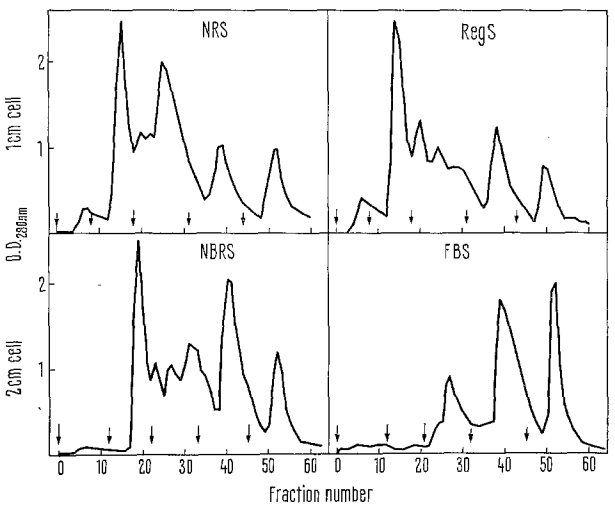
No inhibitory effects on growth of rat embryonic kidney cells could be detected, which grew prolifically in 10% concentrations of all serums. Mouse embryonic liver cells were, however, inhibited by NRS.

Since the evaluation of serums by means of primary embryonic liver cultures is tedious, other systems were tested. Growth of an established line of human kidney T-cells was slightly inhibited in media containing NRS or RegS when compared to FBS or new-born calf serum, while 3T3 cells hardly grew at all in NRS or RegS. For both cell lines the promoting effect of RegS over NRS was no longer significant. Tests based upon the promotion of DNA synthesis, as measured by the incorporation of ^{3}H-TdR, were also tried. Incorporation into rat or mouse thymocytes in *tris*-10 m*M* phosphate medium[19] was appreciably depressed by NRS or RegS compared to FBS. DNA synthesis in new-born rat liver fragments, as measured by the glass fibre disc method[20], was not significantly different for the 3 types of serum tested. All systems however, failed clearly to differentiate NRS from RegS as was found for the growth-promoting properties using embryonic liver cell cultures.



The chromatography on DEAE-cellulose of the proteins from different sera. The sera were dialyzed against 5 m*M* phosphate buffer pH 8.0 and 5 ml separated by step-wise elution from the columns. The arrows indicate the points of addition of the following buffers: 0.02*M* NaH_2PO_4; 0.05*M* NaH_2PO_4; 0.05*M* NaH_2PO_4 + 0.02*M* NaCl; 0.05*M* NaH_2PO_4 + 0.05*M* NaCl; 0.05*M* NaH_2PO_4 + 0.1*M* NaCl.

A biochemical characterization of the proteins in the different serums has been attempted. The total protein contents of RegS and NBRS was appreciably lower than for NRS while FBS had an even lower total protein content (starvation for 48 h did not reduce the serum protein content). The albumin contents of NRS and RegS was, however, proportionately less than for FBS or NBRS. This effect of partial hepatectomy has been noted by other investigators[21]. The separation patterns of proteins obtained by column chromatography on DEAE-cellulose, also differ significantly as is seen from the Figure. FBS contains appreciably less of the early eluted globulin fractions than does NRS. Further testing of the individual fractions will, however, be required to locate the non-dialyzable inhibitory factor in NRS.

Thus, in summary, normal rat serum contains a high MW inhibitor which retards the growth of rat embryo liver cells in vitro. The activity of this substance is decreased after partial hepatectomy and is essentially non-existent in the serum of new-born rats, or of foetal calves. It does not affect the growth of embryo kidney cells in vitro and hence may be a chalone-like material[22].

Zusammenfassung. Es wird gezeigt, dass Serum normaler Ratten einen Hemmstoff hohern Molekulargewichts enthält, der das Wachstum embryonaler Leberzellen der Ratte in vitro verlangsamt.

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Conversion of γ-Hydroxybutyrate to γ-Aminobutyrate by Mouse Brain in vivo[1]

γ-Hydroxybutyrate (GHBA) causes CNS depression in animals, including man[2-4] and has been used as an anaesthetic adjuvant in man[5,6]. GHBA may act indirectly since CNS effects occur slowly after systemic administration and since it apparently exerts no depressant effect on single neurones when applied iontophoretically[7]. GHBA reduces the biosynthesis of γ-aminobutyrate (GABA) from glucose in vivo[8] without affecting the total amount of GABA in the brain, and a direct conversion of GHBA to GABA by homogenates of brain has been reported[9]. It seemed of interest, therefore, to test whether GHBA is converted to GABA in vivo.

