Table 2. Tissue weights, tissue protein contents and lysozyme levels

Dietary treatment	Serum Protein content (mg/ml)	Lysozyme (units/ mg protein)	Spleen Total tissue wt (g)	Protein content (mg/g)	Lysozyme (units/ mg protein)	Kidney Total tissue wt. (g)	Protein content (mg/g)	Lysozyme (units/ mg protein)
5 ppm Iron	74.2 ± 3.1	0.34 ± 0.03	0.38 ± 0.05	112.1 ± 10.4	4.83 ± 0.63	0.75 ± 0.02	76.1 ± 1.1	42.85 ± 2.34
300 ppm Iron	91.3 ± 7.3 NS	0.30 ± 0.02 NS	0.36 ± 0.03 $p < 0.005$	137.5 ± 7.9 NS	5.25 ± 0.33 NS	0.97 ± 0.03 $p < 0.001$	83.2 ± 3.7 NS	31.65 ± 1.36 $p < 0.001$

Data are expressed as means ± SE of 7 animals. NS, not significant.

The hitherto unreported effect of iron-deficiency on increasing the level of lysozyme activity in the kidney is clearly documented in this study. However, the exact biological significance of these findings is unknown at this time. It has been established that the kidney plays an important role in the regulation of plasma lysozyme levels. This regulation is believed to occur by way of the lysozyme protein becoming entrapped in the proximal tubules and degraded there[20]. The increased levels of lysozyme observed in the kidneys of iron-deficient rats may, then, reflect a homeostatic mechanism to maintain normal serum levels. Perhaps, the iron-deficient animal is producing more lysozyme which is being degraded in the kidney. The resulting pattern of serum and spleen lysozyme levels is therefore the same in iron-deficient and control animals. One possible explanation may be that the iron-deficient organism, with a lower immunological capacity[21], requires more lysozyme for its antibacterial functions. The increased kidney levels may be a reflection of a greater lysozyme turnover required for this antibacterial role.

Generally, the interrelationships between nutritional status and lysozyme action merits further study. The effect of iron-deficiency tissue lysozyme levels is particularly pertinent since iron deficiency anemia is a common nutrition problem[21].

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Large regional and strain differences in rat brain sialic acid and 2-deoxyribose

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Summary. Sialic acid, a very polar component of glycolipids and glycoproteins that is exposed on membrane surfaces, was observed in rat brain to vary in the descending order: forebrain, midbrain, cerebellum and medulla. Levels of 2-deoxyribose were also differentially distributed, with about 3.5 times as much in the cerebellum and nearly equal amounts elsewhere. Similar results were obtained with another genetic strain, but clear quantitative differences were evident for both chemicals.

Both lipid-bound (gangliosidic) and protein-bound sialic acid have been known to be associated with cell membranes[1,2], with a significant amount occurring on mitochondrial membranes[3]. SA's highly reactive polar characteristic of the carboxyl group and its exposed position on the membrane surface make it a possible reaction site (receptor) for cationic groups of many drugs and hormones[4]. Since much of the research has dealt with the total brain SA-analyses, it seemed necessary to study the SA-distribution in different areas of the brain. If SA-differences occurred, such data could have relevance in focusing the search for potential mechanisms of action of certain psychoactive drugs and neurohormones.

Methods. Rats were killed by decapitation, and the brains were removed and cut into 4 sections within 2 min; the sections were then frozen in liquid nitrogen. The sections were identified as: 1. forebrain (cut was immediately behind optic chiasma and extended through the hippocampus); 2. midbrain (caudal border of inferior colliculi); 3. medulla; 4. cerebellum.

Rat brains were obtained from young adult rats (250–300 g) of both sexes from different litters. An initial set of experiments was performed on 6 rats of the Wistar strain, and replication experiments were performed on 6 Sprague-Dawley rats.

Tissue content of total sialic acid was determined by a continuous-flow colorimetric method[5], which basically combined the 2 established procedures, by Warren[6] and by Delmotte[7], with a 2-channel Auto Analyzer. In this

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analysis, 2-deoxyribose interferes with the color reaction, but calculations were corrected by exploiting the differences in the characteristic absorption maxima for sialic acid and 2-deoxyribose (DR) (530 and 550 nm, respectively). Samples of mixed standards and of brain were prepared as hydrolysates as described by both Warren and Delmotte. Sialic acid standards of 20, 40, 80 and 100 mg/l and DR-standards of 1, 2, 4 and 8 mg/l were prepared in trichloracetic acid (50 gm/l of physiological saline). Mixed standards were prepared by mixing equal volumes of SA- and DR-standards. Brain samples were prepared by mixing 0.2 g of sonified or homogenized brain in 9.8 ml in 5% trichloroacetic acid solution. All standards and tissue samples were hydrolyzed in glass-stoppered centrifuge tubes for 1 h at 80 °C.

