lation of transfused platelets. The marked variation in the rise of circulating platelets in different animals given transfusions from a single suspension, or from a different suspension prepared in identical fashion at another time, cannot be explained at present.

When complexed with calcium, Sequesterene is relatively non-toxic(3). Proescher has shown the usefulness of Sequesterene as an anticoagulant for humans(4). In our limited experience Sequesterene has been non-toxic as well as non-pyrogenic. Although large numbers of platelets were removed from the circulation of the experimental animals within 30 minutes to 2 hours of transfusion, no evidence of massive thrombosis or embolism has been found.

Summary. (1) A simple method is presented for the efficient separation and concentration of platelets from human, dog, and guinea pig blood in quantities sufficient for transfusion. (2) This method depends upon the use of Sequesterene Na_2 as an anticoagulant, which prevents the development of platelet stickiness and clumping in shed blood. (3) Platelets prepared by this method and transfused into the thrombopenic, irradiated dog remain in the circulation, reverse the coagulation defect, and prevent hemorrhage.

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γ-Aminobutyric Acid Content and Glutamic Decarboxylase Activity in Developing Mouse Brain. (19224)

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γ-Aminobutyric acid is found in the free form in large amounts in brain and spinal cord(1-4). This amino acid and the enzyme which catalyzes its formation from L-glutamic acid by alpha decarboxylation are present in uniquely high concentrations in the tissue of the central nervous system(5,6). The enzyme requires pyridoxal phosphate as a coenzyme, which can be synthesized by brain from adenosinetriphosphate and pyridoxal(5,6). When rats were fed a diet deficient in pyridoxine there was a decrease of approximately 50% in the degree of saturation of apoenzyme with coenzyme, but the content of apoenzyme was normal(7). Refeeding pyridoxine to rats previously made deficient resulted in a return of the activity to a normal level(7). In the present experiments a study was made of the content of γ-aminobutyric acid and the activity of glutamic decarboxylase in the brains of mice at various stages of development in an effort to delineate the role of this system in brain metabolism. Some correlative observations were made on the nervous tissue of cats, rats, dogs, and rabbits.

Experimental. Animals, preparation of tissues, and analyses. All mice, rats, and rabbits employed were killed by dislocation of the cervical vertebrae. The cat and dogs were killed by intravenous nembutal. Whole mouse and rat brains were used for analysis. Alcoholic extracts of the tissues were analyzed for the content of γ-aminobutyric acid by a paper chromatographic method(3) and the maximal potential glutamic decarboxylase activity was determined by a manometric pro-

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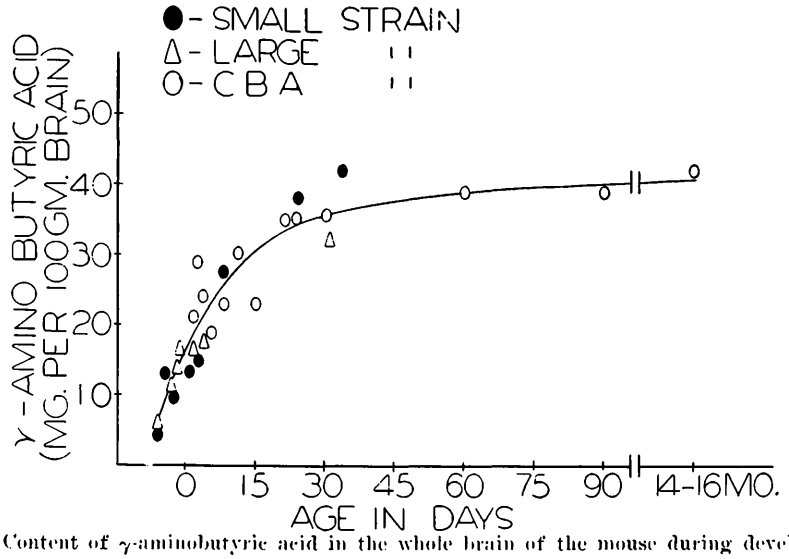

FIG. 1. Content of γ-aminobutyric acid in the whole brain of the mouse during development.

