 3 mm. Similar injections with trypan blue showed that the dye was distributed throughout the subarachnoid space almost immediately after injection; radioautography of brain from animals sacrificed 30 min after injection showed labeling of nuclei in areas remote from the site of the injection. When the labeling of RNA in liver and kidney was studied, *intraperitoneal* as well as *intracranial* injections of 2 μc of C^{14}-uridine and 10 μc of H^3-uridine were given. The mice were coded, and 30 min after injection one of the pair was trained for 15 min while the other served as a control. The animals were sacrificed by decapitation 0–30 min after training and the brains were quickly removed in the cold.

Training apparatus: The training apparatus consisted of a box constructed of 1.9-cm-thick pine, 19 cm high, 25 cm wide, and 45 cm long, with two compartments sharing a continuous grid floor mounted 1 cm above the base. This grid consisted of $^3/_{32}$-in. brass rods, the centers of which were separated by 8.5 mm. An escape shelf, 2.5 cm wide, was mounted 7.3 cm above the floor and ran completely around the inside walls of one compartment of the apparatus. The second compartment lacked the escape shelf but was otherwise identical to the first. Each compartment was covered with a removable plastic lid, and the whole device rested on 2.5-cm-high supports.

Pressing a microswitch on a Hunter timer actuated a buzzer and a 60-watt lamp for 3 sec. If the mouse did not jump, a response timer and a shock generator (Grayson-Stadler model E10664-ES) were activated for the duration of the trial. The buzzer, attached to the outside of the box at the juncture of the two compartments, made a sound and vibrated the box. The light was mounted above the buzzer and illuminated the two compartments equally.

Training procedure: Mice, trained individually, were given 30 sec to explore the escape shelf and 30 sec to explore the grid floor. The light and buzzer (conditioned stimuli, CS) were presented for 3 sec. Termination of the CS was followed by the onset of an electric shock of 0.2 ma at 340 v d-c delivered through the grid floor. The shock remained on until the animal jumped from the grid floor to the escape shelf. The animal was allowed to remain on the shelf for 15 sec, while the timer was reset and the escape latency recorded. The animal was then placed on the grid floor for the next trial. The average interval from the start of one trial to the start of the next was approximately 25 sec. Animals were judged to have made an avoidance response if they jumped to the escape shelf before the onset of the shock. Training was continued for 15 min. The intracranial injection had no significant effect on performance. Three types of control animals were used: (1) *Yoke control* mice were kept in the second compartment of the box while the experimental animals were being trained. Yoke control mice received light, buzzer, and electric shocks at the same time as the trained animals, and were handled to the same degree, but in a random manner. (2) *Quiet control* mice were kept in their home cages as undisturbed as possible after the injection. (3) *Shock control* mice were shocked 30 times during the 15-min period.

Tissue fractionation: The fractionation procedures (modified from ref. 7) were performed at 0–4°C. Brains from the two lightly anesthetized mice were quickly removed, sectioned just behind the cerebellum, and homogenized together in 7 ml of medium A (0.005 *M* Tris-HCl, 0.025 *M* KCl, 0.005 *M* $MgCl_2$, and 0.25 *M* sucrose, pH 7.6) containing 10 μg per ml of polyglucose sulfate (kindly supplied by Dr. Peter Mora) or polyvinyl sulfate (General Biochemicals, Inc.). The homogenate was centrifuged at 900 $\times$ *g* for 10 min. The supernatant solution was saved and the sediment was dispersed in 6 ml of medium A and centrifuged again for 10 min at 900 $\times$ *g*. The sediment (I) was the source of nuclei as described below. The supernatant solutions were

combined and centrifuged at 15,000 × *g* for 10 min and the sediment was used as the crude mitochondrial fraction. The supernatant solution was stirred for 5 min with 1/9 vol of freshly prepared 5% sodium deoxycholate in 0.03 *M* Tris-HCl, pH 8.2, and centrifuged at 105,000 × *g* for 90 min. The resulting pellet was the source of ribosomes,while the supernatant solution was used to prepare soluble RNA. Nuclei were partially purified by dispersing sediment I in 1.5 *M* sucrose and centrifuging for 1 hr at 30,000 × *g*.[8]

