tongue were found to have desulfurase activity but similar preparations from the anterior portion of the tongue and from the liver were inactive. The posterior portion of sheep tongue differs from the anterior portion by containing the circumvallate papillae and taste buds in addition to the glands of Von Ebner. The source of the activity in this area is not known.

The sheep thyroid particulate enzyme described by Maloof and Spector(1) is similar to the salivary enzyme in requiring the 2 cofactors thiocyanate and ascorbic acid, but the salivary enzyme has stricter requirements for ascorbic acid. Both ascorbic acid and thiocyanate have been detected in normal human saliva. The salivary enzyme is for the most part water soluble whereas the sheep thyroid enzyme is particulate(1). The pH optima differ. Sheep thyroid desulfurase is inhibited by iodide, sulfate and dinitrophenol whereas the salivary enzyme is not. Both enzymes are inhibited by cysteine, reduced glutathione, and thiosulfate(1). Neither enzyme was inhibited by urea or perchlorate (1).

The hypothesis that thioamide desulfuration represents a biochemical link between genetically inherited PTC non-tasting and abnormal thyroid metabolism has not been supported by these studies.

*Summary.* A soluble enzyme system from human saliva which desulfurates thiourea has been shown to be heat-labile, non-dialyzable and temperature and pH dependent. Thiocyanate and ascorbic acid are necessary cofactors. Propylthiouracil appears to behave like a non-competitive inhibitor. The products of this reaction are protein bound sulfur, thiosulfate or sulfite, and sulfate. Lamb and lion cub saliva desulfurate thiourea, but rattlesnake venom and calf saliva do not. Preliminary studies of saliva from phenylthiocarbamide tasters and non-tasters have shown no differences in desulfuration activity.

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## Relation Between Myelination and Binding of Sodium and Potassium in Rat Brain.* (27945)

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Using a dialysis technic to measure extent of binding, Hayden *et al.*(1) suggested that such binding of sodium and potassium in guinea pig brain homogenate may be related to its myelin content. In view of conflicting reports by other investigators(2-5), it was felt that the study should be verified in another species and in animals of various ages. Differences in technic and different uses of the term "binding" undoubtedly account for most of the discrepancies reported. Stone and Shapiro(2), for example, filtered brain and muscle homogenates through a cellophane membrane under negative pressure at 3-4°C and found that 20% of potassium would not diffuse through the cellophane

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membrane, under their experimental conditions, whereas 100% of sodium was freely diffusible. They used flame photometry for electrolyte determinations. Pappius and Elliott(3) noted that potassium content of rat cerebral cortex slices under anaerobic conditions in a Warburg apparatus failed to come to equilibrium with the medium when potassium concentration of the medium was high. They suggested that this disequilibrium might be due to binding of a large portion of intracellular potassium. Yannet(4) used an IP glucose injection technic for depletion of extracellular electrolytes in the cat to demonstrate that potassium is bound within the brain tissue of this species.. Folch (5) reported that 30% of brain-tissue cations, including both sodium and potassium, were combined with extracts of brain lipids, a type of combination not found in other tissues. Hayden and coworkers(1) reported 3-4% of bound sodium and potassium in guinea pig brain homogenates after dialysis through cellophane at room temperature. In view of these variations in procedure, in specific tissues studied, in species, and in endpoint, reliable comparison of quantitative or even qualitative results from these experiments seems improbable.

In the present study, a relationship is shown between amount of histologically demonstrable myelin in tissue sections and degree of $K^{42}$ and $Na^{24}$ binding in brain homogenates.

