We have not investigated the mechanism of this "sustaining effect." Casual examination of the living cultures did not reveal any slowing of growth due to hydrocortisone, but this possibility deserves investigation since conditions which retard cell metabolism may improve longevity of cultured cells when the medium is not renewed(13), and inhibition of mitotic rate by about 50% has been reported with prednisolone at 1 to 10 γ/ml in short term human leukocyte cultures(14).

The initial objective of these studies was to see if the synthetic progestins would cause a selective inhibition of the AdenoCx 1 cell line, since these drugs have clinical value in treatment of endometrial cancer(15) and this cell line is presumably a neoplastic endometrial (endocervical) cell. No such selective effect was observed, but generalization must be avoided since the exact nature of this cell line is not certain and the clinical responsiveness of the patient from whom it came is unknown.

Summary. The effect of 8 steroid hormones on 12 lines of human cells was studied in tissue culture. The steroids included estrogens, androgens, progestins, and hydrocortisone. The cell lines included amnion, epidermoid carcinomas, adenocarcinomas, and sarcomas. Cytotoxic effects were not acute, but developed after several days if at all. No consistent relationship was apparent between minimum cytotoxic dose and endocrinologic characteristics of the steroids or type of cell. Testosterone and androsterone were toxic to most cell lines at 10 γ/ml. The ovarian and cervix adenocarcinoma cells were sensitive to most of the steroids at 10 γ/ml. Toxicity at 1.0 γ/ml was rare. Hydrocortisone at 2.5 to 10 γ/ml greatly extended the duration of good growth in cell lines HEp 1 and RPMI-41.

The authors are also indebted to Dr. T. F. Gallagher of this Institute, Dr. E. C. Reifenstein, Jr., Squibb Inst. for Medical Research, New Brunswick, N. J., and Dr. S. S. Stubbs, Upjohn Co., Kalamazoo, Mich., for supplying steroids used for these studies.

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Received September 3, 1963. P.S.E.B.M., 1964, v115.

Influence of Repeated Subtotal Hepatic Resections on *in vitro* Survival of Rat Liver Cells. (28843)

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Subtotal hepatic resection in rats is followed by rapid regrowth of liver tissue, apparently due to an increased rate of cellular division in the hepatic remnant(1). Such liver regeneration has been studied by changes in gross liver size(2), liver weight (3) and liver DNA and RNA content(4,5, 6). Rate of liver regrowth varies inversely

TABLE I. Cell Yields of (a) Liver Tissue Obtained from Initial Resection (Control) and, (b) Liver Tissue Obtained from Multiple Resections (Experimental).

Wt of rats, g	No. of rats	No. of cell preparations	No. of plates	No. of hepatic excisions	Avg cell count/plate at 16 days
		Control			
30– 45	12	1	20	1	110,000
45– 60	15	1	20	1	58,000
95–115	18	2	40	1	130,000
140–160	4	1	20	1	0
195–210	6	1	20	1	45,000
230–250	4	1	20	1	0
290–350	6	1	20	1	0
		Experimental			
150–230	9	1	20	2	40,000
230	2	1	20	3	180,000
293	1	1	20	4	410,000
305–320	3	1	20	5	380,000

Standard error for the final 2 experimental groups, *i.e.* 410,000 cells/plate and 380,000 cells/plate were ± 82,000 cells and ± 69,600 cells respectively. Standard error could not be calculated for the control and other experimental groups, since small yields required pooling of cells.

with age of the animal(3) and is complete in approximately 21 days in mature rats(7). Rat liver can be cultivated *in vitro,* but progressively less successfully as rats age(8). At any age, a prior single hepatic resection increases the success of cultivation(9).

The purpose of the present study was to detect differences in cultivatability *in vitro* among (a) rat hepatic tissue following serial subtotal hepatic resections, (b) tissue resulting from a single resection and (c) normal (control) rat liver. The results indicate that a marked increase in *in vitro* cell yield occurred in hepatic tissue obtained following repeated hepatic resections.

Method. The technique of hepatic resection consisted of the removal of approximately 60% of the liver under sterile conditions at each operation. Estimation of the percent resected is admittedly difficult. During the course of repeated resections the remaining liver formed a spherical mass. Liver regrowth appeared complete at each laparotomy except following the fifth resection when the volume remaining appeared to be smaller than previously.