RNA preparation: All steps were carried out at 0–4°C. The nuclear fraction was suspended in 2 ml of salt medium (medium A lacking sucrose) containing 50 μg per ml of deoxyribonuclease I (electrophoretically purified, from Worthington Biochemical Corp.) and *gently* stirred for 5 min. Sodium lauryl sulfate (from Fisher Scientific Co., purified by the method of Crestfield *et al.*[9]) was added to a concentration of 1%, and *gentle* stirring was continued for an additional 25 min. An equal volume of phenol saturated with water (the only brand which gave good results was Baker-Adamson) was added and *gentle* stirring was continued for 30 min. The phases were separated by centrifugation at 30,000 × *g* for 10 min and the interphase layer was extracted with salt medium for 15 min and centrifuged. The combined aqueous phases were extracted again with fresh phenol for 10 min. The interphase layer contained radioactive material with the same ratio of isotopes as that found for the extracted RNA. The ribosomal fractions were treated similarly, except that no deoxyribonuclease treatment was used, there was only a 15-min treatment with 0.5% sodium lauryl sulfate, and the first phenol extraction lasted 15 min. The supernatant solution after centrifugation at 105,000 × *g* was extracted once with phenol for 30 min.

RNA was precipitated from each fraction by the addition of 0.2 vol of 10% NaCl and 2 vol of 95% ethanol. Precipitation time was 1 hr at −10°C. Nuclear RNA was dissolved in 2 ml salt medium and the other fractions were dissolved in 0.01 *M* acetate buffer, pH 5.0. The nuclear fraction was digested for 10 min at 0°C with 50 μg per ml of deoxyribonuclease I. Following precipitation with salt and ethanol, the RNA from each fraction was dissolved in 1 ml of 0.01 *M* acetate buffer, pH 5.0, for sucrose gradient centrifugation or for assay of radioactivity.

Radioactivity incorporated into the crude mitochondrial fraction was determined after digestion with 1 *M* $HClO_4$ for 40 min at 100°C.

Sucrose density gradient centrifugation: RNA in 1 ml of 0.01 *M* acetate buffer, pH 5.0, was layered over a 30-ml linear 5–15% sucrose gradient containing 10^{-4} *M* EDTA in 0.01 *M* acetate, pH 5.0, and centrifuged for 18 hr at 25,000 rpm in the SW 25.1 rotor of a Spinco model L ultracentrifuge. Rotor temperature was 8°C. Fractions were obtained by piercing the bottom of the centrifuge tube and collecting the drops. Absorbance at 260 mμ was determined on each fraction, and radioactivity was assayed[10] in a Packard Tri-Carb liquid scintillation spectrometer. The cpm of H^3 and C^{14} were calculated as described by Kabara *et al.*[11] The efficiency of C^{14} counting was set to a value five times that of the H^3 efficiency; for this reason the injected dose of H^3 was five times that of C^{14}.

Determination of radioactivity in uridylic and cytidylic acid of brain: Uridylic and cytidylic acids were separated from the soluble supernatant fraction after alcohol precipitation of the sRNA. This fraction was evaporated to a volume of 5 ml and then extracted 6–10 times with ether to remove phenol, and an equal volume of 2 *M* $HClO_4$ was added. It was then treated with 100 mg of charcoal (Norit A, acid-washed) which was removed by centrifugation and washed. Adsorbed nucleotides were eluted from the charcoal with 10% pyridine in 50% aqueous ethanol.[12] The eluate was evaporated to dryness under a stream of air. The residue was then dissolved in 20 μl of water, and the nucleotides from a 5-μl aliquot were separated on thin layers of polyethyleneimine cellulose.[13] Isotope assays were performed on eluates of the uridylic and cytidylic acid spots in order to determine the ratio of C^{14} to H^3. The ratio of the isotopes in this pool was taken as an indicator of the relative amounts of isotope which had penetrated into the brain.

