Effects of sera and liver extracts from partially hepatectomized rats on liver slice DNA synthesis[1]

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Summary. Sera from partially hepatectomized rats (PH) compared to sera from control rats (C) enhance liver slice DNA synthesis but depress kidney slice DNA synthesis. Alone, liver extracts from PH do not affect DNA synthesis; but adding sera to PH extracts stimulates, suggesting that sera and liver factors from PH may participate in compensatory growth.

Liver regeneration following mass loss may be controlled, to some extent, by circulating hepatotropins[2-7]. Some studies suggest that liver may be their source[8,9]. We sought evidence to support the existence of humoral and liver factors affecting DNA synthesis in hepatocytes using in vitro methodology that previously demonstrated a renotropic system[10-12].

Materials and methods. We performed $^2/_3$ partial hepatectomies (PH), or sham-operations (Sham) between 14.00 and 15.00 on 140–250 g Sprague Dawley rats and 20 h later (10.00–11.00) blood was drawn from the lower aorta and/or the livers removed. Sera for assay of DNA synthesis (incorporation of ^{3}H-thymidine into DNA) were prepared as described previously[11]. Livers were homogenized in cold isotonic medium (1 g liver/5 ml medium). After homogenization and centrifugation, the supernates were collected and used immediately.

Organs of rats that received no operation supplied the tissue to be tested. Liver and kidney slices were cut into small portions[13] and randomly distributed among 25 ml Erlenmeyer flasks, so that a flask contained 3–4 pieces. The weight of the slices in each flask approximated 50–75 mg. Slices were incubated on a Dubnoff Shaker in 2 ml medium composed of the following: K^+ 110, Ca^{++} 10, Mg^{++} 20, Cl^- 145, and HCO_3^- 25 mmoles/l, and gassed with 95% O_2 and 5% CO_2. 2 μCi of methyl ^{3}H-thymidine (spec. act 15.2 Ci/mmole) were added to the flasks. When sera and/or liver extracts were studied, 0.2 ml of each replaced an equal volume of medium. DNA determinations and isotope counting were performed in a manner previously described[11]. We ran a minimum of 4 flasks for each control and test, and an average of each difference in specific activity was used for the final result. Statistics were analyzed by paired analysis using Student's t-test (double tail). Statistical significance was set at $p < 0.05$.

Results and discussion. Our preliminary studies showed that incorporation of ^{3}H-thymidine into DNA was uniform over a 90 min incubation, and by autoradiography, that the major incorporation was nuclear. In 6 experiments, the addition of sera from control rats (10% v/v) to incubating liver slices significantly enhanced DNA synthesis over the slices incubating in medium alone (+15.5%) (table 1). In 4 experiments, slices incubated in extract from Sham (10% v/v) compared to slices incubated in medium alone showed no difference in DNA synthesis. There was also no difference when extracts from the remaining liver segment 20 h after $^2/_3$ partial hepatectomy were investigated. With either extract, the small decrease in ^{3}H-thymidine uptake into liver DNA represented less than a 1% change from control. In 4 experiments DNA synthesis by liver slices incubating in both sera (10% v/v) and liver extracts (10% v/v) from Sham compared to DNA synthesis in liver slices incubating in medium alone showed some stimulation (+9.8%).

Our primary intent was to demonstrate whether sera from PH compared to Sham stimulate ^{3}H-thymidine incorporation into liver slice DNA. In 20 experiments, there was a small but significant stimulation to DNA synthesis by PH sera over the 90 min incubation (+9.9%, $p < 0.01$) (table 2). While this stimulation by PH sera was relative to the effects of Sham sera, actual stimulation rather than less depression was present, for normal sera stimulate liver slice DNA synthesis above control

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2 N. L. R. Bucher, T. R. Schrock and F. L. Moolten, Johns Hopkins med. J. *125*, 250 (1969).
3 J. W. Grisham, Cancer Res. *22*, 842 (1962).
4 D. M. Hays, I. Tedo and Y. Matsushima, J. surg. Res. *9*, 133 (1969).
5 J. U. Levi and R. Zeppa, Ann. Surg. *174*, 364 (1971).
6 K. E. Paschkis, Cancer Res. *18*, 981 (1958).
7 R. L. Smythe and R. O. Moore, Surgery *44*, 561 (1958).
8 D. R. LaBrecque and L. A. Pesch, J. Physiol. *248*, 273 (1975).
9 G. F. Leong, J. W. Grisham, B. V. Hole and M. L. Albright, Cancer Res. *24*, 1496 (1964).
10 H. G. Preuss and H. Goldin, Med. Clins N. Am. *59*, 771 (1975).
11 H. G. Preuss and H. Goldin, J. clin. Invest. *57*, 94 (1976).
12 H. G. Preuss, E. F. Terryi and A. I. Keller, Nephron 7, 459 (1970).
13 W. C. Stadie and B. B. Riggs, J. biol. Chem. *154*, 687 (1958).