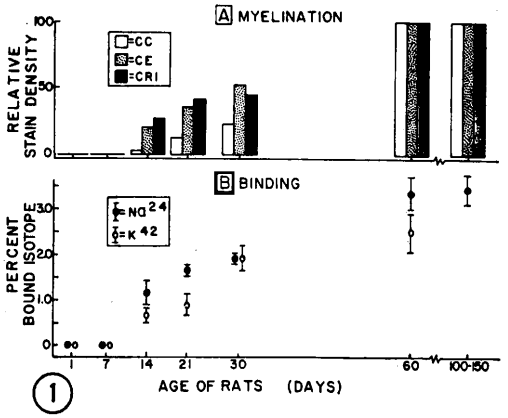
*Myelination.* The chief non-aqueous constituents of the adult brain are lipids, which account for over 60% of the brain's dry weight. Watson(6) has described in detail the development of myelin in the rat brain. Koch and Koch(7) in a study of the chemical differentiation of the rat brain at different ages, demonstrated a correlation between formation of cerebrosides and myelination. Donaldson(8) showed that the progressive diminution of water content of the brain was mainly due to accumulation of myelin. Waelsch, Sperry, and Stoyanoff(9), using deuterium as an indicator, demonstrated that many lipids formed in the rat brain from the 15th to 19th day of life were synthesized within the brain itself. White *et al.*(10) showed that most brain proteins are linked with lipids in various combinations. Koenig (11), using extraction methods, demonstrated that the histologic appearance of the neurokeratin network is an effect of tissue preparation on proteolipids within the myelin sheath.

Recently, Katzman and Wilson(12) extracted lipid-bound cations, including sodium and potassium, from brain tissue slices at –45°C. This low temperature was used to prevent appreciable exchange between lipid and inorganic cation. The total amount of cation extracted was found to be the same as the amount combined with the phosphatidyl serine in the extract.

*General methods.* Rats (Long-Evans) of 7 different age groups were used (Fig. 1). Preliminary experiments showed no sex differences; males and females were used without distinction. Sodium ($Na^{24}$) and potassium ($K^{42}$) were obtained as chlorides in HCl (Oak Ridge National Laboratories), brought to neutrality with NaOH, and diluted with normal saline for injection. Animals were injected IP with one of the isotopes in doses of approximately 2 $\mu$c/g. They had access to food and water *ad lib.*, or were returned to the nursing mother if not yet weaned.

After a 12-hour delay for distribution of the isotope, the animals were sacrificed by guillotine. The skull was split in the midline, the brain was removed and divided in the mid-sagittal plane. Brain stem, basal ganglia, and choroid plexi were removed and meninges stripped off with fine forceps, leaving the cerebral cortex and some underlying white matter free of blood. The tissue utilized in most of the experiments was that superficial to the dashed line in Fig. 2-E. To obtain sufficiently large samples, it was necessary to combine tissue from 5 neonatal brains and from 2-3 brains of 7-day rats. One brain per sample was the rule for older animals.

The samples were transferred to tared Potter-Elvehjem homogenizer tubes and weighed. Standard mammalian Ringer's solution was added and homogenization was carried to completion. Smears of homoge-



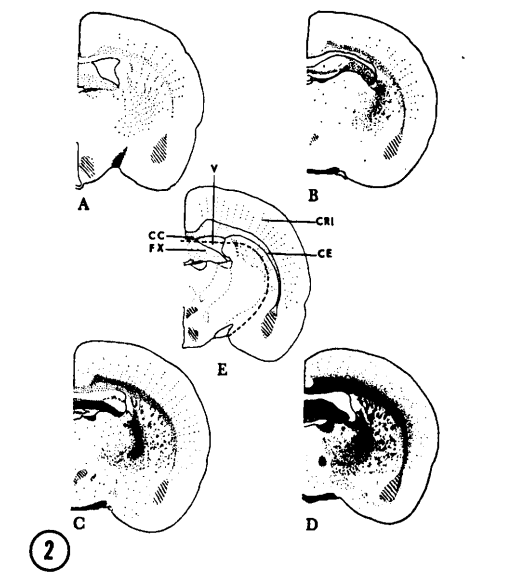




FIG. 1. A: Degree of myelination, based on measurement of relative stain density of Weigert-stained coronal brain sections; density of myelin in sections of 150 g adult brain was designated as 100. CC = corpus callosum; CE = external capsule; CRI = cortical fibers radiating from internal capsule. B: Development of electrolyte binding in the rat brain. Each point represents the arithmetic average of 6-13 separate determinations ± standard deviation.