Results.—Table 1*a* summarizes the data obtained using 25 pairs of mice sacrificed immediately after the 15-min training period. Each pair consisted of a trained mouse and its yoke control. In all cases the RNA from the brain of the trained mouse had a greater amount of radioactivity than the RNA from the brain of the yoke control. Trained mice had 6.5–119 per cent more label in RNA from nuclei, and 7–180 per cent more label in RNA from ribosomes. Using this increase as a criterion, it was possible to identify correctly the trained mouse in

TABLE 1

INCORPORATION OF URUDINE INTO RNA FROM BRAIN, LIVER, AND KIDNEY OF TRAINED AND CONTROL MICE SACRIFICED IMMEDIATELY AFTER TRAINING

Mice	Isotope in trained	Ratio of Labels (Trained:Control) RNA Ribosomes	RNA Nuclei	UMP	Increase, % (Corrected for Pool When Available) Ribosomes	Nuclei
(*a*) *Brain*						
7/8*	H^3	2.63	1.62	—	163.0	62.0
9/10*	H^3	1.14	1.10	—	13.9	9.5
11/12	C^{14}	1.70	1.06	—	70.1	6.5
23/24	H^3	1.39	1.45	—	39.2	44.6
35/36	C^{14}	—	1.57	—	—	57.1
37/38*	H^3	—	1.27	—	—	26.9
41/42*	H^3	1.73	—	—	72.7	—
45/46	C^{14}	1.14	1.53	—	14.0	53.0
49/50*	H^3	2.66	2.19	—	165.7	119.0
53/54*	H^3	1.68	—	—	68.2	—
67/68*	H^3	2.79	—	—	179.5	—
83/84	C^{14}	1.17	1.38	—	17.1	38.4
89/90	H^3	1.54	1.13	—	53.8	12.7
95/96*	H^3	1.71	1.94	—	71.1	93.9
115/116	C^{14}	—	1.20	—	—	20.0
121/122	C^{14}	1.30	1.24	0.77	61.0	68.8
127/128	H^3	1.55	1.25	1.01	55.0	25.0
141/142	C^{14}	—	1.08	0.88	—	18.5
147/148	C^{14}	1.45	1.39	1.03	40.7	34.9
153/154	H^3	2.59	1.87	1.50	72.7	24.7
159/160	C^{14}	—	1.11	1.00	—	11.0
165/166	H^3	—	1.00	0.66	—	51.5
245/246	C^{14}	0.92	0.88	0.65	41.5	35.4
251/252	C^{14}	1.48	1.53	1.31	16.8	12.9
253/254	H^3	1.56	1.53	1.43	7.0	9.1
(*b*) *Liver*						
147/148	C^{14}	1.00	0.98	1.00	0	−2.0
153/154	H^3	0.71	0.67	0.70	1.4	−4.3
245/246	C^{14}	0.83	0.94	0.81	2.4	16.0
251/252	C^{14}	1.47	1.34	1.43	2.8	−6.3
253/254	H^3	0.33	0.43	0.40	−17.5	7.5
(*c*) *Kidney*						
141/142	C^{14}	0.84	—	0.85	−1.1	—
147/148	C^{14}	1.00	0.98	1.00	0	−2.0
153/154	H^3	0.74	0.72	0.70	5.7	2.8
247/248	C^{14}	1.22	1.25	1.19	2.5	5.0

As described in *Materials and Methods*, one mouse was given an intracranial injection of H^3-uridine, and another mouse was given C^{14}-uridine. Those mice in which liver and kidney were used were given an additional injection intraperitoneally. After 30 min, one mouse was trained while the other served as a control; the mice were coded following the injection, and the animal to be trained was selected at random. The training experience lasted 15 min, after which the brains were quickly removed and homogenized together. Ribosomes and nuclei were isolated, and the RNA was extracted from each. The amount of C^{14} and H^3 was determined. In addition, UMP was isolated from the supernatant solution after removal of nuclei and ribosomes, and the amount of C^{14} and H^3 in it was determined. The degree of labeling of the UMP and of the RNA isolated from nuclei and ribosomes is presented as the ratio of the radioactive labels in trained:control mice.