Swiss albino mice (20–30 g) were injected i.p. with 500 μC/kg labelled GHBA-Na^+ (Schwarz BioResearch Inc.; specific radioactivity, 5.5 mc/mmole; 1-^{14}C-carboxyl-labelled) plus 500 mg/kg unlabelled GHBA-Na^+ (Brickman and Co.); the injected volume was about 0.6 ml per mouse (pH 7.4). Animals were decapitated 15–180 min after injections and their brains were quickly removed, weighed and homogenized in 3 ml of 80% ethanol (V/V). Homogenates were centrifuged for 30 min at 15,000 × *g* and supernatants were acidified to pH 2.0 and applied to columns of the cation exchange resin, Dowex 50W-X2 (hydrogen form). GHBA was eluted separately from the 'amino acid fraction'[9]. Aliquots of the amino acid fraction were separated chromatographically and quantitated spectrophotometrically for individual amino acids[10]. Radioactivity was measured with a Packard liquid scintillation counter, and corrections for quenching were made by a method of external standardization.

Results in Table I show that labelled amino acids were synthesized from the labelled GHBA in all experiments, for the proportion of the total radioactivity eluted from the resin by the NH_4OH was far greater than could be accounted for by impurities not eluted by the water

Table I. Content of ^{14}C in amino acid and non-amino acid fractions of mouse brain extracts following i.p. injections of 1-^{14}C-GHBA

Time killed after GHBA (min)	Disintegrations/min ($\times 10^{-3}$) per g of brain: Water wash	NH_4OH wash	Radioactivity in NH_4OH wash (%)
15	482	39.0	7.5
	345	22.8	6.2
Mean	414	30.9	6.9
60	564	72.1	11.3
	399	36.9	8.5
	385	34.1	8.1
	485	95.2	16.4
	484	42.8	8.1
	351	41.5	10.6
	392	51.6	11.7
Mean ± S.E.	437 ± 28	53.5 ± 8.5	10.7 ± 1.0
120	282	74.4	20.9
	237	40.6	14.6
	215	45.9	17.6
Mean ± S.E.	245 ± 20	53.6 ± 10.5	17.7 ± 1.8
180	50	22.2	30.8

Water wash and NH_4OH wash refer to fractions eluted from the cation exchange resin and correspond to 'non-amino acid' and 'amino acid' fractions, respectively.

wash. The radioactivity in the amino acids was about the same in brain extracts from mice killed at 60 or 120 min after the GHBA and was appreciable 180 min after injection. The specific radioactivities of GABA, α-alanine and glutamate are presented in Table II; radioactivity in these 3 amino acids accounted for 37 ± 11% (mean ± S.D.) of the total radioactivity in the amino acid fraction. It is clear from the results presented herein that GABA becomes labelled after injection of labelled GHBA, and that the total amount of GABA in the brain is not changed[11]. There was no simple correlation between the labelling of GABA and the depression caused by the drug, but the 'water wash' (containing unchanged GHBA and its lactonized form) contained more radioactivity when animals were severely depressed (at 60 min after injection of GHBA). 'Severe depression' refers to the state in which animals did not move or right themselves; however, all behaviour was not assessed on a 'quantitative basis' and is presented only as ancillary observations.

Zusammenfassung. γ-Hydroxybutyrat wird im Maushirn in γ-Aminobutyrat und in verschiedene α-Aminosäuren umgewandelt.

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Table II. Incorporation of ^{14}C from injected 1-^{14}C-GHBA into individual amino acids of brain

Time killed after GHBA (min)	GABA: μmoles/g	dpm/g	dpm/μmole	Glutamate: μmoles/g	dpm/g	dpm/μmole	α-Alanine: μmole/g	dpm/g	dpm/μmole
15	3.9	1700	436	6.4	2800	438	0.9	1214	1315
	4.0	1840	460	6.3	1770	281	0.6	1174	1960
Mean	4.0	1770	448	6.4	2285	360	0.8	1194	1655
60	3.2	6170	1900	11.3	1640	145	1.0	885	885
	4.7	4780	1020	6.7	6800	1015	1.0	2490	2490
	3.8	3370	890	6.5	6100	940	0.6	2000	3330
	4.1	3260	795	6.8	2920	430	0.9	1910	2120
	3.7	5020	1360	6.0	5220	870	0.7	2910	4160
Mean ± S.E.	3.9 ± 0.3	4520 ± 565	1193 ± 199	7.5 ± 0.9	4536 ± 978	680 ± 169	0.8 ± 0.1	2039 ± 347	2597 ± 545
120	3.3	9350	2790	13.0	6100	470	0.9	2060	2290
	4.0	12,670	3160	7.1	4800	670	1.4	2760	1970
Mean	3.7	11,010	2975	10.0	5450	570	1.2	2410	2130
180	4.0	3400	710	6.9	2280	331	0.6	1620	2700

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