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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[3] G. BARTHOLINI, A. PLETSCHER, and K. F. GEY, Exper. *17*, 541 (1961).
[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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Results. (1) Spectrophotofluorimetric measurements show that reserpine causes a progressive decrease of 5HT in the platelets within 2 h, which is accompanied by an increase of 5HT as well as of 5-hydroxyindolacetic acid in the buffer. Ro 4-6861 also markedly diminishes the platelet-5HT within 2 h; in the incubation medium a corresponding increase of 5HT, but not of 5-hydroxyindolacetic acid, occurs (Figure 1).

(2) According to paper chromatography, three different compounds with the fluorescence typical for indols are present in the Tyrode medium after incubation with reserpine. In the basic fraction two derivatives with Rf values identical with those of authentic 5HT and N-acetyl-5HT occur. In the acidic fraction two indolyl compounds with the Rf values of 5-hydroxyindolacetic acid and N-acetyl-5HT appear. One-dimensional paper chromatography with three solvent systems, two-dimensional paper chromatography (with butanol-acetic acid-water and butanol-potassium acetate), and thin-layer chromatography confirm that the three compounds found in the Tyrode solution behave like 5HT, N-acetyl-5HT, and 5-hydroxyindolacetic acid respectively (Figure 2). After incubation of platelets with Ro 4-6861, one single derivative with the characteristics of 5HT occurs in the Tyrode medium. In platelets before incubation as well as in platelets incubated for 2 h with reserpine or Ro 4-6861, only 5HT can be found.

Discussion. The metabolism of 5HT released from isolated platelets by reserpine differs from that of 5HT liberated by Ro 4-6861. On incubation with reserpine, 5-hydroxyindolacetic acid and another metabolite appear besides 5HT in the Tyrode medium. Paper chromatography using various solvent systems as well as thin-layer chromatography indicate that the second metabolite might be N-acetyl-5HT. After incubation with Ro 4-6861 no 5HT metabolites and only 5HT can be found. Similar differences in the formation of 5-hydroxyindolacetic acid have been shown *in vivo*, since after i.p. injection Ro 4-6861 in contrast to reserpine did not increase the content of 5-hydroxyindolacetic acid in the brain[1,2]. Furthermore, an interesting parallel with the metabolism of catecholamines *in vivo* is evident. Thus, norepinephrine released by reserpine undergoes oxidative deamination, whereas norepinephrine liberated by sympathomimetic amines is not primarily deaminated[7,8]. Formation of a neutral metabolite, e.g. N-acetyl-5HT, probably explains why the 5HT which disappears from the platelets incubated with reserpine cannot be fully recovered as 5HT and 5-hydroxyindolacetic acid in the incubation medium (Figure 1). N-acetyl-5HT is extracted only to a minor degree into the final aqueous phase used for the spectrophotofluorimetric assay of 5HT and 5-hydroxyindolacetic acid.

The finding that on incubation with Ro 4-6861 no 5-hydroxyindolacetic acid appears in the Tyrode medium is possibly the consequence of monoamine oxidase inhibition. Although Ro 4-6861 has been shown to be a weak inhibitor of this enzyme[9], a competitive inhibition of the

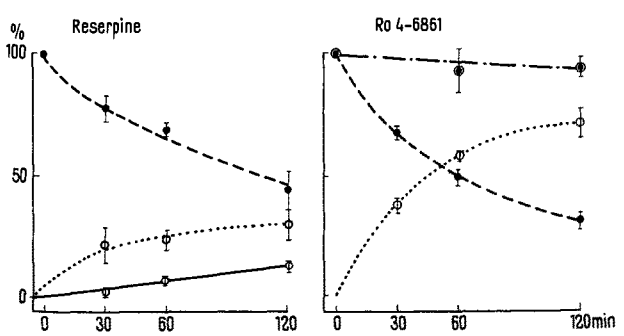

Fig. 1. Effect of reserpine and Ro 4-6861 on the metabolism of 5-hydroxytryptamine (5HT) in rabbit blood platelets suspended in a modified Tyrode solution. Ordinate: 5HT or 5-hydroxyindolacetic acid (in μM) contained in platelets of 1 ml suspension or in 1 ml incubation medium, expressed as percent of the 5HT of the platelets present before incubation with the drugs. Abscissa: minutes of incubation with the drugs (2.5 γ per ml reserpine; 100 γ Ro 4-6861-HBr per ml Ro 4-6861). ----- 5HT of platelets; ..... 5HT of incubation medium (Tyrode solution); —— 5-hydroxyindolacetic acid of incubation medium; ·-·-· 5HT of platelets incubated without drugs. The points represent averages ±S.E. of 2–7 experiments.