Results. Marked regional concentration differences for both compounds were noted. For SA, high levels were found in the forebrain and midbrain, with less in the cerebellum, and still less in the medulla (means and SEs shown in figure 1). This qualitative difference in regional distribution was replicated in another strain of rats; differences among brain regions were statistically significant for both strains (Wistar: $F = 60.88$, $p < 0.01$; Sprague-Dawley- $F = 145.27$, $p < 0.01$). Moreover, clear quantitative differences between the 2 strains were evident for each brain region ($p < 0.01$, t-test).

A different pattern of regional difference was evident for DR, which was about 3.5fold more concentrated in the cerebellum than in other brain regions (figure 2). This observation was replicated in the 2nd strain of rats, and differences among brain regions were again statistically significant (Wistar: $F = 117.47$, $p < 0.01$; Sprague-Dawley: $F = 908.86$ $p < 0.01$). Significant differences between the 2 strains were evident for the medulla and midbrain and, in an opposite direction, for the cerebellum ($p < 0.01$, t-test).

Discussion. Our whole-brain SA-values, 3.75 μmoles/g (averaged over the 4 brain sections), compare well with the values of 3.34 and 3.41 μmoles/g that were reported in previous studies of whole-brain SA in Wistar rats[8,9]. The early belief was that SA is associated only with membranes of neurons and that distribution would therefore parallel the distribution of grey and white matter. However, substantial amounts of lipid-bound SA do occur in the myelin of white matter[10–15].

The amount of protein-bound SA in glia is probably even more substantial[16]. Thus, despite the quantitative discrepancies in the literature, it seems safe to conclude that our data do not simply reflect the distribution of grey and white matter. For that matter it is not obvious what the relative distribution of grey and white matter is in the 4 brain sections which we used.

The regional and potential genetic differences that we observed in total SA should stimulate subsequent research on differences in the various protein- and lipid-bound species of SA. Analysis of more restricted anatomical structures is also called for.

Previous reports are not directly comparable to this present research. For example, one of the more relevant studies[9] focused only on the SA of glycoprotein origin, dealt with only 2 specimens, and analyzed only selected portions of bovine brain. Another study[17] focused on only the lipid-soluble fraction of SA, with selected samples taken from 3 human brains at necropsy. One study of lipid-bound SA found a range of differences among the various brain nuclei examined of almost 15fold[15]. The areas chosen did not correspond with the ones which we used, but the highest levels of lipid-bound SA were in the forebrain (frontal cortex, caudate nucleus, motor cortex). Several nuclei in the brainstem had the lowest levels. Lipid-bound SA normally accounts for about $^2/_3$ of the total SA in rat brain[18], and it will be necessary to establish whether the regional and strain differences in total SA reported here are attributable mainly to protein- or lipid-bound SA.

While it is not clear what functions are subserved by SA, sialocompounds have all the attributes of receptors, specifically: a) SA is a strongly negatively charged, exposed terminal group that projects out from the lipid or protein moiety that is anchored in the cell membrane, b) there is a wide variety of SA-species, both protein and lipid, which provides a rich diversity of conformational forms needed for steric specificities, and c) there

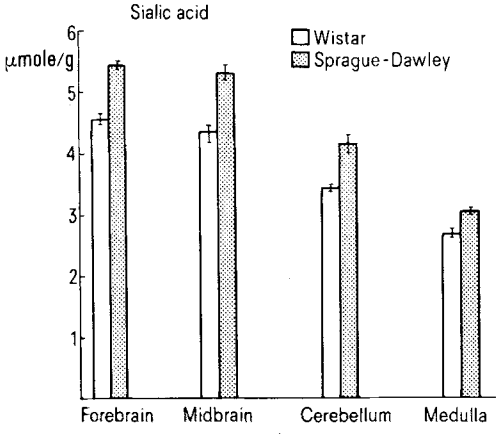

Fig. 1. Regional and strain differences in sialic acid.