* The control mouse of these pairs was a quiet control.

all 25 pairs, even though the correction for the ratio of C^{14} and H^3 in the UMP precursor pool was not always available. To test whether this increase is specific to brain, liver and kidney as well as brain were analyzed in four pairs of mice (Table 1*b* and *c*). No consistent differences were observed between the labeling of RNA from livers and kidneys from trained mice and yoke controls.

The loss of tritium by H^3-uridine and its retention by mitochondria obscure any differences that might exist in this fraction. The crude mitochondrial fraction is of interest because it also includes nerve endings.[14] Work is continuing, using added RNA as carrier. The ratio of H^3 to C^{14} in soluble RNA was in all cases similar to the ratio of label in the UMP or CMP pool.

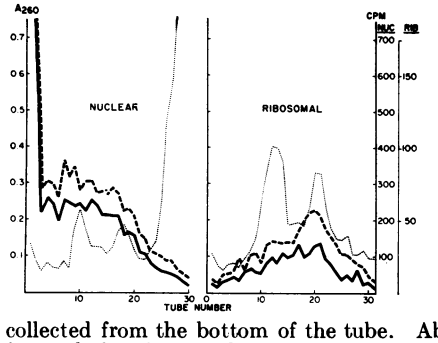

FIG. 1.—Sucrose gradient centrifugation of RNA from brains of trained and yoke control mice sacrificed immediately after training. As described in Table 1 and in *Materials and Methods*, one mouse (no. 153) was injected intracranially and intraperitoneally with H^3-uridine, and another mouse (no. 154) similarly with C^{14}-uridine. After 30 min, the former mouse was trained while the latter served as the yoke control. The training experience lasted 15 min, after which the brains were quickly removed and homogenized together. Ribosomes and nuclei were isolated and RNA was extracted from each fraction. This RNA was centrifuged in a 5–15% sucrose gradient in 30 ml in the Spinco SW 25 rotor for 18 hr at 25,000 rpm, and 1-ml fractions were collected from the bottom of the tube. Absorbance at 260 mμ, C^{14}, and H^3 were determined for each fraction. The results were corrected for the ratio of H^3:C^{14} in the UMP pool (see Table 1). *Solid line* = C^{14}, *dashed line* = H^3, *dotted line* = absorbance at 260 mμ.

Sedimentation patterns on sucrose gradients were obtained for nuclear and ribosomal RNA samples isolated from 22 of the pairs shown in Table 1*a*. Figures 1 and 2 show typical sedimentation patterns of RNA isolated from ribosomes and nuclei of brain and liver taken from the same animals (nos. 153 and 154). Not shown here is the pattern of RNA from kidney, which resembles that of liver except that the rapidly labeled RNA from kidney nuclei seemed degraded. It can be seen that there are no significant differences in amounts of radioactivity in RNA from liver, but that noticeable increases exist in RNA from the brains of the trained mice. The patterns resemble the sedimentation pattern of rapidly labeled RNA of liver reported by others.[15, 16] When nuclear or ribosomal RNA was digested with ribonuclease before gradient sedimentation, over 95 per cent of the radioactivity and absorbance was found at the top of the gradient.

