no samples were tested between early convalescence and 2 years. The absence of a concomitant rise in CF antibody would support this hypothesis. If the changes in antibody levels were the result of infection with antigenically related viruses, a discrepancy in booster antibody response between neutralizing and CF antibodies would have to be postulated.

It is possible that the levels of antibody in the patients studied were maintained by inapparent infections with antigenically related enteroviruses. Detection of changes in antibody titer resulting from such a mechanism would require more frequent sampling of sera. Further investigation will be necessary to determine whether the pattern of immunologic response found in Boston Exanthem disease is also applicable to other ECHO virus infections.

Summary. Persistence of complement fixing and neutralizing antibodies to ECHO 16-like viruses was studied in 15 patients convalescent from Boston Exanthem disease. CF titers fell moderately within 1 or 2 years after infection, but remained demonstrable for 3 to 6 years. Levels of neutralizing antibody persisted without significant decline for a similar period of time.

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Nucleic Acid Content of Various Areas of the Rat Brain.* (25204)

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The present investigation was initiated to provide control values of NA concentrations in local areas of rat brain. Previous reports have, for the most part, concerned themselves with NA content of the whole brain ignoring the differences between the various areas of the brain. Since these areas differ from each other functionally, morphologically, metabolically, and in chemical composition, and since physiological stresses or agents are known to act differently on these various cell groups, it becomes necessary to establish regional control values.

Methods. Adult, white, male rats (300-450 g) were used. The rats were decapitated after receiving a stunning blow on the head and the brain dissected on a cold surface. The tissue was washed with ice-cold distilled water to remove surface blood and blotted on filter paper. More reproducible results were obtained if the tissue was blotted to remove surface water (Table I). The excised tissue was then placed in 10% trichloracetic acid (TCA) or stored in the deep-freeze. A maximum period of 15 minutes elapsed between sacrificing of the animal and the storage or treatment of the tissue with TCA. When tissue weight was 100-200 mg, the homogenates were prepared with a close fitting stainless steel pestle in 16 × 150 mm test tubes. When the hypothalamic and thalamic tissues (20-50 mg) were analyzed for total nucleic acid (TNA), the tissue was homogenized in a 12 ml centrifuge tube using a plastic pestle prepared according to the method of Darbé(1). It was necessary to pool these tissues from 5 animals to obtain enough material for analysis of deoxyribonucleic acid (DNA) using the standard procedure. Enzymatic assays were made following the procedure of McDonald(2) except that it was run at 37°C and different

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TABLE I. Nucleic Acid Contents of Different Areas of Rat Brain.

Area	Preparation	mg P/100 g wet wt TNA	DNA	RNA	RNA/DNA
Cerebral cortex	Frozen (unblotted)	21.2 ± 7.1 (7)*	6.9 ± 1.0 (8)	14.3 ± 7.2	2.1 ±1.1
	Frozen (blotted)	22.1 ± 2.1 (9)	7.5 ± .1 (6)	15.6 ± 2.3	2.0 ± .3
	Fresh	22.5 ± 1.3 (6)	7.0 ± .6 "	15.5 ± 1.5	2.1 ± .3
White matter	Frozen	17.0 ± 1.4 (8)	8.8 ± 2.3 (7)	8.2 ± 2.7	.93 ± .4
	Fresh	18.4 ± 2.7 (6)	8.4 ± 1.1 (8)	10.0 ± 2.9	1.2 ± .4
Cerebellum (whole)	Frozen	50.0 ± 1.5 "	39.4 ± 4.5 (7)	10.6 ± 4.7	.27 ± .12
	Fresh	58.3 ± 2.8 "	38.2 ± 4.0 (8)	20.1 ± 4.9	.53 ± .14
Hypothalamus	Frozen	18.2 ± 3.6 (4)		7.9 ± 4.3	.77 ± .46
	Fresh	29.8 ± 5.1 (8)	10.3 ± 2.4 (3)	19.5 ± 5.6	1.9 ± .7
Thalamus	Fresh	20.4 ± 2.3 "	8.3 ± 1.1 "	12.1 ± 2.6	1.5 ± .4
Medulla	Frozen	13.9 ± 2.3 (6)			
	Fresh	13.0 ± 1.7 "	9.4 ± 2.4(14)	3.6 ± 2.9	.38 ± .3

