Action de la saponine. Les résultats sont semblables à ceux obtenus avec la digitonine: il ne nous a pas été possible de solubiliser plus de 20% de l'activité initiale.

Nous avons fait varier la concentration en saponine de 0,5% à 5% et le pH de 7 à 8,5. La concentration optimum nous paraît être 2% et le pH 7.

Action du tween 20 (dérivé polyoxyalkylénique du monolaurate de sorbitol): Les surnageants obtenus se sont révélés inactifs bien que particulièrement riches en protéines; les culots sont également inactifs, et ceci semble dû à une action dénaturante importante du Tween 20.

Action du laurylsulfate de sodium. Nous avons essayé le laurylsulfate de sodium à diverses concentrations (0,05 à 0,5%), entre pH 7 et 8,5 et pendant des temps variables. Les conditions suivantes nous ont donné la meilleure solubilisation: concentration, 0,2% pendant 1 h; pH 7, température +2°C; centrifugation, 135000 *g*/1 h. Le surnageant contient alors 30% de l'activité initiale totale, avec une activité spécifique légèrement inférieure à celle des mitochondries.

Le culot a une activité spécifique 2 à 3 fois plus élevée que l'initiale. En retraitant le culot par le laurylsulfate dans les mêmes conditions on obtient de nouveau un surnageant actif. L'activité totale des surnageants réunis représente suivant les opérations 40 à 50% de l'activité totale. Un troisième traitement du culot ne nous a pas donné de surnageant actif, quelques soient les modifications de pH et de concentration en laurylsulfate apportées; mais on observe alors une nette diminution de l'activité des culots traduisant soit une dénaturation soit une inhibition de l'enzyme (Tableau).

Le surnageant actif, de couleur jaune, conserve son activité au moins 15 jours à −15°C et au moins 24 h à +4°C.

Comparativement aux autres tensioactifs essayés, le laurylsulfate nous a donc donné les meilleurs résultats. Il permet d'obtenir, avec un rendement satisfaisant, une solution à partir de laquelle peut être tentée une purification de l'enzyme.

Summary. The monoamineoxidase of the mitochondria of rat liver may be dissolved up to 50% of the initial activity by treatment with a tensioactive agent followed by ultracentrifugation. The best experimental conditions are obtained with 0.2% of sodium laurylsulphate (pH 7) during 1 h at +2°C.

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Glutamate Decarboxylase and γ-Aminobutyrate Transaminase in Developing Rat Brain

Maturational Changes in Cerebral Cortex IV.

Apart from the detailed investigations by Himwich[1] on the changes in glutamate decarboxylase (GAD, E.C.4.1.1.15) in the developing mammalian brain, no information is available on other enzymes of the glutamate-γ-aminobutyrate pathway. This report deals with an analysis of the activity of glutamate decarboxylase (GAD) and γ-aminobutyrate-α-ketoglutarate transaminase (GABAT, E.C.2.6.1.–.) during postnatal development of the rat brain. This study is part of a programme on maturation of changes in the cerebral cortex.

Wistar albino rats were killed by decapitation. The brain was quickly removed. 20% (w/v) homogenates were prepared in a Potter homogenizer. Water-mercaptoethanol (0.01) or water-sucrose (0.25) -mercaptoethanol (0.01) was used as a medium. Both enzymes were determined in the same homogenate. GAD was determined according to Hilgersom[2] and GABAT according to Salvador and Albers[3] with slight modifications.

After a 45 min incubation period (37°C, shaking, aerobic conditions), the reaction was stopped and the protein denatured by heating for 5 min at 100°C. Thereupon 2 ml of 0.25 *M* 3,5-diaminobenzoic acid (Aldrich), pH 6 (with K_2CO_3), and 1 ml of 0.5 *M* phosphate buffer, pH 5.8, were added and the mixture heated for 60 min at 60°C. After a brief centrifugation the resultant supernatant was diluted with 0.01 *M* phosphate buffer, pH 6.0. Fluorescence was measured with a Unicam SP 500 spectrophotometer with fluorometer attachment and a filter (Schott Jena PIL, 212017). The excitation wavelength was 418 mμ and the fluorescence wave-length 505 mμ. By using succinic semialdehyde synthesized, according to Schenck[4], calibration curves were made. The purity of the succinic semialdehyde used was controlled by determinations of the two functional groups.

The activities of the two enzymes in water homogenates are presented in Table I. The most striking result is the approximately constant ratio of the two enzymes, particularly during the greatest change in enzyme activity.

Table I. Activity of enzymes in water homogenate

Age (days)	GAD-act ± S.D.	GABAT-act ± S.D.	No. of determinations	GABAT/GAD ratio
0	4	13	1	3.3
5	4	17	1	4.3
10	11 ± 2	32 ± 5	4	2.9
15	20	49	2	2.5
20	27 ± 2	75 ± 2	4	2.8
25	29	84	1	2.9
30	34 ± 2	99 ± 10	4	2.9
40	35	88	1	2.5
Adult (± 3 months)	36 ± 3	115 ± 15	4	3.2

The activities are expressed as μmol product formed per g wet weight per h.