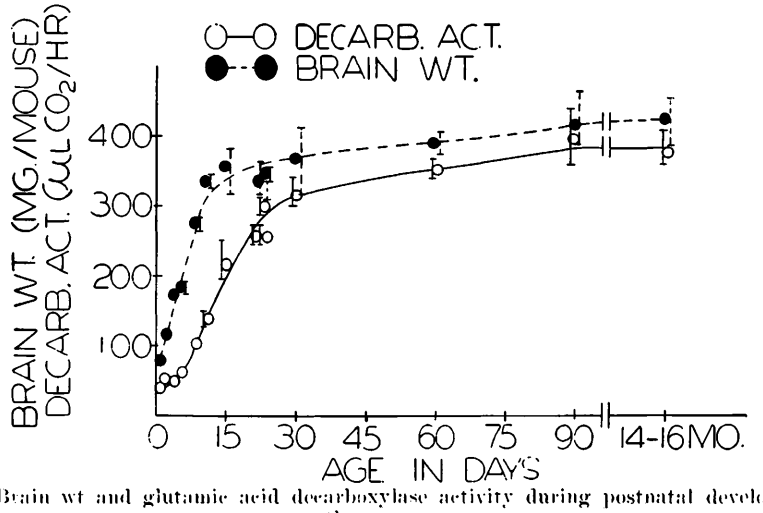

FIG. 2. Brain wt and glutamic acid decarboxylase activity during postnatal development in the mouse.

cedure(7) using homogenates of fresh brain in the presence of an excess of pyridoxal phosphate. Analyses for γ-aminobutyric acid and the decarboxylase assays were performed on aliquots of the same homogenates prepared from several brains. Most of the mouse brains used were from animals of the CBA/Jax strain. Brains from 154 mice of this strain were employed in making 49 individual determinations of both γ-aminobutyric acid and the enzyme. Each point shown in Fig. 1 and 2 is the average of from 2 to 7 determinations. Analyses for γ-aminobutyric acid were also made on 11 pooled samples of brain from Small strain mice and 10 pooled samples from the Large strain mice. The latter two strains of mice are maintained by Dr. M. Runner at the Jackson Memorial Laboratory. The rabbits used were of the New Zealand White Stock and Sandy Flemish strains. The dogs used were a 7-year-old Springer Spaniel and two 1-2-year-old Shetland Sheep dogs. The rats were of the Sprague-Dawley strain. In order to follow the histological changes which match the chemical changes observed, frozen sections in the sagittal plane at comparable

levels were cut of brains of CBA strain mice taken at postpartum intervals of 3 hours, 5, 8, 15, 19 and 22 days and 1, 2, and 3 months. These sections were stained for nucleic acids with thionin buffered to pH 5.0, for myelin by Spielmeyer's method, and for neurofibrillae by a Cajal technic. Also available for comparison were several series of other strains of mice and transverse and sagittal rat material stained for cells and fibers.

Results. γ-Aminobutyric acid content, glutamic acid decarboxylase activity, and fresh weight of developing mouse brain. All of the three variables studied increased greatly during postnatal development, attaining their maximal levels by 90 days (Fig. 1 and 2). The increases in γ-aminobutyric acid and decarboxylase are shown as increases in amounts per unit of fresh weight of tissue. The increase of decarboxylase activity lagged behind that of the weight and the γ-aminobutyric acid during the earlier period. Half-maximal values were attained at the following times: γ-aminobutyric acid, 3 days; brain weight, 6 days; glutamic decarboxylase, 15 days. At 15 days of development the γ-aminobutyric acid content and weight were 75 and 81%, respectively, of their final levels. The steadily rising concentration of γ-aminobutyric acid during the early stages of development shows that the rate of production of this amino acid by the decarboxylase exceeds the rate of its utilization during this period. The fact that a constant concentration of γ-aminobutyric acid is maintained after 60 days, when the decarboxylase activity is high, suggests that the system or systems utilizing γ-aminobutyric acid are operating at a higher level later in development. The nature of the transformations which γ-aminobutyric acid might undergo in nervous tissue is not known yet. This subject is under further investigation in the St. Louis laboratory.

