STIMULATION OF REGENERATION OF THE LIVER BY COMBINED TREATMENT WITH ADRENALIN, GLUCAGON, AND THEOPHYLLINE

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Administration of adrenalin with theophylline or of glucagon with theophylline for several days regularly increases the weight of the regenerating liver in rats compared with the control. Combined treatment with adrenalin, glucagon, and theophylline produces maximal stimulation. This treatment leads to a decrease in the glycogen concentration in the regenerating liver and to an increase in the number of nuclei and of binuclear cells. The DNA concentration was significantly increased in the regenerating liver of rats receiving combined treatment with adrenalin, glucagon, and theophylline.

During the first few days after partial hepatectomy the glycogen reserves are sharply reduced in the regenerating liver [1, 4, 5]. The reduction in the glycogen concentration in the liver precedes the maximum of DNA synthesis [10]. These facts suggest that the energy released by the breakdown of glycogen is utilized for regeneration. Glycogenolysis is readily induced by adrenalin and glucagon. These hormones activate the adenyl cyclase of the liver [2], with a consequent increase in synthesis of cyclic 3,5-adenosine monophosphate (CAMP), which participates in glycogenolysis [6, 7].

The present investigation was carried out to study the possibility of stimulating regeneration in the liver by administration of these hormones, especially in conjunction with theophylline, which promotes the accumulation and preservation of CAMP.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats. After ligation, the middle and left lateral lobes of the liver, accounting for about 70% of its mass, were removed. The hepatectomized animals were divided into groups. Some received adrenalin or adrenalin with theophylline, while other received glucagon* and theophylline or a combination of adrenalin, glucagon, and theophylline. Rats receiving no treatment or injections of physiological saline acted as the control. Theophylline was given by mouth as an aqueous suspension twice or three times a day in a daily dose of 10 mg/100 g body weight. Adrenalin was injected subcutaneously twice or three times a day in doses of 0.5 ml of the 0.01 or 0.02% solution. Glucagon was injected subcutaneously three times a day in a daily dose equivalent to 33 or 75 μg per rat. Administration of the substances began on the second day after the operation and continued through the sixth day of the experiment. On the seventh, the animals were decapitated. The weight of their liver and, in some experiments, the content of glycogen and DNA in the liver were determined. To estimate the DNA concentration pieces of liver weighing 500 mg were dried and defatted with anhydrous alcohol and acetone, and then ground into a powder. DNA was determined in 50 mg of the powder by the modified diphenylamine method [4]. Pieces

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TABLE 1. Effect of Adrenalin, Glucagon, Theophylline, and Their Combinations on Regeneration of the Liver in Rats (M±m)

Expt. No.	Treatment	No. of rats	Final body weight (in g)	Mean weight of regenerating liver: mg	mg/100 g body weight	in %	Glycogen concentration (in mg/g wt. of liver)
1	1. Control (no treatment)	12	206	6137±249	2979±80	100	—
	2. Theophylline	18	208	7444±102	3507±88	120	—
				$P_{1,2} < 0,001$	$P_{1,2} < 0,001$		
2	1. Control (no treatment) *	14	238	7139±124	2999±110	100	—
	2. Theophylline + adrenalin	12	215	8850±216	4109±149	137	—
				$P_{1,2} < 0,001$	$P_{1,2} < 0.001$		
	3. Theophylline	15	220	6793±185	3088±170	103	—
	4. Adrenalin*	14	229	7714±316	3368±186	111	—
				$P_{1,4} < 0,05$	$P_{1,4} < 0,05$		
3	1. Control (no treatment)	12	218	6652±202	3051±135	100	—
	2. Adrenalin† + theophylline	12	191	7704±333	4033±118	132	—
				$P_{1,2} < 0,02$	$P_{1,2} < 0,001$		
4	1. Control (no treatment)	8	141	4665±106	3308±182	100	38,7±5,4
	2. Adrenalin† + theophylline	8	144	6240±124	4333±182	130,9	25,2±1,9
				$P_{1,2} < 0,001$	$P_{1,2} < 0,002$		$P_{1,2} < 0,05$
	3. Glycogan‡ + theophylline	10	147	6500±303	4422±228	133,6	19,1±2,9
				$P_{1,3} < 0,001$	$P_{1,3} < 0,01$		$P_{1,3} < 0,01$
5	1. Control (injections of physiological saline)	9	151	5528±273	3661±163	100	40,5±3,8
	2. Adrenalin‡ glucagon** + theophylline	8	156	8194±239	5266±184	143,8	24,3±2,5
				$P_{1,2} < 0,001$	$P_{1,2} < 0,001$		$P_{1,2} < 0,01$

* Adrenalin twice a day, 0.5 ml of 0.01% solution each time.
† Adrenalin three times a day, 0.5 ml of 0.02% solution each time.
‡ Glucagon three times a day in daily dose of 33 μg.
** Glucagon three times a day in daily dose of 75 μg.