Similar analyses were carried out on 28 pairs of mice sacrificed 15 min after

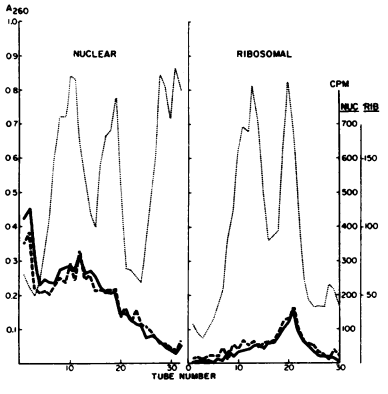

FIG. 2.—Sucrose gradient centrifugation of RNA from the livers of trained and yoke control mice sacrificed immediately after training. The livers from the same mice shown in Fig. 1 were treated exactly as the brains were. The RNA from nuclei and ribosomes were sedimented in a similar gradient, and all procedures were as described for Fig. 1. The *key* is the same as Fig. 1.

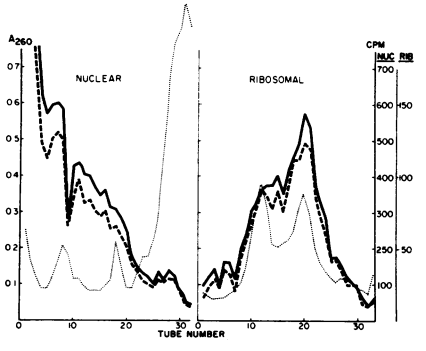

FIG. 3.—Sucrose gradient centrifugation of RNA from brains of trained and yoke control mice sacrificed 15 min after training. All procedures were as described in Table 1 and in Fig. 1, except that the trained mouse had been injected intracranially with C^{14}-uridine and the yoke control with H^3 (no intraperitoneal injection was given). In addition, the mice were not sacrificed until 15 min after the training experience ended. The *key* is the same as Fig. 1.

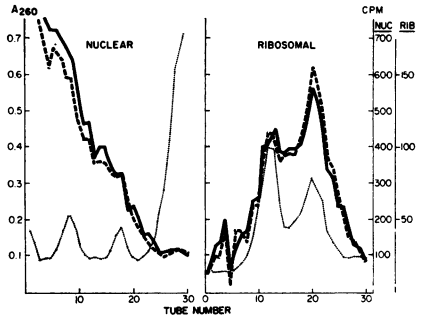

FIG. 4.—Sucrose gradient centrifugation of RNA from brains of trained and yoke control mice sacrificed 30 min after training. All procedures were as described in Table 1 and Fig. 1, except that the trained mouse had received an intracranial injection of H^3-uridine (no intraperitoneal injection was given). In addition, the mice were not sacrificed until 30 min after the training experience ended. The *key* is the same as Fig. 1.

training. Figure 3 shows that the differences in RNA labeling between trained and yoke control mice were reduced, especially in the ribosomal fractions. This is most likely due to the increase of labeling of the ribosomal peaks. It is of interest that 23 of these 28 pairs were identified correctly with respect to which mouse was trained. Unfortunately, the radioactivity in UMP was not examined, so we do not know whether this correction would have prevented these errors. Twelve pairs of mice were sacrificed 30 min after training (Fig. 4). None of these pairs showed consistent differences in labeling of RNA between trained and control mice; it should be noted, however, that any differences that might have persisted this long would be obscured by the heavy labeling of the ribosomal RNA.

To test whether the phenomenon observed was actually an *increase* in labeling of the RNA of the trained mouse, or a possible *decrease* in the yoke control, the yoke control mouse was compared with a mouse that had been put back into its home cage after the injection of the radioactive uridine, and that had not been disturbed until sacrificed 45 min later (quiet control). The yoke control mouse was treated as usual; it was placed in the appropriate compartment of the training apparatus while another mouse was being trained. The trained animal was then discarded. Table 2 shows that in four such experiments, no consistent differences in labeling of RNA from the brains of quiet and yoke controls were observed nor were significant differences observed in sedimentation patterns. We conclude that the differences between the trained mouse and its yoke control represent an increase in the labeling of RNA in the brain of the trained mouse and not a decrease in the yoke control. This conclusion is also supported by the results obtained when trained animals were compared with quiet controls (Table 1*a*).

