The Specificity of the Regulation of Organ Growth: The Effect of Tissue Extracts on the Incorporation of Tritiated Thymidine in Liver and Kidney (35828)

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It has been suggested that cell proliferation is regulated by inhibiting factors described as "chalones" (1). Evidence has been furnished that the chalone model may be valid for the skin (2–5), hematopoietic system (6, 7), lung (8), liver (9), and kidney (10, 11). The synthesis and concentration of "chalones" are thought to be dependent on the degree of cellular differentiation, and cell growth is regulated by an organ-specific feedback mechanism as was first suggested by the "template–antitemplate" theory of growth control of Weiss (12).

Experimental data on the multiplication of cells in the liver and the kidney often fit well into this hypothesis. Our work has been concerned with an attempt to demonstrate these factors in the liver and the kidney and, in particular, to investigate their organ-specific action. An experimental model was chosen in which the DNA synthesis of both organs could be measured and compared.

Materials and Methods. Liver and kidneys of adult Sprague-Dawley rats, weighing 500 g, were removed under sterile conditions and homogenized (Braun) for 5–10 min in cold buffered solution (0.2 *N* Tris buffer (pH 7.4); 0.25 *M* saccharose; 0.005 *M* $MgCl_2$ at a ratio of 5:4:1). For each gram of tissue, 1 ml of cold buffer was used. The homogenate was centrifuged (16,000*g*; 16 hr; 2°) and the supernatant was lyophylized. The powder was dissolved in ice-cold distilled water and precipitated serially with ethanol (denatured by 5% methanol) at concentrations of 10, 50, 65, and 80%. After 12 hr of stirring, the precipitates were centrifuged (2°) at 2000*g* for 25 min and lyophylized (preparation by Dr. W. Hondius-Boldinger, NV Organon).

Pregnant Sprague-Dawley rats were housed in Macrolon cages and kept under controlled conditions (Hope Farms diet, water *ad libitum*). The young from at least 2 litters were divided into groups of 5 animals. Experimentals (22 days old) were treated subcutaneously with organ extracts and their precipitates at 5 geometrically progressing dose levels (12.5 mg to 200 mg in 2 ml of 0.9% saline), 10 hr before intraperitoneal injection of ^{3}H-thymidine (250 μCi/kg of body wt). Controls were treated subcutaneously with the solvent and/or beef albumin (Serva-Entwicklungslabor Heidelberg: Cohn fraction V, lyophylized). Two hr later (10 a.m.), the animals were killed and bled. Liver and kidneys were removed and homogenized (Potter-Elvehjem) in cold 0.9% saline. After a dilution of 1:25 in 0.9% saline, the uptake of ^{3}H-thymidine of the entire organ was quantitatively measured [method (13, 14a, b)].

Incorporation values are calculated from the mean value of 3 activity measurements in relation to the protein content (mg). The effect of treatment on the ^{3}H-thymidine incorporation is expressed as a percentage of the control. The results were statistically analyzed, and significant differences ($p = 0.01$) compared with controls are indicated in Figs. 2–4.

Results and Discussion. Reviewing the findings of the last 10 years, numerous contradictory results were reported after administration of organ homogenates. Inhibitory effects on liver cell multiplication (15–17) were observed as well as stimulation (18–20)

or no effects (21). A similar spectrum of results exists for the kidneys: inhibition (22–24), stimulation (25). The findings were formerly reported to be organ specific but more recently discussed as unspecific effects (22, 24, 25). A histone dependent inhibitory effect on the DNA-synthesis has been observed (26), and inhibition by liver arginase has been demonstrated (27, 28). However, other factors were shown to be mainly responsible in the liver (29). Isolation of inhibitory substances revealed that the liver actually contained some cytotoxic factors (30).

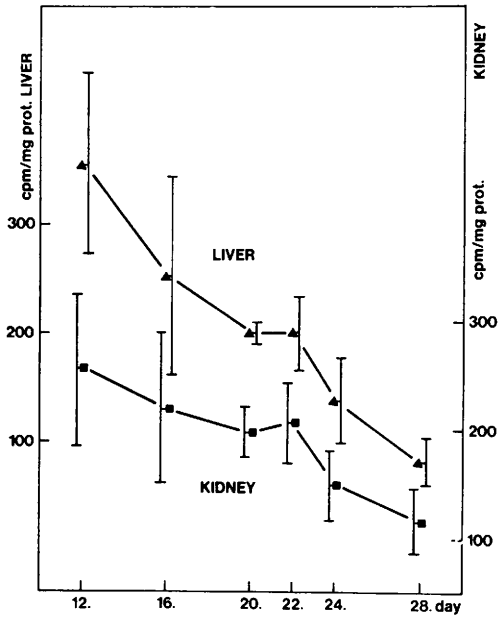

FIG. 1. Incorporation of ^{3}H-thymidine in the liver and the kidneys of 12- to 28-day-old rats (mean values and standard deviations).