Twenty-one day intervals were allowed between each resection. The experimental and control groups were fed a standard diet and were operated upon on alternate weeks using the same equipment, personnel and techniques.

A single subtotal hepatic resection was performed on a control group of 65 male Long-Evans rats of varying weights. These animals were divided by weight into 7 graded groups. The smallest group was 30-45 g in weight, and the largest 290-350 g (Table I).

The experimental group consisted of 15 male Long-Evans rats (Table I). In 9, specimens for tissue culture were taken at the second hepatic resection; in 2 at the third hepatic resection; in one at the fourth hepatic resection; and in 3 at the fifth hepatic resection. Weights of these rats varied from 150 to 320 g during the time span of the 5 resections.

Liver tissue was obtained under sterile conditions, minced, trypsinized and cultivated in Petri plates (1.8×10^6 cells/plate) in Eagle's medium supplemented with 10% calf serum at 37°C in 5% CO_2. The medium was changed 4 times at 3-day intervals. The cells were trypsin-dispersed and counted in a hemocytometer on the 16th day following inoculation.

Results. Moderate cell yield in tissue culture (58,000-130,000 cells/plate) was noted in liver tissue from the 3 groups of control rats with weights less than 125 g. In the 4 groups of larger control rats, there was minimal cell yield (45,000 cells/plate) in only one group (195-210 g). There was no cell survival from tissue obtained from any of the controls which weighed more than 230 g.

In the experimental group, cell yield was relatively large (180,000 cells or more/plate) in all 3 groups of rats having more than 2 hepatic resections. Rats in these 3 groups all weighed more than 230 g. In the groups having 4 and 5 hepatic resections (weights 290-320 g) the cell yield was heavier (380,000 cells/plate and 410,000 cells/plate respectively), which is more than 3 standard deviations greater than the control group.

In a corollary experiment, in our laboratory, autotransplants of regenerating hepatic tissue to subcutaneous tissue and to peritone-

al surfaces did not survive in a recognizable form either grossly or histologically.

Discussion. Histologically, regenerating hepatic tissue contains a dense concentration of nuclei and evidence of increased mitotic activity(1,10) but no apparent change in the relative numbers of stromal and parenchymal cells(11). Liver DNA content is elevated(5) and there is increased p32 uptake(12).

In the present studies the liver remnant tripled its size in 3 weeks, following massive hepatic resection. This occurred repeatedly in the same rats for at least 4 successive resections with some suggestion of a diminution of the response at the fifth resection.

Increased cell yield *in vitro* was apparent in liver from all animals that had undergone more than 2 hepatic resections. Multiple prior resections superceded relative maturity of the animal as the dominant factor in influencing cell yield *in vitro*.

As cell yield was measured in these experiments rather than cell growth rate, it is possible that a change in the ability of the hepatic cells to survive and initiate growth might account for the observed results. Such a change could be the result of an unusual proliferation of fibroblasts in the regenerating liver tissue after repeated resection. This has not been apparent in histological sections, however. Although it would seem unlikely, such a change could also result from increased resistance of the liver cells to trypsin following serial resections.

The nature of the factor stimulating hepatic regeneration is controversial(13,14) and the attempts to identify it as a humoral agent employing parabiotic rats are contradictory(2,14). The present study suggests that a humoral agent is not a factor in increased *in vitro* cell survival as it would have been diluted out during the trypsinization and cultivation procedures. Studies using serum from partially hepatectomized rats in an isolated tissue culture system would contribute additional evidence.

Summary. Hepatic tissue from immature rats demonstrates minimal cell yield in tissue culture while hepatic tissue from mature rats (> 230 g) does not survive under the conditions employed. Mature rat (> 230 g) hepatic tissue obtained following repeated subtotal hepatic resections in the same animal demonstrates relatively large cell yield in tissue culture, using the same technique. The yield in tissue culture using hepatic tissue from mature rats following repeated resection is greater than in any of the control groups, including the most immature (weanlings) rats, and markedly greater than in tissue obtained following single hepatic resection.

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Received September 3, 1963. P.S.E.B.M., 1964, v115.