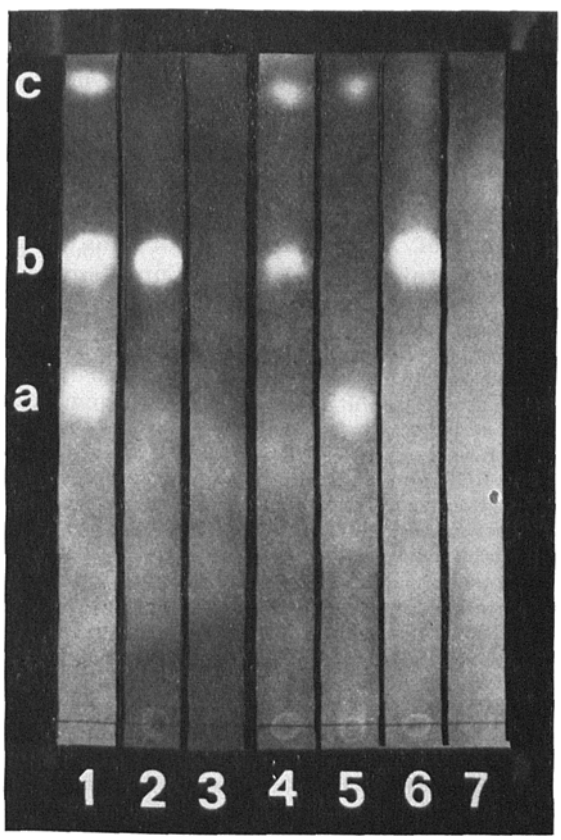

Fig. 2. Paper chromatography of the indolyl compounds before and after incubating isolated blood platelets of rabbits with reserpine or Ro 4-6861 at 37°C for 2 h. (1) Standards: (a) 5-hydroxyindolacetic acid, (b) 5-hydroxytryptamine, (c) N-acetyl-5-hydroxytryptamine; (2) before incubation: basic extract of platelets; (3) before incubation: acid extract of platelets; (4) reserpine: basic extract of incubation medium (Tyrode solution); (5) reserpine: acid extract of incubation medium; (6) Ro 4-6861: basic extract of incubation medium; (7) Ro 4-6861: acid extract of incubation medium. Solvent system: *n*-propanol-NH_3 1 *N* (5:1). Paper: Schleicher & Schuell, No. 2043. The fluorescence of the spots was developed by acidifying the paper with 4 *N* HCl and exposure to the low UV (254 mμ).

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5HT degradation cannot be excluded because of the relatively large amount of Ro 4-6861 present in the platelet suspension (approximately 0.4 μM/ml Ro 4-6861 compared with 0.05–0.08 μM/ml 5HT). It is, however, also conceivable that Ro 4-6861 releases 5HT by a different mechanism than reserpine, whereby the metabolizing enzymes of the platelets are, for instance, by-passed.

In preliminary experiments, other phenylalkylamines like tyramine and amphetamine as well as chlorpromazine caused 5HT liberation similar to Ro 4-6861, whereas the benzoquinolizine derivative Ro 4-1284[10] behaved like reserpine[11].

Zusammenfassung. Nach Einwirkung von Reserpin auf gewaschene, isolierte Thrombocyten werden neben 5-Hydroxytryptamin, 5-Hydroxyindolessigsäure und eine Substanz mit den Characteristika von N-Acetyl-5-hydroxytryptamin im Inkubationsmedium (modifizierte Tyrodelösung) gefunden. Das Phenyläthylaminderivat Ro 4-6861 setzt 5-Hydroxytryptamin ohne Bildung dieser Metabolite frei.

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Medizinische Forschungsabteilung, F. Hoffmann-La Roche & Co. AG, Basel (Switzerland), April 17, 1964.

