Liver Homeostasis: an in vitro Evaluation of a Possible Specific Chalone

The elucidation of the biochemical mechanisms controlling cellular proliferation, which are fundamental for tissue homeostasis in the organs of the adult animal, poses one of the most challenging problems of contemporary biological research. The implications for an understanding of the nature of, and possible control of malignant growth are obvious. From this point of view, considerable interest has focused on the investigation of tissue specific growth-inhibitors called chalones, which have been described for granulocytes and erythrocytes[1], epidermis[2], kidney[3], endometrium[4] and lung tissues[5].

These inhibitory growth mechanisms sometimes even remain operative in established lines of cultured cells exhibiting contact inhibition of growth[6,7]. Other endogenously produced growth regulators, possibly ketoaldehydes, have also been reported[8], as well as less well defined products[9].

The hyperplasia of rat liver following partial hepatectomy offers a suitable biological system to investigate changes in homeostatic growth mechanisms. Normal adult rat serum has been claimed to contain a growth inhibitor for liver which is decreased following partial hepatectomy[10,11]. On the other hand, the serum of partially hepatectomized rats has been claimed to contain a growth promoter[12]. Liver hyperplasia has been correlated with changes in serum proteins[10,13,14], and has been shown to be affected by liver macerates[11,15], specific proteins isolated from liver tissue[16], as well as non-specific enzymes such as arginase[17].

The present work was aimed at the in vitro evaluation and properties of the growth-regulating substance occurring in normal rat serum, by means of its effect on primary cultures of rat liver embryonic fibroblasts.

Experimental. Male albino rats 180–200 g were partially hepatectomized (left and median lobes) by a standard technique under ether anaesthesia. Blood was removed by cardiac puncture, using a plastic syringe, 48 h postoperatively, also under ether anaesthesia. The serum was recovered by centrifugation from the chilled clotted blood, pooled, and sterilized by Millipore filtration. Blood obtained by decapitation gave identical results, indicating that possible traces of ether remaining in the serum were non-toxic. Control rats were sham operated.

Suspensions of liver cells were prepared by trypsinizing several livers obtained from one litter of approximately 15-day-old rat embryos. Approximately $2-5 \times 10^5$ cells were inoculated into small Carrel flasks containing 5 ml of lactalbumin phosphate medium (LP)[18] with 10% of the appropriate serum under test. After 3–4 days the unattached cells were removed and fresh medium added until 6–7 days, when the cells were fixed and stained with haematoxylin. Cultures exhibited the typical patterns of growth of parallel oriented fibroblasts, although the morphology of the colonies was sometimes affected by the different serums. Cell growth was scored by counting cells per field in several complete traverses across the diameter of the flask using an inverted microscope.

Results. The effect of different serums on the growth of rat embryonic liver fibroblasts is shown in Table I. Prolific growth is found in media containing foetal bovine serum (FBS) or new-born rat serum (NBRS) (less than 14 days old). Normal rat serum (NRS) is however strongly inhibitory, while serum from rats 48 h after partial hepatectomy, regenerating serum (RegS) is significantly less inhibitory. Better growth of cultures was obtained in flasks precoated with preparations of rat tail collagen but in these cases the differential effect of the serums on growth tended to be obscured. Dialysis of the serums against LP medium, or heating to 56 °C for 1 h further reduced the growth-supporting properties of the serums. This presumably indicates the presence of dialyzable or thermolabile growth factors. The inhibitor in NRS is however non-dialyzable, while FBS contains a non-dialyzable growth-promoting factor, possibly fetuin, as is shown in Table II.

Table I. The effect of different serums on the growth of rat embryonic liver fibroblasts

Serum	Cells/mm^2 Mean ± S.D.	%
Foetal bovine serum (FBS)	357 ± 35	100
New-born rat serum (NBRS)	321 ± 30	90
Normal rat serum (NRS)	136 ± 12	38
Heated NRS	68 ± 8	19
Regenerating (48 h) serum (RegS)	218 ± 25	61
Heated RegS	89 ± 9	25

LP medium containing 10% of the different sera. Mean ± S.D. for 5 different experiments of cell growth after 6–7 days.

Table II. The growth of rat embryo liver cells in media containing different proportions of dialyzed sera

Growth (% of control)			
NRS 10% + FBS 0%	NRS 10% + FBS 10%	NRS 10% + FBS 25%	NRS 10% + FBS 50%
25	41	54	60
FBS 10% + NRS 0%	FBS 10% + NRS 10%	FBS 10% + NRS 25%	FBS 10% + NRS 50%
54	42	23	18

All sera were dialyzed at 4 °C to equilibrium with LP medium. Control was undialyzed FBS 10% with a growth of 345 cells/mm^2. Results are the mean of 2 experiments.

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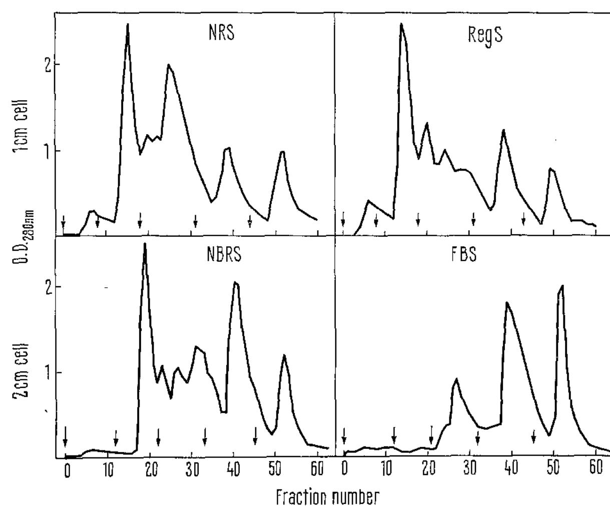
No inhibitory effects on growth of rat embryonic kidney cells could be detected, which grew prolifically in 10% concentrations of all serums. Mouse embryonic liver cells were, however, inhibited by NRS.

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