FIG. 2. Tracings (6×) of cross sections of rat brains showing degree and sites of histologically demonstrable myelination. A. 14 days; B. 21 days; C. 30 days; D. 100 days; E. anatomical key [following de Groot's atlas(13)]. CC = corpus callosum; CE = external capsule; CRI = cortical fibers; FX = fornix; V = ventricle.

nized centrifuged tissue showed no intact cells when examined microscopically. Samples were further diluted with Ringer's solution to the proportion 1:20.

An aliquot of this mixture was taken for pre-dialysis radioactivity determination; a duplicate 10 ml aliquot was placed in a cellophane dialysis bag and dialyzed for 2 hours at room temperature against 200 ml of modified Ringer's solution, constituted to imitate the cationic content of the diluted brain homogenate(1).

At the end of 2 hours, samples were taken from inside and outside the dialysis bag for weighing, drying, and radioactivity determination‡ (Geiger-Müller counter, Scott type). Appropriate corrections for decay, coincidence, and self-absorption were made.

Three control procedures were also carried out. The dialysis bag was filled with modified Ringer's solution to which had been added a small amount of tracer, without brain homogenate. In the second procedure, a small amount of radioactive tracer was mixed with non-radioactive brain homogenate in the dialysis bag. In the third control series, the bag contained radioactive liver homogenate from the same experimental animals.

The amount of bound isotope was determined by calculating the difference between the radioactivity remaining in the dialysis bag after control dialysis and the amount remaining after the experimental procedures.

For histologic studies, brains were removed from representative animals and sectioned within 30 to 50 minutes by the frozen technic, or stored in 10% formalin; after mounting, the sections were stained by the Weigert-Pal-Erhart method or according to Pulliam's modification of Lillie's variant of the Weil-Weigert stain (de Groot, personal communication). Timing of the staining procedures was standardized so that the various groups could be compared in evaluation of myelin distribution and content. Sections A-D (Fig. 2) were prepared by tracing projected images of Weigert preparations; location and degree of myelination were indicated by stippling. Section E was labelled according to de Groot's atlas(13). The tis-

‡ We are grateful to the staff of the Radioactivity Research Center, Univ. of California San Francisco Medical Center, for generous help throughout these studies.

sues studied in these experiments were superficial to the dashed line in Section E, and included cortical fibers (CRI), most of the corpus callosum (CC), some fornix fibers (FX), the external capsule (CE), and lateral portions of the basal ganglia.

Quantitation of the amount of stained myelin was obtained by densitometry. The amount of light, from a constant source, which passed through the Weigert-stained sections was read with a photoelectric cell (Weston Model 3 light meter). Since staining intensity of the cellular background varied in sections from animal to animal, the primarily cellular areas were masked with opaque material prior to density readings. The density of myelin in 150 g adult brain was designated as 100 (*i.e.*, "complete" myelination).

*Results.* Dialysis of the control solutions (isotope added to Ringer's solution and isotope added to non-radioactive brain homogenate) showed no evidence of binding. Similarly, neither sodium nor potassium were bound in whole liver homogenates from animals injected with radioactive tracers. Dialysis of radioactive brain homogenates indicated 3.4 $\pm$ 0.5% bound potassium for adult animals. Very young animals showed no binding. The curves of progressive increase in binding with increasing age are graphed in Fig. 1-B.

Fig. 2, sections A, B, C, and D, respectively, summarize location and extent of myelination in rats at 14, 21, 30, and 100 days of age.

To give a more coherent picture of the process of myelination, myelin-stained sections were also prepared from animals of other ages. Myelination was first detected in faint traces in the fornix (FX), external capsule (CE) and lateral olfactory tracts of the 11-day rat. On the 13th day, these same structures were more heavily myelinated; no additional areas of myelin were demonstrable. On the 14th day (Fig. 2-A), a few irregularly distributed groups of myelinated fibers were noted in the corpus callosum and in the corpus striatum. A few faintly stained fibers also could be detected radiating to the cortex from the internal capsule (CRI).