[10] 2-Hydroxy-2-ethyl-3-isobutyl-9, 10-dimethoxy-1, 2, 3, 4, 6, 7-hexahydro-11bH-benzo[a]quinolizine.

[11] *Addendum.* While this paper was in print, it could be shown that the second metabolite (indicated as possible N-acetyl-5HT) is probably 5-hydroxytryptophol (5-hydroxy-3-(β-hydroxyethyl)-indole). N-Acetyl-5HT and 5-hydroxytryptophol have the same Rf values in all the chromatographic systems used in this investigation. The two compounds can, however, be separated by thin layer chromatography on silica gel using ethylacetate as solvent system.

The Effect of Aggregated γ-Globulins on Immune Cytolysis: the Role of Complement[1]

Earlier experiments[2] have shown that heat-aggregated human γ-globulin completely inhibits the immune reaction in an *in vitro* system consisting of isolated rat peritoneal mast cells, rabbit anti-rat γ-globulin serum (ARGG) and heat-labile serum factors (C'). From the observation that after washing away the aggregated HGG, the addition of ARGG and C' allows cytolysis to proceed, the assumption was made that this reaction cannot take place in the presence of aggregated human γ-globulin because the available complement is exhausted. The present experiments were performed to test this assumption.

Rat peritoneal mast cells were isolated and incubated with ARGG and 20% fresh guinea-pig serum (C') as described earlier[3]. Under these conditions 80% of the total histamine contained in the mast cells is released. Standard human γ-globulin SRK (16%; i.m.) was aggregated by heating for 20 min to 62°C. Bovine γ-globulin (BGG) was obtained by washing bovine serum with DEAE-cellulose[4]; part of this globulin was further purified on a DEAE column; the γ-globulin fractions obtained in this manner were freeze-dried and dissolved in the physiological buffer solution used for suspending the mast cells and part of it was aggregated by heating for 20 min to 62°C. Complement activity (C' H_{50}) was determined by the classical methods[5].

First, the effect of aggregated human γ-globulin on the anaphylactic release of histamine from isolated mast cells in the above *in vitro* system and on complement activity was compared. In earlier experiments it was found that aggregated human γ-globulin (HGG) in final concentrations of 0.1–1.0%, irrespective of whether or not heat-labile serum components are present, neither changes the appearance of the mast cells nor releases their histamine[2]. The results, which are graphically represented in Figure 1, show the close parallelism that exists between the effects of different concentrations of aggregated HGG on complement activity and on the anaphylactic release of histamine.

It is known from ISHIZAKA's experiments[6] that aggregated BGG has no complement-fixing properties. Therefore the effect of normal and aggregated bovine γ-globulin on the anaphylactic release of histamine from mast cells and on complement consumption in the immune reaction was examined. The results (Figure 2) show that neither untreated nor aggregated BGG are effective. Untreated BGG *per se* has no histamine-releasing effect on mast cells and, in its presence, the histamine-releasing effect

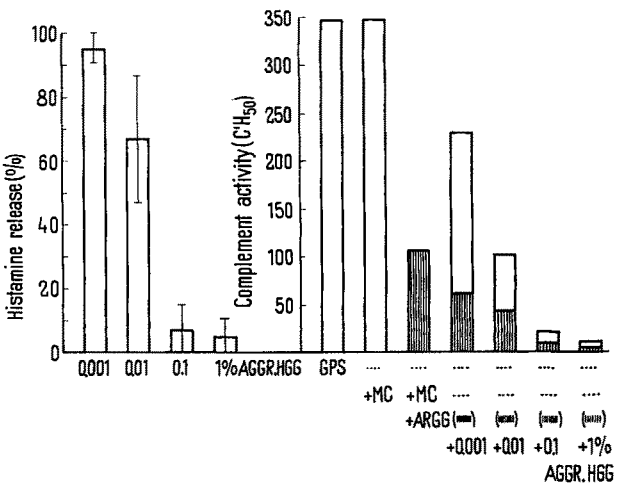

Fig. 1. The effect of heat-aggregated human γ-globulin (HGG) on histamine release from isolated rat mast cells (MC) by anti-rat γ-globulin serum (ARGG) and on complement activity (C' H_{50}). GPS = fresh guinea-pig serum.

[1] Supported by the Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung.

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