These experiments also indicate that light flashes, buzzers, and the average of 12 electric shocks which the yoke control receives do not by themselves lead to an increase in labeling of RNA in brain. This finding led us to test the effects of excessive aversive stimulation on RNA synthesis. A mouse was placed in the half of the training box that lacked an escape shelf. A total of 30 shocks was given at random intervals for 15 min; each time the shock was applied, the mouse was observed to jump (shock control). Incorporation of radioactivity into RNA of brain was compared between shock control and quiet control animals. Four pairs of mice were so treated. Table 3 indicates that there are no significant differences in the incorporation of radioactive uridine into RNA in the brains of these animals. No differences were seen in sedimentation patterns.

Discussion.—Most of the indications that RNA metabolism is related to learning have been indirect or they have required prolonged periods of training of experimental animals. Owing to the reliability of the double labeling technique, the

TABLE 2

INCORPORATION OF URIDINE INTO RNA FROM BRAIN OF YOKE CONTROL AND QUIET CONTROL MICE SACRIFICED IMMEDIATELY AFTER TRAINING

Mice	Isotope in quiet control	Ratio of Labels (Yoke:Quiet Control) RNA Ribosomes	Nuclei	UMP	Increase, % (Corrected for Pool) Ribosomes	Nuclei
129/130	H^3	0.82	0.76	0.79	3.9	−3.9
265/266	H^3	1.39	1.39	1.40	−0.7	−0.7
267/268	C^{14}	1.40	1.12	1.27	10.2	−11.8
269/270	H^3	0.91	0.93	1.05	−13.3	−11.0

The procedures used were exactly as described in Table 1, except that the pair consisted of yoke control and quiet control mice.

approach described in this paper has the advantage of being able to provide direct evidence of changes in RNA metabolism of brain *while the animal is undergoing training*, and offers a means of studying the RNA that is associated with this response to the environment. We have been able to show that 15 min of conditioned avoidance training increases incorporation of radioactive uridine into RNA of the brain. The increased incorporation occurs in RNA that sediments in a sucrose gradient like rapidly labeled RNA of liver and other tissues.[16] Although the increase occurs throughout the gradient, the greatest per cent of increase in label occurs in the RNA which is isolated from brain ribosomes and which sediments at about 18*S*. This corresponds to fractions of liver RNA reported to stimulate amino acid incorporation into acid-insoluble polypeptides.[15, 17] We are now working on the base composition of this fraction and on its ability to stimulate amino acid incorporation in an *in vitro* system. Similar results have been found in the goldfish.[18]

Two obvious possibilities can account for the increased labeling which we observe: either increased synthesis of RNA or changes in specific activity of the pool of RNA precursors. These pool changes can be due to changes in permeability to the injected uridine or to an increase or decrease of *de novo* synthesis of the pool. All of these would affect RNA labeling in the brain. There are reasons for believing that these differences are probably not due to changes in the precursor pool. First, there is no evidence for large changes in the ratio of C^{14} to H^3 in UMP and CMP of the soluble fraction (Table 1). While it is true that this ratio is obtained after the training period ends, we have found that this ratio is not changing significantly throughout the training period. Second, there is no significant difference in the ratio of C^{14} to H^3 that is incorporated into soluble RNA. Although we feel that the increase in labeling that we observe in trained animals probably represents an increase in RNA synthesis, this is not unequivocally established because we have not specifically examined the direct nuclear pools of RNA precursors. Light may

TABLE 3

INCORPORATION OF URIDINE INTO RNA FROM BRAIN OF SHOCK CONTROL AND QUIET CONTROL MICE SACRIFICED IMMEDIATELY AFTER TRAINING