TABLE 2. Effect of Combined Treatment with Adrenalin, Glucagon, and Theophylline on Number of Binuclear Cells, Number of Nuclei, and DNA Content in Regenerating Liver

Expt. No.	Treatment	Wt. of regenerating liver (in % of control)	No. of binuclear cells in 100 fields of vision	No. of nuclei in 30 fields of vision	DNA concentration (in extinction values)
2	1. Control (no treatment)	100	15,3±1,6	14,1±0,8	—
	2. Theophylline	103	17,0±1,7	18,0±2,0	—
			$P_{1,2} > 0,05$	$P_{1,2} > 0,05$	
	3. Adrenalin	111	19,0±0,6	21,8±1,6	—
			$P_{1,3} < 0,001$	$P_{1,3} < 0,001$	
	4. Theophylline + adrenalin	137	22,5±1,3	29,1±1,5	—
			$P_{1,4} < 0,001$	$P_{1,4} < 0,001$	
4	1. Control (no treatment)	100	22,5±1,3	18,3±0,4	0,85±0,05
	2. Adrenalin + theophylline	130,9	26,7±1,7	24,9±0,9	1,09±0,03
			$P_{1,2} > 0,05$	$P_{1,2} < 0,001$	$P_{1,2} > 0,05$
	3. Glucagon + theophylline	133,6	29,3±1,2	24,8±0,4	1,15±0,05
			$P_{1,3} < 0,002$	$P_{1,3} < 0,001$	$P_{1,3} < 0,001$
5	1. Control (injections of physiological saline)	100	20,8±1,2	18,7±0,5	0,86±0,08
	2. Adrenalin + glucagon + theophylline	126,3	29,3±1,2	26,7±0,6	1,28±0,08
			$P_{1,2} < 0,001$	$P_{1,2} < 0,001$	$P_{1,2} < 0,01$

of liver from each rat were also embedded in paraffin wax, and sections cut to a thickness of 6μ were stained with hematoxylin-eosin. Under an immersion objective the number of nuclei in 30 fields of vision and also the number of binuclear cells in 100 fields of vision were counted.

EXPERIMENTAL RESULTS

The experimental results are given in Tables 1 and 2.

As Table 1 shows, adrenalin in conjunction with theophylline increased both the absolute and the relative weight of the regenerating liver in all the experiments. Glucagon had a similar action in conjunction with theophylline (experiment 4). However, the greatest effect was obtained by combined treatment with adrenalin, glucagon, and theophylline: the weight of the liver of the experimental rats was 43% higher than that of the control, partially hepatectomized animals (Table 1, experiment 5).

The increase in weight of the regenerating liver observed in the rats receiving glycogenolytic hormones with theophylline was accompanied by a decrease in the glycogen concentration and an increase in the number of nuclei and the number of binuclear cells. In the animals receiving glucagon with theophylline and, in particular, in the rats treated with adrenalin, glucagon, and theophylline, there was a substantial increase in the DNA concentration in the liver.

The results confirm that adrenalin and glucagon, in conjunction with theophylline, and especially when all three are given together, stimulate proliferation in the regenerating liver.

This conclusion that glucagon stimulates regeneration of the liver is in agreement with the work of Lieberman and Short [8], who showed that glucagon, in conjunction with triiodothyronine, L-amino acids, and heparin, stimulates proliferation in the intact liver. However, Price et al. [9] came to a different conclusion, namely, that glucagon inhibits DNA synthesis and hyperplasia of the liver. It will be noted that this conclusion was drawn from experiments carried out under highly artificial conditions, on eviscerated dogs whose life was maintained for a few days by intravenous injection of digests of proteins and carbohydrates and of insulin.

Glucagon and adrenalin, hormones stimulating glycogen breakdown, at the same time stimulate regeneration of the liver, as is exhibited particularly well if they are given with theophylline. However, it must be pointed out that the degree of this stimulation does not always correlate directly with the decrease in the liver glycogen concentration (Table 1, experiments 4, 5). The possibility cannot be ruled out that CAMP, which accumulates in the liver during the action of these hormones and theophylline, stimulates regeneration of the liver not only by increasing glycogen breakdown, but also in some other way.

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