By the 21st day (Fig. 2-B) there was a noticeable increase in myelination of the corpus callosum. The external capsule and fornix had become more heavily myelinated, and the fibers extended further ventrally than at 14 days. There was, however, only a slight increase in myelination of fibers radiating into the cerebral cortex. In the 30-day rat (Fig. 2-C) there was increased myelination in the external capsule and fornix; the corpus callosum at 30 days contained only slightly more stainable myelin than at 21 days. Extent of myelination of intra-cortical fibers was continuing to increase and in a peripheral direction.

In adult rats (100 days of age, Fig. 2-D), the corpus callosum, fornix, and external capsule were heavily myelinated, and there was very extensive myelin investment of fibers radiating into the cortex. There was no detectable increase of stainable myelin in older rats (150 days, Fig. 1-A).

*Discussion.* These observations on the development of histologically demonstrable myelin are in good agreement with the description given by Watson(6) for the white rat. Luse(14) has, however, pointed out that the first electron microscopically demonstrable myelination occurs as early as the fourth post-natal day. Assuming a relationship between myelination and binding, the dialysis method employed here (standard deviations of $\pm$ 0.2 to 0.5% in Fig. 1-B) would not be expected to demonstrate any electrolyte binding as early as day 7, four days before the faintest trace of myelination was detected by classical histologic methods.

These data indicate that the rat brain binds sodium and potassium in ratios similar to those previously reported for the guinea pig(1). In the case of sodium the value for the guinea pig was 3.1 $\pm$ 0.5%, for the rat, 3.4 $\pm$ 0.5%. Potassium was bound in guinea pig brain to 4.1 $\pm$ 0.9% and in the rat to 2.5 $\pm$ 0.5%; this latter difference is possibly significant ($p = 0.05$).

Our values for binding of K in the adult brain were lower than the 20% reported by Stone and Shapiro(2) and by Bergen, Stone, and Hoagland(15). It was thought that this might have resulted from differences in the

relative amounts of cortical (grey) and subcortical (white) tissue used in the studies; we used cortex with central structures largely removed, whereas Stone *et al.* used "whole brain." To check this we carried out a series of experiments using the entire cerebral hemisphere plus part of brain stem and cerebellum. With these tissue samples, the proportion of bound $K^{42}$ increased from 2.5 ± 0.5% (Fig. 1) to 5.0 ± 0.4%. This does not approach the value reported by the investigators referred to, which must, therefore, arise from other differences in technic or interpretation. The increased potassium binding in these specimens containing larger proportions of white matter, however, supports the idea that potassium binding, as here defined, is related to the presence of myelin in the tissue. The suggested relationship between the ontogenetic development of myelination and the degree of binding (as seen on comparison of Fig. 1-A and 1-B with brain slices of Fig. 2), may be correlated with the observation of Katzman and Leiderman(16) that potassium was not bound in the brains of rats younger than 35 days of age. Their failure to detect bound electrolyte in younger rats was probably due to the different technic used.

Attempts to correlate our values for binding of sodium with the data reported in the literature have been even less fruitful than for potassium binding, because of the differences in approach and technics. Yannet(4) found that sodium was completely diffusible. In contrast, Folch(5) reported that 20% of brain sodium was bound to phosphatidyl serine.

Comparison of the data on changes in percent of electrolyte binding to changes in myelin content of Weigert-stained sections led to the definite conclusion that the increase in percent of sodium and potassium binding closely parallels the increase in histologically demonstrable myelination. This evidence, while strongly suggestive of a relationship between the two phenomena, does not eliminate the possible role of cytoplasmic constituents (*e.g.*, mitochondria) in the observed binding. The technic is being applied to extracts of brain tissue in attempts to define the physical components involved.

*Summary.* Dialysis of brain homogenates from adult Long-Evans rats given intraperitoneal injections of radioactive tracers revealed that 2.5% to 3.4% of brain potassium and sodium were "bound," *i.e.*, had not passed a cellophane dialysis membrane after 2 hours' dialysis at room temperature. These values are similar to those we have previously observed in the guinea pig. No binding was demonstrated by this technic in newborn or 7-day-old rats. Extent of binding progressively increased from 14 days to adulthood. The development of myelination was studied in the Long-Evans rat and was found to parallel the development of sodium and potassium binding. These observations support the view that electrolyte binding, as here defined, is related to the presence of myelin.

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