* Mean ± stand. dev. No. in parentheses is No. of determinations.

time intervals were used. RNA and 5′ adenylic acid were used as substrates to measure the activities of ribonuclease (RNase) and 5′ nucleotidase respectively. TNA and DNA were determined by using the ultraviolet absorption method of Logan *et al.*(3) and RNA content was calculated as the difference between TNA and DNA. The value of the absorptivity, a_p, at 268 mμ used in this investigation was 320 expressed in terms of mg NAP(3). The atomic extinction coefficient, ϵ(P), is 30.98 times a_p or 9814. All phosphorous analyses were made using the Fiske-Subbarow method(4).

Results. In the course of determining DNA, the residue from the lipide extraction is treated with KOH to hydrolyse RNA. A residue was inevitably found after hydrolysis. The only previous report of a residue is that found during the analysis of bone(5). The quantity of material which absorbed at 260 mμ using perchloric acid extracts of the residue(6) amounted to approximately 0.5% of the DNAP in the white matter and cerebellum.

Handling of the tissue after removal from the brain affects the NA concentrations to different extents in different areas of the brain (Table I). More variable results were obtained when the tissue was not blotted after washing with ice-cold water to remove surface blood. Samples of cerebellum and hypothalamus which had been stored in the deep-freeze had significantly lower TNA contents than tissue analysed immediately after the animal had been sacrificed ($p < 0.01$). The differences in the other areas of the brain were not statistically significant ($p = 0.10$). DNA analyses in all areas gave the same values whether the tissue was fresh or frozen. RNA content was reduced by 47% in the cerebellum and by 60% in the hypothalamus as a result of storage in the deep-freeze. If the tissue was dissected without being on a cold block, TNA was reduced by 20% in the cortex, 27% in white matter, and 37% in the cerebellum.

Several preliminary experiments were performed to determine whether enzymes which act on RNA were present to a greater extent in the cerebellum than the cerebral cortex. Extracts of these tissues prepared with phosphate buffer, pH 7.3, were incubated overnight with RNA. Changes in absorbance at 260 mμ were 2.5 times greater with cerebellum than with cerebral cortex per g tissue. Additional studies were performed with tissue homogenates at different time intervals and using RNA and AA as substrates. The results are given in Table II. About 17% per organic P was liberated by the action of cerebellar homogenates on RNA than with cortical homogenates although changes in absorbance at 268 mμ were greater with cortical homogenates than with cerebellar homogenates after 2 hours. Cerebellar homogenates liberated 44% more inorganic P than cortical homogenates with AA as the substrate.

TABLE II. Enzyme Studies on Rat Cortex and Cerebellum Homogenates.

Substrate	Ribonucleic acid						5′ adenylic acid	
Tissue	Cortex			Cerebellum			Cortex	Cerebellum
Measurement	A_{260}†	Inorg. P*	Organic P*	A_{260}†	Inorg. P*	Organic P*	Inorganic P*	
Time, min.								
10	.03	.02	.08	.00	.05	.07	.04	.04
30	.08	.05	.24	.02	.14	—	.17	.14
120	.25	.25	.98	.12	.20	1.15	.39	.56

Values represent change from t = 0. Solutions containing 1 ml homogenate (40-60 mg tissue/ml acetate buffer, pH 5.0) and 1 ml substrate (0.5 mg RNAP or 0.5 mg 5′ adenylic acid/ml buffer) incubated at 37°C.

* μg P/mg wet wt. † $\triangle$A/mg wet wt.