Table 1. Effects of sham sera and liver extracts from sham and partially hepatectomized (PH) rats on liver DNA synthesis

Incubation conditions	No. of experiments	*Spec act ± SEM** (DPM/μg DNA)	p	%
Sham sera	6	23.7 ± 6.0	<0.02	15.55
Sham extract	4	− 0.5 ± 3.9	NS	− 0.9
PH extract	4	− 1.0 ± 6.6	NS	− 0.8
Sham sera and Sham extract	4	8.9 ± 3.3	<0.1 >0.05	9.8

*Average difference between 4 sets of slices incubating under the test conditions listed compared to 4 sets of slices incubating in medium alone. **Additions were 10% v/v. NS, not significant.

Table 2. Effects of sera and liver extracts from Ph and Sham rats on liver DNA synthesis

Conditions	No. of experiments (positive)	*Spec act ± SEM	P	%
PH sera vs Sham sera	15/20	+12.4 ± 4.3	< 0.01	+ 9.9
PH extract + sera (PH or Sham) vs Sham extract + sera (PH or Sham)	16/20**	+23.4 ± 6.8	< 0.01	+19.0

*Spec act, difference in specific activity. **Results using PH and Sham sera are combined.

(table 1). This stimulation was somewhat specific. When the effects on renal DNA synthesis of sera from PH were compared to sera from Sham, there was significant depression in 8 experiments (–11%, $p < 0.05$).

Could liver be the source of circulating hepatotropin? Liver extracts from PH placed alone in medium failed to stimulate. LaBrecque and Pesch[8] also did not find any increase in ^{3}H-thymidine incorporation into DNA of liver slices or isolated liver cell suspensions by their hepatic extract which had previously stimulated DNA synthesis when injected in vivo. They felt that this was because a longer time than that used for in vitro incubation was necessary to show stimulation. However, in our previous studies on the renotropic system[11], we showed stimulation of DNA synthesis by renal extracts only when sera was present. Although liver extracts from PH rats did not stimulate DNA synthesis in slices when alone, they did increase this parameter in the presence of PH sera or Sham sera (table 2).

Our assay uses incorporation of ^{3}H-thymidine into DNA as an estimate of hyperplasia. While the use of slices offer similar initial intracellular precursor pools for DNA, non-specific extracellular dilution of tracer by sera could increase isotope incorporation into DNA in the test slice despite no actual change in DNA synthesis. However, this would appear with slices from any organ, and sera from PH actually decrease DNA synthesis in rat kidney slices. Similarly, finding that neither extracts from regenerating nor control livers affect DNA synthesis alone and that extracts from regenerating livers cause a relative stimulation to hepatic DNA synthesis only in the presence of sera also suggests that our results are not secondary to non-specific isotope dilution by extracts.

These in vitro findings are consistent with a proposal[5] that it may take hours for stimulators to develop, but a considerably shorter period of time for them to stimulate DNA synthesis. As a first approximation, we suggest that after loss of liver mass, there is a rise in a humoral factor and a liver factor that occurs within 20 h. Since the latter works only in the presence of sera, it could activate the serum factor which, in turn, stimulates DNA synthesis. If the liver is the only organ containing specific activator, this could explain why PH sera enhance liver slice DNA synthesis not renal.

Pharmacology and function of the myoepithelial cell in the eccrine sweat gland[1]

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Summary. Both acetylcholine and a Ca-ionophore, A23187, are comparatively strong stimulants of eccrine sweat secretion in vitro. Nevertheless, the contraction of the secretory coil was seen only after stimulation with acetylcholine but not with alpha or beta adrenergic drugs or with A23187. It was thus inferred that the myoepithelial contraction may not be playing an indispensable role in the overall process of eccrine sweat secretion.

The secretory coils of human, monkey, cat and other mammalian eccrine sweat glands consist of 3 cell types: clear, dark and myoepithelial cells. Because of its resemblance to other fluid and electrolyte transporting cells, the clear cell is generally believed to be responsible for secretion of water and electrolytes by the sweat gland[3]. The function of the dark cell is unknown, although secretion of a mucoid substance in sweat is often regarded as being its function. The myoepithelial cell is spindle-shaped, lies on the basement membrane, and is filled with masses of myofilaments. It is rather infrequent that foot processes of 2 opposing myoepithelial cells are bound to each other, and for this reason the myoepithelial cells are generally regarded as discontinuous cells[3]. The function of the myoepithelial cell is not clear, but the following hypotheses have been postulated: a) myoepithalial contraction expulses the preformed sweat onto the skin surface; b) the myoepithelial cell is a suppotive structure for the tubule; c) myoepithelial contraction opens the intercellular channel of the secretory cells[3]. The present study is thus intended to clarify the role of the myoepithelial cell in eccrine sweat secretion using an isolated single segment of the secretory coil. An isolated eccrine sweat gland responds to a number of sudorific agents in varying degrees[4]. If there is a dissociation between myoepithelial contraction as analyzed photomicrographically

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3 R. A. Ellis, in: Handbuch der Haut- und Geschlechtskrankheiten, vol. 1: Normale und pathologische Anatomie der Haut. Ed. J. Jadassohn. Springer-Verlag, Berlin, Heidelberg, New York 1967.

4 K. Sato, Rev. Physiol. Biochem. Pharmac., in press (1977).