Decarboxylase determinations of some samples of rat brain gave the following average values: 3 days, 41 μl CO_2; 7 days, 67 μl CO_2; 60-90 days, 290 μl CO_2. It is thus apparent that the postnatal changes in enzyme level in this species are similar to those found in the mouse.

Cytological observations. The changes occurring during postnatal development discussed in the preceding section are paralleled by drastic alterations in the brain morphology. The mouse brain at birth has largely completed the process of differentiation of neuroblast to neuron and is passing over into a period of growth and maturation of the neuronal elements themselves. Thus, in the first week it is possible to delineate all of the general cytoarchitectonic areas of the brain, although the constituent neurons are only partially differentiated and the fiber tracts substantially unmyelinated. During the second and third weeks the cell bodies increase in size, become separated from one another by markedly expanded dendritic processes, exhibit an increased prominence of Nissl granules and an apparent corresponding decrease in nuclear DNA. The axons of the major tracts become myelinated and the neuropil as revealed by silver stains grows particularly abundant. These changes occur throughout the brain but appear in a somewhat serial manner; being most noticeable first in the hindbrain, and later in the higher centers. These modifications continue, but at a diminished rate, until the end of the first month and appear to be completed within the following two months at an ever decreasing rate. On the basis of these histological observations we are led to conclude that the period of greatest increment in features which are related to maturation of the nuclear masses and fiber tracts of the CNS corresponds to the time of maximum increase in activity of the glutamic acid decarboxylase.

Correlation with previous work. A comparison of the postnatal morphological changes in the mouse with those in the rat reveals that the two species are similar. This conclusion is useful since it allows a comparison of our results with enzymatic determinations carried out in the rat(8), and the utilization of the careful quantitative measurements of Smith (9) on rat neocortex. Potter *et al.*(8), measured succinic dehydrogenase and ATP-ase activity in whole rat brains from 5 days prepartum to adult stages. The general form of curves obtained by these workers resembles that observed for glutamic acid decarboxylase in the mouse with the exception that the

changes from the first to the fourth week in rat brain represent a smoother rise and do not exhibit the spurt of activity observed during the second and third week in the brain of the mouse. Smith(9) measured cells per unit volume and total cells, volume, area, and thickness of neocortex in postnatal rats and found that the cells per unit volume diminished as the other parameters of maturation increased. Smith's curves for the quantitative changes in rat neocortex and those recorded by Peters and Flexner(10) in the guinea pig cortex are similar if the postnatal period of the rat corresponds to the prenatal period in the guinea pig; an assumption also consistent with the behavior of the two species. On this basis it should be possible to graph the enzymatic changes in whole brain of mouse and rat and in cerebral cortex of guinea pig (11) on a comparable time scale. When this is done, it is found that those enzymes which exhibit a marked increase in activity do so at a somewhat earlier maturation period in the brain as a whole than in the frontal cerebral cortex, a finding in agreement with the serial nature of morphological changes referred to above.

The activities of choline esterase(12) and carbonic anhydrase(13) in the rat, and of cytochrome oxidase, succinic dehydrogenase and ATP-ase in rat, pig, and guinea pig (8,11,14,15) all increase during the period of brain development which corresponds to the postnatal interval in the mouse. The general form of the curves for these enzymes is rather similar to that for glutamic acid decarboxylase, with the interesting exception of choline esterase. The activity of this enzyme reaches a maximum at about the same period that glutamic acid decarboxylase begins to level off, but this peak is followed by a sharp relative decrease and a secondary rise of smaller dimension. Since the highest brain centers continue to mature in the period following the climax of choline esterase activity and since histochemical localization of the enzyme indicates that it is deficient in adult dog cerebral and cerebellar cortices(16), one interpretation of this difference might be that choline esterase is more restricted in its CNS action than glutamic acid decarboxylase and the other enzymes which continue to increase in activity as long as the brain continues to enlarge and undergo differentiation.