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Table II shows the results of the influence of 0.25 *M* sucrose homogenate on the GAD activity. The findings suggest that the GAD and GABAT activity in sucrose homogenate is approximately 50% and 60% of the activity in water homogenate respectively.

The parallelism between the two enzyme activities in water homogenates during development is obvious. Calculation of the regression curve confirmed this ($p < 0.005$). This fact can be expressed in the following formula:

$$[\text{GABAT}] = a[\text{GAD}] + b \qquad (b \text{ may be zero}).$$

The interpretation of these results remains difficult. Only a few data are known about other enzymes of the glutamate-γ-aminobutyrate pathway during development. The same is true for the substrates of these enzymes. Nothing is known about a possible cellular constancy of the GABAT/GAD ratio during development. SALVADOR and ALBERS[3], and ALBERS and BRADY[5] found different ratios in various parts of the central nervous system of adult rhesus monkeys. A parallelism similar to that found by us was reported by SISKEN et al.[6] for the optic lobe of the chicken. They reported, however, a ratio of approximately 1. A discussion on the effect of the sucrose medium is given by HILGERSOM[2]. A significant interpretation of this effect during development must await further experimentation.

Table II. Activity of enzymes in 0.25 sucrose homogenate

Age (days)	GAD-act ± S.D.	GABAT-act ± S.D.	No. of determinations	GABAT/GAD ratio
10	6 ± 1	15 ± 8	4	2.5
15	10	32	1	3.2
20	13 ± 1	46 ± 7	4	3.5
30	17 ± 1	57 ± 11	4	3.4
Adult (± 3 months)	22 ± 2	81 ± 13	4	3.7

Résumé. Le décarboxylase de glutamate (GAD) et le transaminase γ-aminobutyro-α-cétoglutarique (GABAT) ont été étudiés dans le cerveau du rat pendant le développement postnatal. Le résultat le plus frappant de cette recherche a été de montrer que le rapport de ces deux enzymes est à peu près constant au cours du développement. Ce rapport (GABAT/GAD) est de 2,5 à 3,7.

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Two Types of 5-Hydroxytryptamine Release from Isolated Blood Platelets

4-Chloro-N-methyl-amphetamine (Ro 4-6861) has been reported to decrease cerebral 5-hydroxytryptamine (5HT) and 5-hydroxyindolacetic acid without inhibiting the decarboxylase of aromatic amino acids[1,2]. The hypothesis was put forward that the compound liberates 5HT which, in contrast to 5HT released by reserpine, is not transformed to 5-hydroxyindolacetic acid. In order to further investigate the differences in the mechanism of action of Ro 4-6861 and reserpine, experiments with isolated blood platelets of rabbits have been carried out and will be reported in this paper.

Experimental Procedure. Platelets of rabbits were isolated as previously described[3], washed twice with physiological saline, and resuspended in a modified Tyrode solution[4] corresponding to the original plasma volume. The platelet suspension was incubated at 37°C under gentle shaking with or without reserpine (dissolved in saline containing 0.05 ml/l glacial acetic acid) and Ro 4-6861 (dissolved in saline).

Measurements of 5HT and 5-hydroxyindolacetic acid in the platelets and in the incubation medium were carried out with spectrophotofluorimetric methods[1,2,5]. For the chromatographic determinations, part of the platelet homogenates (with 1 *N* HCl) and of the incubation medium were brought to pH 10 (with K_2CO_3) and extracted with ethyl acetate (basic extract). The other part of the acid platelet homogenate and of the incubation fluid (acidified with HCl to about pH 1) was extracted with ethyl ether (acid extract). These extracts were evaporated to dryness at 25°C and redissolved in 0.5 ml methanol. For the paper chromatography (Schleicher & Schuell, No. 2043) the following solvent systems were used: butanol-potassium acetate, pH 5 and 7 (10:1, v/v), *n*-propanol-NH_3 1 *N* (5:1), butanol-acetic acid-water (5:1:4). The spots were developed either with 4 *N* HCl inducing the typical yellow fluorescence of indolyl compounds in the low UV or with Folin-Denis reagent producing the characteristic blue colour of hydroxylated phenols. Thin-layer chromatography was carried out on silica gel using the following solvent systems: isopropanol-methyl acetate-NH_3 25% (35:45:20), butanol-acetic acid-water (5:1:4). The spots were developed with formaldehyde inducing a sensitive yellow fluorescence in the UV[6].

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[4] NaCl 7.60 g/l (0.130 *M*), KCl 0.42 g/l (0.006 *M*), Versene 0.80 g/l (0.002 *M*), $NaH_2PO_4 \cdot 2H_2O$ 0.14 g/l (0.001 *M*), $NaHCO_3$ 2.10 g/l (0.003 *M*), Glucose 2.00 g/l (0.111 *M*), Saccharose 4.50 g/l (0.010 *M*).
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