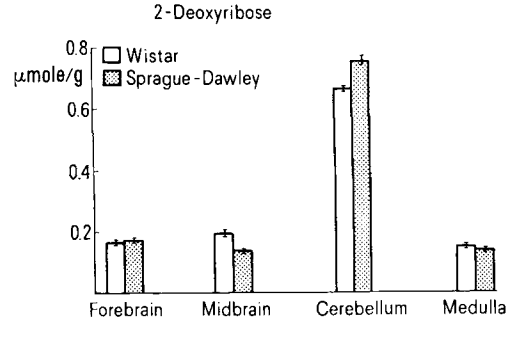

Fig. 2. Regional and strain differences in 2-deoxyribose.

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are vast numbers of SA-residues present in the nervous system, estimated at 10^{11} per cell[19]. Good evidence for receptor action of sialocompounds has been presented for such bacterial toxins as tetanus and botulinum[20] and for lactins, viruses, mycoplasma, some hormones, and antibodies[21]. SA may also be involved in cationic binding of calcium, with an associated influence on neurotransmitter release and uptake[22].

Since DR a major constituent of DNA, the elevated DR-level in cerebellum would suggest a corresponding concentration of DNA in the cerebellum. The cerebellum contains exceptionally large amounts of DNA in the rat, rabbit and cat, with the amount exceeding that in the cerebral cortex by a ratio of more than 6 to 1[23]. This large amount is attributed to the extreme cell density of the cerebellar granular layer. Our research on rats is to our knowledge the first demonstration of genetic differences in brain SA and DR; such differences for SA confirm the speculation many years ago[24] that SA should be readily affected by genetic variables because gangliosides have a high degree of individual character, including the number of SA-residues. In subsequent research, we have replicated the differential distribution of both SA and DR in Wistar rats[25]. Moreover, the steady state concentration of both substances seems to be highly responsive to experimental variables; we have demonstrated significant effects of age and/or experience, and effects of both acute and chronic administration of ethanol[25].

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Thermoregulatory effects on the sheep of intracerebroventricular injections of L-glutamic acid

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Summary. L-glutamic acid injected in doses of 200–1000 nmoles · kg^{-1} into the cerebral ventricles of sheep had dose-dependent thermoregulatory effects: an increase in heat production and/or a decrease in respiratory frequency, and a rise in rectal temperature. A dose of 800 nmoles · kg^{-1} had effects comparable with those of a similar injection of 3 nmoles · kg^{-1} carbamylcholine.

When some of the putative transmitter substances are injected into a lateral cerebral ventricle of the unanaesthetized sheep through a previously implanted ventricular cannula, they cause distinct and quite regular patterns of change in the thermoregulatory effectors with resultant changes in rectal temperature (T_{re}) as summarized in the 1st 4 columns of the table[2–4]. These apparently orderly changes in effector activities have been interpreted as evidence that these substances, although applied in a rather gross and diffuse way, each act at particular points at the central nervous interface between the afferent paths from thermosensors and the efferent paths to thermoregulatory effectors.

Other possible transmitter substances tested in our laboratory at an early stage in this continuing study were: histamine, L-glutamic acid, taurine, glycine and γ-aminobutyric acid. At dose levels of up to 200 nmoles · kg^{-1} these substances were not found to cause thermoregulatory effects when injected into a lateral cerebral ventricle at 0 and 40 °C ambient temperature (T_a)[5]. In other species, however, some of these substances have been found to elicit thermoregulatory effects or, at least, to cause changes in body temperature when applied centrally[6,7]. The differences between the doses of 5-HT, CCh, NA and DA (table) which were required to elicit thermoregulatory effects could relate to differences in the rate and extent of diffusion into brain tissue from the ventricles, and differences in the accessibility of the target synapses, as well as to differences in receptor densities. We could, therefore, have missed the thermo-

Thermoregulatory changes induced by ICV injections of drugs

Responses	Drugs and doses 5-HT 40 nmoles · kg^{-1}	NA 100 nmoles · kg^{-1}	DA 200 nmoles · kg^{-1}	CCh 3 nmoles · kg^{-1}	Glut 800 nmoles · kg^{-1}
Panting (at high T_a)	↑	↓	↓	↓	↓
* Ear skin temperature	↑	↓	↓	↓	↓
Shivering (at low T_a)	↓	↓	↓	↑	↑
Rectal temperature (T_{re})	↓	↑↓ **	↑↓ **	↑	↑

5-HT, 5-hydroxytryptamine; NA, noradrenaline; DA, dopamine; CCh, carbamylcholine (carbachol); Glut, L-glutamic acid; T_a, ambient temperature; ↑, increase; ↓, decrease; * indicates state of peripheral vasomotor tone; ** ICV NA or DA causes a rise in T_{re} at high T_a and a fall in T_{re} at low T_a.