It appears that the increase in glutamic acid decarboxylase activity is correlated with maturation of function in the CNS. Since γ-aminobutyric acid and glutamic acid decarboxylase are especially characteristic of brain and spinal cord(1-4,17) our observations lend credence to the hypothesis that the production of γ-aminobutyric acid from glutamic acid as catalyzed by glutamic acid decarboxylase is of importance in CNS activity: neuronal, interneuronal, or both.

Observations on nervous tissue of other species. A number of two-dimensional chromatograms of white and gray matter obtained by gross dissection of spinal cord of rabbits showed that there was higher concentration of γ-aminobutyric acid in the gray matter. Subsequent analyses of portions of cat brain gave the following results: gray matter (frontal tip of cortex), 64 mg % of γ-aminobutyric acid and 293 μl CO_2 per hour in the decarboxylase assay; gray matter of thalamus, 41 mg % and 326 μl CO_2 %; pooled gray matter, 43 mg % and 250 μl CO_2; pooled white matter, 28 mg % and 49 μl CO_2. These results show clearly that the levels of γ-aminobutyric acid and decarboxylase activity are higher in gray matter than in white matter. It is not known yet whether the γ-aminobutyric acid and enzymatic activity found in white matter are actually present in white matter or are a result of the presence of some gray matter in the samples examined. Determination of decarboxylase activity at different levels of rabbit spinal cord gave the following average values: lumbar, 174 μl CO_2; thoracic, 136 μl CO_2; cervical, 104 μl CO_2. Since there is a decreasing ratio of gray to white matter in going from the lumbar to the cervical regions of the spinal cord, these results are in keeping with the finding of a greater enzymatic activity in gray matter.

The failure to detect γ-aminobutyric acid in extracts of the sciatic nerve of rabbits has been reported(2). In the present study two-dimensional chromatograms were made of extracts of freshly dissected samples of cerebral cortex, optic nerve, and brachial plexus from

three dogs. γ-Aminobutyric acid was detected only in the samples of cortex. The present results are consistent with the interpretation that the γ-aminobutyric acid-glutamic decarboxylase system is only operative in the central nervous system, chiefly in the gray matter.

Summary. Brain weights, content of γ-aminobutyric acid, and the activity of glutamic acid decarboxylase were determined in brains of mice at various stages of postpartum development. All of the quantities studied increased greatly during postnatal development, attaining their maximal levels at 90 days. The increase in decarboxylase activity was slower during the first two weeks than that of the weight and the γ-aminobutyric acid. A cytological study revealed that the period of greatest increase in glutamic acid decarboxylase activity is correlated with the period of greatest increment in features which are related to maturation of the central nervous system. Examination of selected samples of nervous tissues of the cat, dog, and rabbit showed that the glutamic decarboxylase-γ-aminobutyric acid system is present chiefly in the gray matter of the central nervous system. The results of the present study were correlated with relevant data from the literature.

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Comparison of Actions of Dromoran (Nu-2206) and Morphine in Production of Vomiting.* (19225)

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Following the synthesis of 3-hydroxy-N-methyl morphinan by Schnider and Grussner (1) in the laboratories of Hoffmann-LaRoche, Inc., Randall and Lehmann(2) determined its analgesic potency in rats. They found it to be about 4 times as strong an analgesic as morphine. When doses having an equal analgesic action were given, the duration of action with Dromoran was 2 to 3 times that with morphine. In an earlier series of experiments a comparison was made of the action of Dromoran on intestinal smooth muscle with the actions of morphine, metopon, methadone, Nisentil, and meperidine(3). The intent of the present work is to compare its tendency to produce vomiting to that of morphine.

Method. Eighty-five dogs weighing between 5 and 44 kg with an average weight of 14.5 kg were used in these experiments. The animals fasted for 18-22 hours prior to each

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