Mice	Isotope in quiet control	Ratio of Labels (Shock:Quiet Control) RNA Ribosomes	Nuclei	UMP	Increase, % (Corrected for Pool) Ribosomes	Nuclei
185/186	C^{14}	1.60	1.71	1.65	−3.1	3.6
211/212	H^3	0.70	0.62	0.64	9.4	−3.1
225/226	C^{14}	—	1.30	1.40	—	−7.1
227/228	H^3	1.42	1.57	1.51	−6.0	4.6

The procedures used were exactly as described in Table 1, except that the pair consisted of shock control and quiet control mice.

be shed on this point by comparing the ability of chromatin isolated from nuclei of the brains of trained and untrained mice to function as a template in an *in vitro* RNA polymerase system. In addition, we are attempting to determine whether an increase in polysomes occurs during the training experience.

It is not clear which aspect of the training experience produces these changes in labeling of brain RNA. The effects of general stimulation and random jumping can be discounted since there was no difference between the various control mice (Tables 2 and 3). One explanation is that the learning process stimulates the synthesis of RNA which is involved in the synthesis of new materials used in memory storage or for repair and maintenance of brain cells, but other possibilities exist. For example, there are many stresses to which the trained animal is subjected that might also trigger the labeling of RNA. These stresses, if they exist, affect the brain but not liver or kidney (Table 1*b* and *c*). For example, the attention of the trained mouse to the light and buzzer increases during training, and these otherwise benign conditioned stimuli may become stressful when they are associated with the shock. In addition, the jumping of the trained mouse with each presentation of the conditioned stimulus is not random, but is directed specifically to reaching the escape shelf. This organized change in locomotor activity is not seen in any of the control mice. These or other factors, instead of learning, could be responsible for the increase in RNA labeling. Until further experiments are carried out, the significance of the increased RNA synthesis to brain function will not be clear. Of great relevance to this problem is the absence of consistent differences between the incorporation of radioactive uridine into RNA of liver or kidney of trained and untrained mice. We consider this type of datum to be critical to analysis of brain functions, but it is one that is lacking in many studies. Although we have not ruled out the possibility that incorporation is also increased in some other tissue of the trained animal, the lack of such changes in liver and kidney rules out the occurrence of a general biochemical response to the training experience and points to a specific biochemical change in the brain. It is important to emphasize that these changes are large, qualitatively reproducible, and found after *only 15 min of training.* This indicates either that a large part of the brain is involved in a process in which the rate of RNA synthesis is perhaps doubled, or that the rate is increased manyfold in a smaller area. One way to interpret these results is to consider them analogous to stimulation of the synthesis of RNA in liver or uterus by hydrocortisone and estrogen, respectively.[19, 20] That is, an early biochemical response of *any* tissue or organ to a specific stimulus is the synthesis of RNA utilized in protein synthesis. In agreement with this interpretation, the sedimentation patterns of rapidly labeled RNA from liver and brain resemble each other and do not change significantly when the appropriate stimulus is administered; only the amount of labeling increases. This is in accord with the idea that there is a sustained increase in RNA synthesis in brain during the training period. Whether this RNA signals the beginning of brain function in relation to learning and memory or to some other mechanism, such as one that has evolved to *maintain or repair* brain cells following stimulation, remains to be seen.

Summary.—Increased incorporation of radioactive uridine into RNA isolated from brain nuclei and brain ribosomes was observed in mice trained for 15 min. No such increases were observed in liver or kidney. We should like to conclude that

the differences in the incorporation of radioactive uridine into brain RNA represents an increase in RNA synthesis that is part of the molecular basis of learning. Good sense, however, compels caution, and alternative hypotheses are considered.

* Partially supported by research grants from the U. S. Public Health Service.

† NSF Cooperative Graduate Predoctoral Fellow. Supported in part by NIH training grants 5-T1-GM-404 and 5-T1-GM-685.