Discussion. The results of NA analyses (Table I) in the various areas of the brain indicate that fresh tissue should be used for TNA analyses. However, it is not necessary to use fresh tissues when DNA concentrations are being measured. The differences between RNA in fresh and frozen samples of cerebral cortex and cerebellum might be explained by either of the 2 following hypotheses: 1) A labile RNA is present in cerebellum which is degraded during the slow freezing and thawing processes or is present to a greater extent in the cerebellum than in the cerebral cortex. Smith(7) found that using dry ice instead of cracked ice during homogenization of tissues increased TNA concentration by 18%. However, the analysis was always the same in frozen liver as in fresh tissue. 2) An enzyme or enzymes are present in greater concentration in the cerebellum than in the cerebral cortex, which degrades RNA during the freezing and thawing process.

There is less RNase activity and more 5′ nucleotidase activity in the cerebellum than in the cerebral cortex (Table II). Similar results have been reported for distribution of RNase in rabbit brain(8) and human brain(9) and for 5′ nucleotidase in rat brain(10). Hence, RNA in the cerebellum may be degraded more rapidly than in the cerebral cortex because of the large concentration of this latter enzyme which acts not upon RNA but on its degradation products. However, the true concentrations of RNase in the various areas may be different from the values found with the assay conditions used in this investigation.

The NA contents of rat cerebral cortex previously reported have been higher than those reported in Table I. These analyses(11) used color tests which have been shown to yield higher results than the ultraviolet absorption method(3).

The amount of DNA is approximately the same in all areas except the cerebellum (Table I). Similar observations have been reported for rabbit(8) and cat(12). Microdeterminations on rabbit and monkey cerebellum have also been reported by Kissane and Robbins (13). However, distribution of RNA is different in rat brain with highest concentrations found in the cerebellum, hypothalamus, and cerebral cortex (Table I). The lowest amount of RNA is in the medulla. Similar results have been found with cat tissues(12).

It is interesting to note that protein content is high in the areas where RNA concentration is high (cerebral cortex, cerebellum, and hypothalamus), but the content of both substances is low in medulla(14).

A knowledge of DNA concentration permits an attempt at calculation of tissue components such as average cell densities, dry weight/average cell, and total number of cells in each brain area (Table III). These calculations are based upon the assumption that amount of DNA/cell is constant in all cerebral cells (see discussion of Nurnberger and Gordon (15)). Although no direct measurements of DNAP/cell have been reported for rat brain, an average value of 65×10^{-8} μg DNAP/nucleus is used in these calculations(16). Comparison of the calculated cell densities from chemical analyses and those obtained by direct measurement(15) indicates close agreement between these two different methods. The difference is less than 25% in most areas, but is 40% for white matter. This disagreement may be due to different definitions of

TABLE III. Cell Densities, Weight/Cell, and Total Number of Cells in Areas of Rat Brain.*

Analyte	Area					
	Cortex	White matter	Cerebellum	Hypothalamus	Thalamus	Medulla
DNAP, μg/g wet wt	70 ± 6§	84 ± 11	382 ± 40	103 ± 24	83 ± 24	94 ± 24
Cell density, cells/g wet wt × 10^5	1.08 ± .09	1.29 ± .17	5.88 ± .62	1.58 ± .37	1.28 ± .17	1.45 ± .37
Nurnberger & Gordon (15)	.96	.78	4.77	1.79	1.21	1.13
Wet wt/cell, 10^3 pg/cell	9.3	7.8	1.7	6.3	7.8	6.9
% moisture	78.8†	80.0‡	78.4†	80.0‡	80.0‡	77.1†
Dry wt/cell, 10^3 pg/cell	2.0	1.6	.37	1.3	1.6	1.6
Wet wt, mg	400	175	250	40	40	100
Total No. of cells/area, 10^7	4.3	2.3	14.7	.63	.51	1.5

* pg = 10^{-12}g cell density = $\frac{\mu g \text{ DNAP/g wet wt}}{65 \times 10^{-6} \mu g \text{ DNAP/nucleus (cell)}}$. Wet wt/cell = 1/cell density. Dry wt/cell = (100 − % moisture) × wet wt/cell. Total No. of cells/area = Cell density × wet wt of area.