Between days 12 and 16 after birth, incorporation of ^{3}H-thymidine into the liver and the kidneys showed great individual variations (Fig. 1). The DNA synthesis decreased more in the liver than the kidneys up to day 20, after which the curves ran almost parallel with nearly identical rates of incorporation and standard deviations. Erythropoiesis in the liver has been reported to alter the thymidine incorporation until day 20 (31, 32). For standardization, the age of animals was therefore chosen as 22 days. Our measurements were always done at exactly

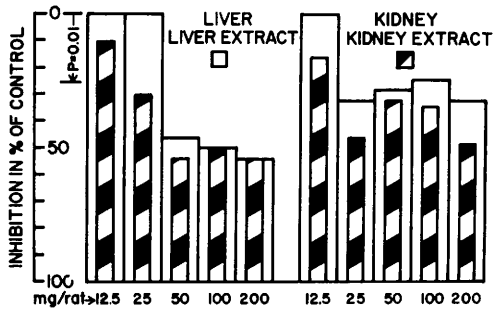

FIG. 2. Incorporation of ^{3}H-thymidine in the liver and the kidneys after treatment with liver or kidney extract.

the same time to avoid diurnal variations in the mitotic index (21, 33). The organ weights showed a significant influence on the ^{3}H-thymidine incorporation. Because of this factor, the animals were specially selected according to body weight, which correlated well with organ weights. The organ extracts used in our experiments were obtained strictly according to the methods described for skin "chalones" (1) to demonstrate similar tissue-specific regulation mechanisms for the liver and kidneys.

After treatment of the rats with the whole liver extract, a dose dependent inhibitory effect in the liver was found reaching a maximum of 50% compared with the controls. However, the same extract decreased the DNA synthesis in the kidneys by 30%. Similar data were found in both organs after application of the whole kidney extract. Therefore, in this model no evidence was seen

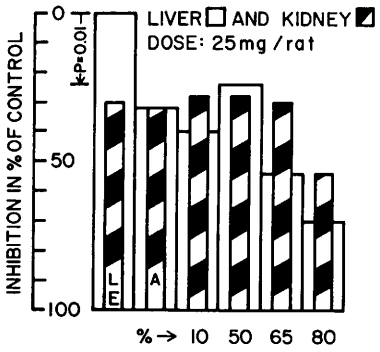

FIG. 3. Incorporation of ^{3}H-thymidine in the liver and in the kidneys after administration of liver extract (LE) alcohol precipitates of liver extract, and albumin (A).

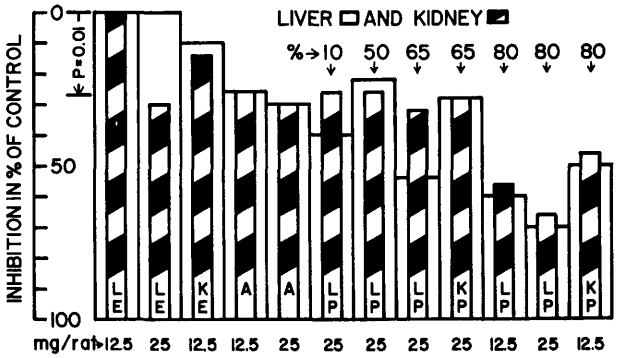

FIG. 4. Incorporation of ^{3}H-thymidine in the liver and in the kidneys after administration of liver (LE) or kidney (KE) extracts and their alcohol precipitates (LP, KP).

for any organ-specific growth inhibitory effect (Fig. 2).

When animals were treated with the alcohol fractions of liver extract the most effective inhibition on the liver was found in the high alcohol precipitates. The 65% fraction showed a good inhibition of liver DNA synthesis, but only a comparatively small effect on the kidney. However, the organ specificity could not be generalized since the 65% alcohol precipitate of kidney extracts inhibited both liver and kidney. The best inhibitory effect on liver DNA synthesis was found by 80% alcohol precipitates of liver extracts, but thymidine incorporation in the kidneys also was substantially decreased. Reversely, the application of 80% alcohol precipitates of kidney extracts resulted in lowered DNA synthesis of the liver and kidneys at almost the same values (Figs. 3, 4).

Albumin (administered at 25 mg/animal) was also seen to inhibit the uptake of thymidine in the liver and kidneys. For the kidneys the same amount of inhibition was found as after treatment with the whole liver extract. In summarizing the results, all our experiments showed a substantial inhibitory effect of liver–kidney extracts on DNA synthesis, but the effect was not found to be organ specific. Further investigations are needed to determine the structure, classification, and the mode of action of these active cell fractions.

Summary. Experiments with liver and kidneys were undertaken in an attempt to demonstrate organ-specific inhibitory factors analogous to epidermal "chalones." Extracts and alcohol precipitates of extracts of liver and kidney were tested for inhibitory action on DNA synthesis in the named organs. Whole organ extracts and high concentration alcohol precipitates had marked inhibitory action, as high as 50%, on DNA synthesis in both organs. However, an organ-specific effect could not be demonstrated.

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