‡ Supported by a Career Development Award (GM-K3-14,911) from NIH.

[1] Dingman, W., and M. B. Sporn, *J. Psychiat. Res.*, **1**, 1 (1961).

[2] Hydén, H., and E. Egyházi, these PROCEEDINGS, **52**, 1030 (1964).

[3] Flexner, L. B., J. B. Flexner, G. de la Haba, and R. B. Roberts, *J. Neurochem.*, **12**, 535 (1965).

[4] Barondes, S. H., and H. D. Cohen, *Science*, **151**, 594 (1966).

[5] Agranoff, B. W., R. E. Davis, and J. J. Brink, these PROCEEDINGS, **54**, 788 (1965).

[6] Kano-Sueoka, T., and S. Spiegelman, these PROCEEDINGS, **48**, 1942 (1962).

[7] Trakatellis, A. C., A. E. Axelrod, and M. Montjar, *J. Biol. Chem.*, **239**, 4237 (1964).

[8] Khan, A. A., and J. E. Wilson, *J. Neurochem.*, **12**, 81 (1965).

[9] Crestfield A. M., K. C. Smith, and F. W. Allen, *J. Biol. Chem.*, **216**, 185 (1955).

[10] Patterson, M. S., and R. C. Greene, *Anal. Chem.*, **37**, 854 (1965).

[11] Kabara, J. J., N. R. Spafford, M. A. McKendry, and N. L. Freeman, in *Advances in Tracer Methodology*, ed. S. Rothchild (New York: Plenum Press, 1963), vol. 1, p. 76.

[12] Tsuboi, K. K., and T. D. Price, *Arch. Biochem. Biophys.*, **81**, 223 (1959).

[13] Randerath, E., and K. Randerath *Anal. Biochem.*, **12**, 83 (1965).

[14] De Robertis, E., A. P. Pellegrino DeIrâldi, G. R. R. R. De Lores Arnaiz, and L. Salganicoff, *J. Neurochem.*, **9**, 23 (1962).

[15] DiGirolamo, A., E. C. Henshaw, and H. H. Hiatt, *J. Mol. Biol.*, **8**, 479 (1964).

[16] Hiatt, H. H., *J. Mol. Biol.*, **5**, 217 (1962).

[17] Henshaw, E. C., M. Revel, and H. H. Hiatt, *J. Mol. Biol.*, **14**, 241 (1965).

[18] Glassman, E., K. Schlesinger, and J. E. Wilson, *Federation Proc.*, **25**, 713 (1966).

[19] Kenney, F. T., and F. J. Kull, these PROCEEDINGS, **50**, 493 (1963).

[20] Gorski, J., W. D. Noteboom, and J. A. Nicolette, *J. Cellular Comp. Physiol.*, **66** (Suppl. 1), (1965).

---

*RADIOMIMETIC BEVERAGES, DRUGS, AND MUTAGENS**

BY KARL SAX AND HALLY J. SAX

DIVISION OF BIOLOGICAL SCIENCES, UNIVERSITY OF GEORGIA, ATHENS

Read before the Academy April 25, 1966

It has long been known that chemical agents as well as ionizing radiation can induce mutations and chromosome aberrations in both plant and animal cells. This early work was reviewed by Auerbach[1] and by Levan[2] in 1951. More recent reviews were published by Gustafsson[3] and by Wilson[4] in 1960, and by Kihlman[5] in 1963. Wilson[4] warned of the possible dangers of the "chemical age," as well as the "atomic age," to modern man. Levan[2] suggested that the simple onion root tip technique be used to test the possible radiomimetic effect of insecticides, fungicides, drugs, and antibiotics. A preliminary survey of radiomimetic agents led us to make a more detailed study of coffee, alcohol, and antinauseants.

Our tests were made by pouring 10 cc of the test solution over five layers of paper toweling in a Petri dish and sowing 100 onion seeds on the moist paper. The root