† Gosselin *et al.*(17). ‡ Assumed value. § Mean ± stand. dev.

the areas or difficulties in selection of pure white matter. It should be noted that all values in Table III represent the composition of the average cell and do not distinguish between neuronal and supporting cells. However, this type of calculation can supply valuable information about changes in composition of the cell produced by altering the physiological state of the tissue.

Summary and Conclusions. Nucleic acid analyses were made on the various areas of the rat brain using the ultraviolet absorption method. It was found necessary to use fresh tissues to obtain correct ribonucleic acid concentrations since analyses in some areas (cerebellum and hypothalamus) were lower when the tissues had been kept frozen. Enzymatic analysis indicated that the higher concentration of 5′ nucleotidase in cerebellum than in cortex might be indirectly responsible for the loss of the ribonucleic acid during the freezing and thawing processes. Deoxyribonucleic acid concentration in the cerebellum is approximately 4-5 times the amount found in the other areas. Ribonucleic acid content of the brain decreased in the order: cerebellum, hypothalamus, cerebral cortex, thalamus, white matter and medulla. Average cell densities, weight/cell, and total cells/area were calculated from the deoxyribonucleic acid concentrations. Close agreement between the calculated cell densities and direct measurements were found.

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Metabolic Alteration of 2-Methylthio-4-Amino-5-Hydroxymethylpyrimidine (Methioprim).*† (25205)

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Amethopterin-resistant mutants of *Bacillus subtilis* and mutants of *Escherichia coli* resistant to purine antagonists show increased sensitivity to the inhibitory effect of methioprim (2 - methylthio - 4-amino-5-hydroxymethylpyrimidine) when compared to the parent organisms, *i.e.,* collateral sensitivity(1). Significant growth suppression of subcutaneous tumors (Ehrlich carcinoma, clone 2 and Krebs) is also observed with this compound(2). Furthermore, chromatography of urine from dogs and rats receiving methioprim reveals, in both cases, the excretion of an additional ultraviolet-absorbing substance with anti-bacterial activity similar to methioprim‡. The present study reports that rat liver readily metabolizes methioprim to at least 3 products.

Materials and methods. Rat liver incubations. Adult male Sprague-Dawley rats were sacrificed by decapitation. Livers were immediately excised and placed in chilled bath of Krebs phosphate buffer, pH 7.4(3). Slices weighing 70-100 mg were cut with a Stadie-Riggs microtome. Three slices were placed in each 20 ml beaker with 3 ml of Krebs buffer solution to which methioprim (20 μmoles/ml) was added. The buffer-substrate mixtures and control beakers were incubated under atmosphere of 95% O_2-5% CO_2 at 36.5°C with constant shaking in Dubnoff metabolic incubator. At end of 5 hours, the reaction was stopped and protein precipitated by rapidly heating in boiling water bath for 5 minutes followed by cooling. The supernatant fluid was collected by centrifugation for analysis of metabolic products. Aliquots of supernatants of incubation mixtures and controls were applied to large sheets of Whatman 3 MM paper and ascending chromatograms were developed. Initial separation of components of incubation mixtures was carried out using top layer of solvent prepared by mixing n-butyl alcohol (500 ml): water (500 ml); glacial acetic acid (16 ml). Chromatograms were examined for metabolic products by using ultraviolet light, and various spray reagents and by bioautographic technic using microbiological assay described below. *Microbiological assay.* Methioprim and some closely related compounds suppress growth of amethopterin-resistant mutants of *Bacillus subtilis* to a greater extent than the parent strain(1). Such compounds have no effect on growth of these strains when 2 - methyl-4-amino-5-aminomethylpyrimidine (the pyrimidine moiety of thiamine) is present in growth medium. For routine purposes, an agar-diffusion method and a chemically-defined medium(4) were used. An amethopterin-resistant mutant of *Bacillus subtilis,* designated as strain 6051/A, was homogeneously seeded into medium with and without a supplement of 2-methyl-4-amino-5-aminomethylpyrimidine. Similar platings of the parent *B. subtilis* 6051 were also made. Either paper discs impregnated with test solutions or strips of paper chromatograms were placed on the

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